カレトラ・ソフトカプセル
カレトラ・リキッド
（ロピナビル／リトナビル）
に関する資料

ダイナボット株式会社

カレトラ・ソフトカプセル

(KALETRA・SOFT CAPSULES)

(Lopinavir／Ritonavir)

輸入承認申請書添付資料概要（英文）

ダイナボット株式会社

カレトラ・リキッド

（KALETRA・LIQUID）

（Lopinavir／Ritonavir）

輸入承認申請書添付資料概要（英文）

ダイナボット株式会社

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Pharmacologic Class, Scientific Rationale, Intended Use, and Potential Benefits

2.2 Pharmacologic Class, Scientific Rationale, Intended Use, and Potential Benefits

2.2.1 Pharmacologic Class

ABT-378/ritonavir is a peptidomimetic inhibitor of both the HIV-1 and HIV-2 proteases. Inhibition of HIV protease prevents cleavage of the *gag-pol* polyprotein resulting in the production of immature, non-infectious virus.

2.2.2 Scientific Rationale

2.2.2.1 HIV Disease Considerations

Human immunodeficiency virus (HIV) infection is a chronic disease with an invariably fatal outcome if untreated. HIV, both directly and indirectly, mediates the destruction of CD4 cells with a profound disturbance of cellular immunity. Untreated, the disease almost always results in characteristic opportunistic infections such as *Pneumocystis carinii* pneumonia, toxoplasmosis, opportunistic fungal infections, Kaposi's sarcoma, and disseminated mycobacterial infections. In its terminal phase, the nearly complete depletion of effective cellular immunity allows for unchecked viral growth and a rapidly downward clinical course.

Although the natural history can be long, including years of clinical latency, it is not a disease of indolent pathology but a consumptive, destructive process involving prodigious rates of viral turnover. The HIV life cycle is characterized by a prolific replication rate (>10^{11} particles per day) and a high mutation rate, the latter a consequence of an inherent error rate of $3x10^{-5}$ in reverse transcription.[1]

2.2.2.2 Therapeutic Considerations

Given the rapid turnover of HIV, intervention with potent drugs that significantly interrupt the replication cycle can produce dramatic changes in the natural progression of HIV. This has been demonstrated with the introduction of HIV protease inhibitors (PI) into clinical practice in the mid-1990s, and the subsequent declines in HIV morbidity and mortality.[2, 3, 4] By the same token, failure to adequately suppress viral replication permits

the rapid selection of drug resistance mutations, viral rebound, and the resumption of disease progression.

These viral dynamics also dictate that effective inhibition of replication requires the continuous presence of suppressive drug concentrations.[5] Since mutational escape is driven by ongoing replication, it is not surprising that the degree of viral suppression predicts the durability of response.[6] Using conservative assumptions for the daily viral production rate, a missed dose early in therapy that allows concentrations to fall below the effective drug concentration required for 50% inhibition of in vitro viral replication (EC_{50}) for 8 hours would result in production of more than $3x10^9$ virions under the conditions of high selective pressure. Although the consequences of missed doses diminish later in therapy when viral load is decreased, the cumulative toll of persistent viral replication under these conditions virtually guarantees the eventual emergence of drug-resistant virus.

These considerations argue that for protease inhibitors, which are lipophilic compounds that do not undergo intracellular metabolism, plasma trough concentrations are critical to antiviral activity.[7] In the context of combination therapy, the pharmacokinetics of individual drugs may be less apparent but no less important, as corroborated by evidence that ongoing replication persists even during "potent" combination treatment.[8] The need for maximizing protease inhibitor trough levels is further supported by the varying levels of adherence seen in the clinical setting, with higher trough levels providing a margin of error for inevitably erratic doses. Substantial evidence has accumulated relating virologic failure with suboptimal protease inhibitor drug levels.[9-14] The need to maximize drug levels must, however, be balanced by considerations of tolerability, toxicity, and pill burden, as poor drug adherence resulting from these therapeutic issues will ultimately defeat superior pharmacokinetics. The concept of a therapeutic margin can thus be defined as the range of drug exposures over which highly suppressive antiviral activity and acceptable tolerability coexist. Drugs with a poor, narrow, or wide therapeutic margin can be represented schematically by Figures 1, 2, and 3, respectively:

Figure 1

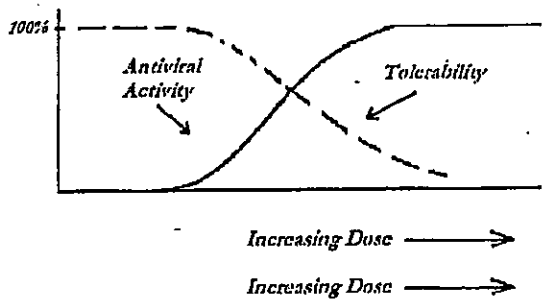


Figure 2

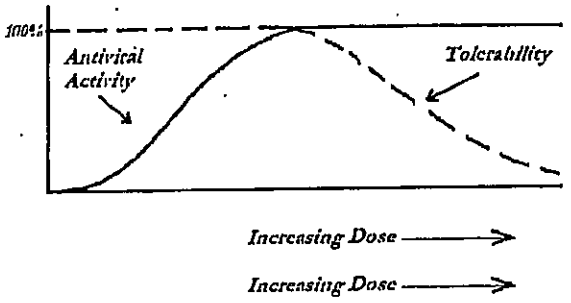
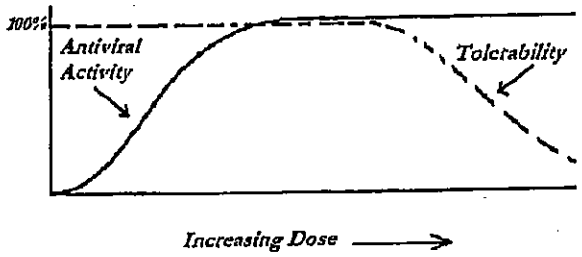


Figure 3



At present, 5 protease inhibitors have been approved for the treatment of HIV infection: saquinavir (Invirase™ and Fortovase™), ritonavir (Norvir™), indinavir (Crixivan™), nelfinavir (Viracept™), and amprenavir (Agenerase™).[15-20] While these compounds are highly potent *in vitro*, all have poor and/or highly variable bioavailability owing to high clearance. Despite frequent administration of high doses, drug levels only modestly exceed estimated *in vivo* inhibitory concentrations. At their indicated doses, the mean trough plasma levels of these drugs are in the range of 1- to 6-fold the protein binding-corrected EC_{50} for wild-type HIV.[21] There is wide variability in cytochrome P450 3A isoform (CYP3A) across subjects and the dispersion (coefficient of variation) of minimum observed plasma concentration (C_{min}) is larger than that of any other pharmacokinetic variable. Furthermore, adherence to medication and timing of dosing are invariably less than perfect. Thus, in the clinical setting, drug levels of these agents

are within the dose-response range for antiviral activity against wild-type HIV. Although pharmacokinetic modulation of existing protease inhibitors with ritonavir can improve their ratio of C_{min} to EC_{50} with corresponding improvement in antiviral activity,[22] substantial increases in overall drug exposures may be associated with increased toxicity.

Thus the available protease inhibitors are hampered by either limited bioavailability or poor-to-narrow therapeutic margins, as defined above. These suboptimal characteristics are reflected in current clinical experience, where it has become clear that the durability of response to highly active antiretroviral therapy (HAART) is far from optimal. In clinical trials of protease inhibitor-based regimens, response in approximately 2/3 of subjects are obtained after 24 weeks, but diminish subsequently.[23] The experience of clinicians and data such as these have dampened the initial enthusiasm for protease inhibitors, and point to the need for drugs with wider therapeutic margins.

2.2.2.3 ABT-378

The critical objectives for the development of a new HIV protease inhibitor were twofold: (1) a high C_{min}/EC_{50} ratio, significantly greater than that of available agents, and (2) a highly acceptable safety and tolerability profile. ABT-378 has approximately 10-fold greater *in vitro* potency than ritonavir, resulting in an EC_{50} of approximately 0.1 μM for wild type HIV in 50% human serum. Pharmacokinetic studies in animals demonstrated relatively unremarkable bioavailability of ABT-378 when dosed alone. Coadministration of ABT-378 with ritonavir, however, substantially improved the pharmacokinetic profile of ABT-378, as a consequence of potent inhibition of CYP3A-mediated metabolism of ABT-378 by ritonavir.[24,25] Based on these early preclinical results, the goal of achieving a high C_{min}/EC_{50} ratio seemed likely, and clinical development was initiated.

2.2.3 Intended Use

ABT-378/ritonavir is indicated in combination with other antiretroviral agents for treatment of HIV-infection.

2.2.4 Potential Clinical Benefits

Results observed in this clinical program have shown ABT-378/ritonavir at the 400 mg/100 mg BID dose to be very well-tolerated and highly effective in suppressing viral replication among both antiretroviral-naive and antiretroviral-experienced HIV-infected subjects. The major therapeutic benefits associated with ABT-378 400 mg/ritonavir 100 mg therapy in HIV-infected subjects are outlined below.

Benefits

- Potent antiviral activity, as reflected by:
  - High response rate in both treatment-naïve and treatment-experienced subjects
  - High response rate in subjects with high viral load and low CD_4 cell count
  - Durability of virologic response, shown through 72 weeks in 2 phase II studies
- Immunologic improvement, as reflected by increased CD_4 cell count in all treatment populations
- Dosing convenience
  - coformulated ABT-378 and ritonavir
  - low pill burden of 3 capsules twice daily
  - food recommendation permits dosing with meals
  - availability of an oral solution
- Overall favorable tolerability

Overall, the therapeutic benefit/risk ratio is very favorable and reflects the wide therapeutic margin provided by ABT-378/ritonavir when administered at a dose of 400 mg/100 mg BID.

ABT-378/ritonavir answers an unmet medical need in the therapy of HIV-infected subjects, and provides substantial therapeutic benefit with relatively little risk.

2.2.5 References

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2. Commercial Marketing (1)

ABT-378/ritonavir

Documents for Application Summary

Section 2.3

Commercial Marketing

2.3 Commercial Marketing Experience

ABT-378/ritonavir is not currently commercially available anywhere in the world.

3. Chemistry, Manufacturing, and Controls Summary (1-40)

ロ. 物理的化学的性質並びに規格及び試験方法
ハ. 安定性

I. 有効成分に関する理化学的知見

1．名　称

(1) 一般名：ロピナビル(lopinavir) / リトナビル(ritonavir)

(2) 化学名：

ロピナビル（JAN)

(-)-(2*S*)-*N*-{(1*S*,3*S*,4*S*)-1-benzyl-4-[2-(2,6-dimethylphenoxy)acetylamino]-3-hydroxy-5-phenylpentyl}-3-methyl-2-(2-oxotetrahydropyrimidin-1-yl)butyramide

リトナビル：(JAN)

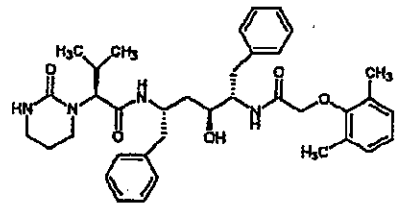
(+)-5-thiazolylmethyl[(α*S*)-α-[(1*S*,3*S*)-1-hydroxy-3-[(2*S*)-2-[3-[(2-isopropyl-4-thiazolyl)methyl]-3-methylureido]-3-methylbutyramido]-4-phenylbutyl]phenethyl]carbamate

(3) 分子式：$C_{37}H_{48}N_4O_5$（ロピナビル）/ $C_{37}H_{48}N_6O_5S_2$（リトナビル）

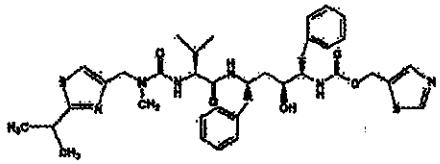
(4) 分子量：628.80（ロピナビル）/ 720.96（リトナビル）

(5) 構造式：

ロピナビル



リトナビル



2．物理的化学的性質（ロピナビル）

(1) 性　状：本品は白色～淡黄色の粉末又は塊である.

(2) 溶解性：本品はメタノール，エタノールに溶けやすく，イソプロパノールにやや溶けやすく，水にはほとんど溶けない.

(3) 吸湿性：95%RH で 4.5～5.0%の重量増加を示した.

II. 製剤学的事項

1．組成

ロピナビル/リトナビル：133.3mg/33.3mg（1カプセル当たり）

80mg/20mg（1mL当たり）

2．性状

ソフトカプセル：本品はだいだい色・長楕円形の軟カプセルである．

リキッド：本品は淡黄色～淡褐色の澄明な液体である．

3．安定性

カレトラ・ソフトカプセル

保存条件	保存期間	保存状態	結果
5℃（長期保存）	12ヵ月	白色ポリエチレン瓶又はブリスター包装	変化なし
25℃ RH60%（加速）	6ヵ月		変化なし

カレトラ・リキッド

保存条件	保存期間	保存状態	結果
5℃（長期保存）	12ヵ月	PETボトル	変化なし
25℃ RH60%（加速）	6ヵ月		変化なし

4. Labeling (1-3)

4.0

LABELING

4.1

DRAFT LABELING

©Abbott

0074395977

Exp.
Lot
DN0603-V1

NDC 0074-3959-77
180 Capsules

PROPRIETARY NAME®

Each soft gelatin capsule contains:
lopinavir .....133.3 mg
ritonavir .....33.3 mg

℞ only

Do not accept if band on cap is broken or missing.

Dispense in original container.

Store in refrigerator between 36°- 46°F (2°- 8°C). Avoid exposure to excessive heat.

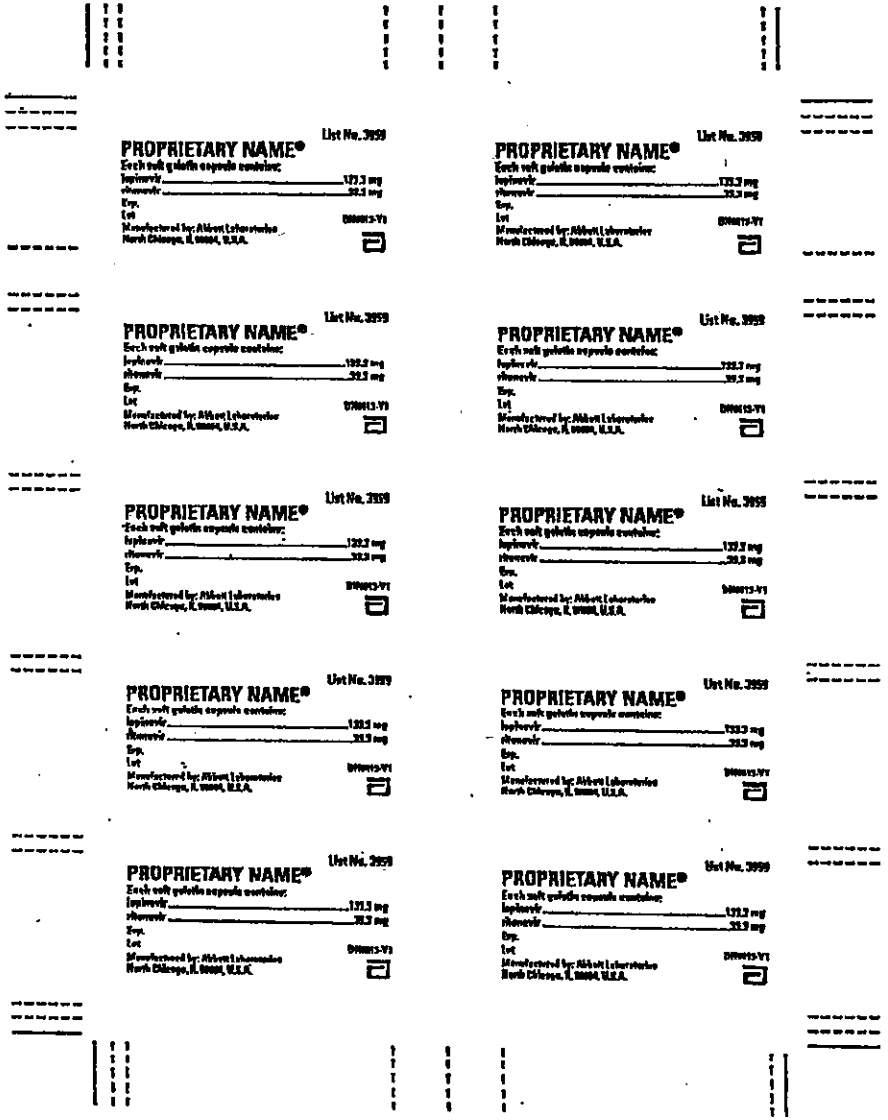
Refrigeration by patient is not required if used within 42 days and stored below 77°F (25°C).

Use by product expiration date.

See enclosure for prescribing information.

Abbott Laboratories
North Chicago, IL 60064,
U.S.A.

INFORMATION COPY



NDC 0074-3959-11
120 Capsules

PROPRIETARY NAME®

Each soft gelatin capsule contains:
lopinavir ....133.3 mg
ritonavir ....33.3 mg

NDC 0074-3959-11
120 Capsules

PROPRIETARY NAME®

Each soft gelatin capsule contains:
lopinavir ....133.3 mg
ritonavir ....33.3 mg

℞ only

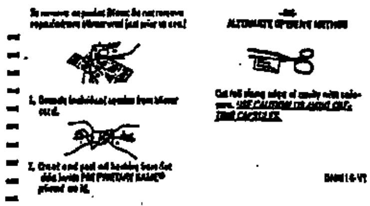
PROPRIETARY NAME®

Each soft gelatin capsule contains:
lopinavir ....133.3 mg
ritonavir ....33.3 mg

Store in refrigerator between 36°-46°F (2°-8°C). Avoid excessive heat.
Refrigeration by patient is not required if used within 42 days and stored below 77°F (25°C).
Use by product expiration date.
See enclosure for full prescribing information.
[illegible]
Abbott Laboratories
North Chicago, IL [illegible], U.S.A.

PROPRIETARY NAME®

Each soft gelatin capsule contains:
lopinavir ....133.3 mg
ritonavir ....33.3 mg



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5. General Pharmacology Overview (1-21)

ABT-378/ritonavir

Documents for Application Summary

Section 2.5.1

General Pharmacology Overview

2.5.1 General Pharmacology

The human immunodeficiency virus (HIV) protease is a constitutive enzyme that processes viral proteins essential for the maturation of infectious virions. Protease inhibitors block viral maturation and are active in acutely and chronically infected cells. The introduction of HIV protease inhibitors into combination antiretroviral regimens has led to a dramatic decline in the morbidity and mortality associated with HIV infection.[1-3] Combination regimens including a protease inhibitor lead to profound and sustained suppression of viral replication.[4,5] At present, five protease inhibitors have been approved for the treatment of HIV infection: saquinavir (Invirase™ and Fortovase™), ritonavir (Norvir™), indinavir (Crixivan™), nelfinavir (Viracept™), and amprenavir (Agenerase™).[6-11] While all of these compounds are highly potent *in vitro*, each of the currently available protease inhibitors is limited by one or more properties that affect its use in HIV-infected subjects. These limitations include moderate or poor oral bioavailability, high protein binding, significant side effects, frequent high doses, and inconvenient dosing schedules with strict dietary requirements.

ABT-378 is a novel peptidomimetic HIV protease inhibitor with approximately 10-fold greater *in vitro* potency than ritonavir. The EC_{50} (effective drug concentration required for 50% inhibition of *in vitro* viral replication) of ABT-378 for wild type HIV in 50% human serum is approximately 0.1 μM. Pharmacokinetic studies in animals have demonstrated relatively unremarkable bioavailability of ABT-378 when dosed alone. Coadministration of ABT-378 with ritonavir, however, substantially improves the pharmacokinetic profile of ABT-378.[12,13] These pharmacokinetic properties are attributable to the rapid cytochrome P450 3A isoform (CYP3A)-mediated metabolism of ABT-378 and its inhibition by ritonavir. Based on these early preclinical results, the

development program for ABT-378/ritonavir was designed to evaluate the safety and antiviral activity of ABT-378 coadministered with ritonavir as a pharmacokinetic enhancer.

ABT-378/ritonavir at the proposed registrational dose has been or is currently being evaluated in 28 clinical studies conducted worldwide in which 2628 HIV-infected subjects have received ABT-378/ritonavir. Results have shown the combination to be effective in producing profound and sustained suppression of viral replication. In addition, the combination has been demonstrated to be well tolerated among both antiretroviral-naïve and antiretroviral-experienced HIV-infected subjects.

To investigate ABT-378 for possible ancillary pharmacologic activity, this compound was evaluated in a battery of receptor binding and ion transport assays as well as in functional assays for the central nervous (CNS) and cardiovascular (CV) systems. In the *in vivo* functional studies, ABT-378 was co-administered with ritonavir to enhance ABT-378 exposure and to model the combined effects that might be expected during clinical administration. A summary of the tests performed and results obtained are listed in Appendix A.

ABT-378, in the absence of serum proteins, was evaluated in various receptor and ion channel binding assays at concentrations of 0.3, 1, 3, and 10 μM. The most notable effects of ABT-378 occurred at the 10 μM concentration which reduced the binding of reference radioligands 47% to 54% of control at the L-type calcium channel, 62% of control at the sodium channel site 2 and 47% of control at the chloride ionophore. Inhibitions of less than 15% occurred in all potassium channels assays and at the muscarinic M_3 receptor site.[14]

ABT-378 was evaluated for interactions at certain classes of potassium (K^+) channels by membrane potential, cation efflux and radioligand binding assays. ABT-378 (0.1 nM to 1 μM) did not alter the membrane potential in a smooth muscle cell line (A10) indicating

that it had no effect on the K^+ channels expressed in this cell line. ABT-378 (10 pM to 10 μM) also failed to displace the binding of [^{125}I]charybdotoxin to rat brain-derived membranes indicating that it had no interaction with the voltage-gated K^+ channels or calcium-activated maxi-K^+ channels labeled by [^{125}I]charybdotoxin. In a preliminary study to assess effects on calcium-activated maxi-K^+ channel function, ABT-378 (1 nM to 10 μM) did not inhibit ionomycin-stimulated $^{86}Rb^+$ influx into C6 glioma cells and did not alter the basal levels of $^{86}Rb^+$ influx into C6 glioma cells.[15]

The combined administration of ABT-378 and ritonavir was evaluated for potential central nervous system (CNS) effects in tests of locomotor stimulation/depression, motor coordination, hypnotic potentiation, pro-convulsant and anticonvulsant activity, and nociception. Effects on rectal temperature were measured and the gross physiological, behavioral and toxic effects were assessed in a preliminary observation test. ABT-378 and ritonavir were administered orally in combination as follows: 10 and 5 mg/kg, 30 and 15 mg/kg, 100 and 50 mg/kg, and 300 and 150 mg/kg of ABT-378 and ritonavir, respectively.[16]

The co-administration of ABT-378 (10 mg/kg, p.o.) and ritonavir (5 mg/kg, p.o.) had no effect in the preliminary observation test in mice. The dose combinations of 30 mg/kg, p.o. and 15 mg/kg, p.o. as well as that of 100 mg/kg, p.o. and 50 mg/kg, p.o. of ABT-378 and ritonavir, respectively, produced loss of traction, stereotypies (chewing) and ptosis in one of four mice examined. The dose combination of 300 mg/kg, p.o. and 150 mg/kg, p.o. of ABT-378 and ritonavir, respectively, produced loss of traction, stereotypies (chewing) and ptosis in two of four mice examined as well as slight sedation and hypothermia.

In the dose range tested, the co-administration of ABT-378 and ritonavir had no effects on locomotor activity of rats or motor coordination of mice, and had no clear effects in the hot plate test in mice, tail-flick test in rats or on rectal temperature in mice. The co-

administration of ABT-378 and ritonavir at 300 and 150 mg/kg, p.o., respectively, significantly increased barbital-induced sleep in mice. The dose combinations of 10 and 5 mg/kg, p.o., as well as of 30 and 15 mg/kg, p.o., significantly increased the effects of ethanol in mice, but no clear effects were observed at the larger dose combinations.

The co-administration of ABT-378 and ritonavir significantly decreased electroshock threshold in mice in a non-dose related manner at the dosing combinations of 10 and 5 mg/kg, p.o., 100 and 50 mg/kg, p.o., and 300 and 150 mg/kg, p.o. The dose combination of 30 and 15 mg/kg, p.o. did not have a significant effect on electroshock threshold. In contrast, the latency and number of convulsions induced by pentylenetetrazol in mice were unaffected by the combined administration of ABT-378 and ritonavir, with the exception that the dose combination of 300 and 150 mg/kg, p.o. tended to increase the number of tonic convulsions.

The cardiovascular profile of ABT-378 co-administered with ritonavir was evaluated in rats instrumented with telemetry transmitters.[17] Conscious male rats were dosed by oral gavage with either vehicle or a fixed combination (2:1) of ABT-378 and ritonavir of 10:5, 30:15, or 100:50 mg/kg, respectively. The dose combination of 10:5 mg/kg had no effect on heart rate or blood pressure, but dose combinations of 30:15 and/or 100:50 mg/kg produced mild, sustained decreases in heart rate. In the vehicle treated animals, heart rate was elevated by 6-7% at 6 hours after dosing whereas heart rate was reduced by 8% in animals receiving the 100:50 mg/kg dose combination. These negative chronotropic effects were observed at peak plasma concentrations of 6.08 ± 0.73 μg/mL of ABT-378 and 2.34 ± 0.75 μg/mL of ritonavir (6 hour time point).

In a second cardiovascular study, pentobarbital-anesthetized male beagle dogs were instrumented to measure both myocardial function and hemodynamic parameters.[17] ABT-378 (2, 6 and 20 mg/kg) and ritonavir (1, 3 and 10 mg/kg) were intravenously infused at a fixed 2:1 ratio in one group while vehicle was infused in a second group. Each of the 3

dosing combinations was administered over a 30 minute time period for a total infusion protocol time of 90 minutes. Intravenous infusion of the low dose combination (2:1 mg/kg, respectively) produced little or no cardiovascular response. After administration of the 6:3 mg/kg dose combination, there were statistically significant reductions in blood pressure and heart rate. However, these changes (which occurred at plasma concentrations of 11.95 ± 0 .92 and 3.68 ± 0.38 μg/mL, respectively) were modest and are not considered to be physiologically relevant. Administration of the 20:10 mg/kg dose combination produced marked and sustained decreases in blood pressure (-12% systolic and -32% diastolic), heart rate (-43%), and left ventricular contractility (dP/dt max, -30%). The decrease in contractility was accompanied by increases in central venous and left ventricular end-diastolic pressures. These negative chronotropic and inotropic responses were observed at peak plasma concentrations of 33.37 ± 1.15 and 17.45 ± 0.64 μg/ml, respectively. The responses were largely maintained for a 60-minute post-infusion period (final plasma concentrations of 21.75 ± 1.33 and 8.33 ± 0.82 μg/mL). It should be noted that plasma levels of sodium pentobarbital were rising in parallel with administration of ABT-378/ritonavir, possibly due to inhibition of CYP3A4 mediated metabolism by ritonavir. The plasma concentrations of sodium pentobarbital were elevated from a control value of 35.6 ± 2.1 to 49.1 ± 4.0 μg/mL at the end of the final infusion period. Therefore, elevated plasma levels of pentobarbital could have been a factor in the etiology of these responses.

To address the possible contribution of anesthesia and/or increased pentobarbital levels to the cardiovascular responses noted above, a third cardiovascular study was performed in conscious beagle dogs chronically instrumented with telemetry transmitters for measurement of systemic arterial pressure and heart rate.[17] The experimental design was a randomized, 7-way crossover using oral administration of 2:1 fixed dose combinations of ABT-378 and ritonavir. The seven treatment groups were 3:1.5, 10:5, 30:15, 100:50, 0:50, 100:0, and 0:0 mg/kg, p.o. of ABT-378 and ritonavir, respectively. In this study,

neither mean arterial pressure or heart rate was altered at doses up to and including 100:50 mg/kg, p.o. Following administration of the highest dose combination, plasma concentrations of ABT-378 and ritonavir reached a C_{max} of 23.47 ± 1.62 and 23.00 ± 2.93 μg/mL at a T_{max} of 4.0 ± 0.7 and 3.7 ± 0.6 hours, respectively. Thus, the combined administration of ABT-378 and ritonavir produced no cardiovascular effects in conscious beagles at plasma concentrations in excess of those achieved by the end of the protocol in the anesthetized dog study.

Finally, as a follow-up investigation of one incident of ventricular tachycardia observed in a phase I clinical study (M97-650), a fourth cardiovascular study was performed in pentobarbital-anesthetized beagle dogs instrumented to measure blood pressure, heart rate and electrocardiographic indices.[18] Intravenous infusion of ABT-378 and ritonavir at 10:1.5 mg/kg over 60 minutes produced modest decreases of 6 ± 2% and 16 ± 3% in mean arterial pressure and heart rate, respectively. The PR interval was increased by 13 ± 4% at the end of the infusion, while QTc (Bazett's formula) was unaffected. These modest hemodynamic and electrocardiographic effects were associated with plasma concentrations of 10.17 ± 0.64 mg/mL and 0.93 ± 0.15 μg/mL of ABT-378 and ritonavir, respectively. When the infusion rate was increased to deliver additional ABT-378 and ritonavir at a dosing ratio of 20:1.5 mg/kg over a subsequent 60 minutes, mean arterial pressure and heart rate were significantly decreased by -14 ± 2% and -37 ± 2%, respectively. These effects were accompanied by a significant increase in PR interval of 37 ± 6%, while QTc interval was unchanged. Plasma concentrations of 22.41 ± 2.91 μg/mL and 1.30 ± 0.28 μg/mL of ABT-378 and ritonavir, respectively, were achieved by the end of this 20:1.5 mg/kg infusion. The prolonged PR interval may be related, in whole or in part, to the decrease in heart rate.[19] In addition, the electrocardiogram was monitored for ventricular ectopic beats and none were seen.

In summary, ABT-378 was found to increase barbital and ethanol sleep times as well as decrease electroconvulsive shock threshold in mice when administered with ritonavir at doses (10 and 5 mg/kg, p.o., respectively) which likely yielded plasma concentrations (low μM) near therapeutic. Only modest, if any, effects on other CNS, CV, receptor or ion channel functions were found at therapeutic to super therapeutic doses/plasma concentrations. These results suggest that CNS and CV adverse effects are not likely to be prominent in clinical studies.

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Appendix A
Tabulation of General Pharmacology Studies

TEST	SPECIES	DOSE, ROUTE	RESULTS
Receptor Binding and Ion Transport Assays	Muscarinic M_3, Ca channel (L and N types), K channel (ATP-sensitive, voltage-dependent, Ca-dependent), Na channel (sites 1 and 2) and Cl ionophore and ion transport [basal activity: Ca channel L-type, Na channel] [stimulated activity: Ca-ATPase, L-type Ca channel, Na channel, Na-K-ATPase, Na/Ca antiport, Na/H antiport, Na/K/Cl cotransport	0.3, 1, 3,10 μM (n = 2)	At 10 μM, binding of reference radioligands reduced 47% to 54% of control at the L-type Ca channel, 62% of control at the Na channel site 2 and 47% of control at the Cl ionophore. Inhibitions less than 15% in all K channel assays.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
Receptor Binding and Ion Transport Assays (cont.):			
Membrane Potential Studies of Potassium Channel Function	Changes in membrane potential assessed using the bis-oxonol dye $DiBAC(4)_3$ in Rat smooth muscle. A10 cell line that express ATP-sensitive K^+ channels and calcium-activated K^+ channels.	0.1 nM to 1 µM (n=3)	No effect on membrane potential in the smooth muscle cell line (A10) within the concentration range tested.
$[^{125}I]$Charybdotoxin binding	$[^{125}I]$Charybdotoxin high affinity (pM) binding to calcium-activated K^+ channels in rodent brain membrane protein including the maxi-K^+ subtype and various K^+ channels including the Shaker family of voltage-gated channels (K_V 1.2, K_V 1.3).	10 pM to 10 µM (n=2)	No displacement of $[^{125}I]$charybdotoxin binding within the concentration range tested.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
Receptor Binding and Ion Transport Assays (cont.): Calcium-activated Potassium Channel Function	$^{86}Rb+$ influx stimulated by the calcium ionophore ionomycin in rat C6 glioma cells.	1 nM to 10 µM (n=1)	No inhibition of ionomycin-stimulated cation influx within the concentration range tested.
CNS Profile:			
Preliminary Observation (Irwin)	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 4)	No changes from control at 10:5 mg/kg dose combination. Loss of traction, stereotypies and ptosis in 1 of 4 mice at dose combinations of 30:15 and 100:50 mg/kg up to 30 and 60 min. after dosing, respectively. Similar effects plus slight sedation and slight hypothermia at the highest dose combination of 300:150 mg/kg up to180 min. after dosing.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Activity Motor (Locomotor) Function)	Rat	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on locomotor activity as compared with the propyleneglycol-ethanol vehicle. Caffeine (16 mg/kg, p.o.) increased locomotor activity by 22% and chlorpromazine (16 mg/kg, p.o.) decreased activity by 71%.
Rotarod (Motor Coordination)	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on motor coordination as compared with the propyleneglycol-ethanol vehicle. Diazepam (8 mg/kg, p.o.) decreased drop-off time 97%.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued): Barbital Interaction	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	Sleep duration was significantly increased at the dose combination of 300:150 mg/kg, p.o., as compared with the propyleneglycol-ethanol vehicle. Diazepam (4 mg/kg, p.o) increased sleep duration 233%, and caffeine (16 mg/kg, p.o.) completely abolished sleep.
Ethanol Interaction	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	The dose combinations of 10:5 and 30:15 mg/kg, significantly increased sleep time as compared with the propyleneglycol-ethanol vehicle. No significant effects were observed at the higher dose combinations, although a similar tendency was apparent at the 100:50 mg/kg, p.o., dose combination. Diazepam (8 mg/kg, p.o.) induced sleep

in all animals.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Electroconvulsive Shock (ECS) Threshold	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 15)	The dose combinations of 10:5, 100:50, and 300:150 mg/kg, p.o., significantly decreased ECS threshold (50%, 25% and 47%, respectively) as compared with the propyleneglycol-ethanol vehicle. The dose combination of 30:15 mg/kg had no effect on seizure threshold. RO 15-4513 (64 mg/kg, p.o.) decreased seizure threshold (18%) and diazepam (8 mg/kg, p.o.) increased ECS intensity from control 154%.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Pentylenetetrazol (PTZ) Induced Seizure	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	The dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg did not clearly affect the number of convulsions and deaths as compared with the propyleneglycol-ethanol vehicle. However, the dose combination of 300:150 mg/kg tended to increase the number of convulsions. RO 15-4513 (64 mg/kg, p.o.) significantly decreased clonic convulsion and death latencies, and diazepam (8 mg/kg, p.o.,) completely antagonized the PTZ-induced convulsions and death.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Hot Plate (Nociception)	Rat	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on foot-lick or jump latencies as compared with the propyleneglycol-ethanol vehicle.. Morphine (64 mg/kg, p.o.) increased latency to foot-lick by 175%.
Tail-Flick (Nociception)	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on tail-flick withdrawal latency as compared with the propyleneglycol-ethanol vehicle.. Morphine (128 mg/kg, p.o.) increased latency to tail-flick 388%.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Rectal Temperature	Mouse	ABT-378/ritonavir dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effects rectal temperature as compared with the propyleneglycol-ethanol vehicle. Chlorpromazine (16 mg/kg, p.o.) significantly decreased body temperature.
Cardiovascular Profile:			
Hemodynamic Evaluation	Conscious Rats	ABT-378/ritonavir dose combinations of 10:5, 30:15, and 100:50 mg/kg, p.o. (n=6/group, 4 groups)	Modest (15% max) reduction in heart rate with higher two doses. Plasma concentrations at peak effect were 6.08 ± 0.73 µg/ml of ABT-378 and 2.34 ± 0.75 µg/ml of ritonavir.

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
Cardiovascular Profile (cont.):			
Myocardial Function and Hemodynamic Evaluation	Anesthetized Dogs	ABT-378/ritonavir dose combinations of 2:1, 6:3, and 20:10 mg/kg, i.v. 30 min. infusion/ dose, 3 doses/ animal (n=6/group, 2 groups)	Low dose produced no effect. Very modest depressor and bradycardic responses after middle dose (plasma concentration of 11.95 ± 0.92:3.68 ± 0.38 µg/ml). High dose produced marked decreases in blood pressure, heart rate, and contractility, which were sustained for ≥60 min. after end of infusions. Peak plasma concentrations were 33.37 ± 1.15: 17.45 ± 0.64 µg/ml, with levels of 21.75 ± 1.33: 8.33 ± 0.82 µg/ml at 60 min. after end of infusions. Plasma levels of pentobarbital were elevated by the end of the protocol (ritonavir inhibits CYP3A4 metabolism).

Appendix A
Tabulation of General Pharmacology Studies (Continued)

TEST	SPECIES	DOSE, ROUTE	RESULTS
Cardiovascular Profile (cont.):			
Hemodynamic Evaluation	Conscious dog	ABT-378/ritonavir dose combinations o 3:1.5, 10:5, 30: 15 100:50, 0:50, 100:0 0:0 mg/kg, p.o. (n=7 crossover)	No effect on mean arterial pressure or heart rate at doses up to and including 100:50 mg/kg (peak plasma concentration of 23.47 ± 1.62:23.00 ± 2.93 µg/ml).
Electrocardiographic Evaluation	Anesthetized Dog	ABT-378/ritonavir dose combinations of 10:1.5 and 20:1.5 mg/kg, i.v. (n=5/ group, 2 groups)	Modest decrease in mean arterial pressure and heart rate at the low dose, moderate decreases at the high dose. PR interval lengthened, but no effect on QTc. No ventricular ectopy.

6. Overview: General Pharmacology Profile of Abbott-157378 (1-29)

Abbott-157378 (ABT-378)

Integrative Pharmacology Report

Overview: General Pharmacology Profile of Abbott-157378

R&D/99/560

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Table of Contents

1.0 Overview

Abbott-157378 (ABT-378), a novel peptidomimetic, is a potent and specific HIV protease inhibitor. Compared with ritonavir and other currently available inhibitors, ABT-378 demonstrates approximately a ten-fold greater *in vitro* potency against the wild-type enzyme (K_i ~ 1 pM) and markedly improved efficacy against common clinical mutant (so-called "resistant") isolates. EC_{50} values in the presence of 50% human serum are between 0.1 μM for wild-type up to 1 μM against mutant isolates. Co-administration of ABT-378 with ritonavir, which acts as a pharmacokinetic enhancer by blocking CYP3A4 mediated metabolism, results in elevated and prolonged serum levels of ABT-378 that support a twice a day dosing regimen.

To investigate ABT-378 for possible ancillary pharmacologic activity, this compound was evaluated in a battery of receptor binding and ion transport assays as well as on functional assays for the central nervous (CNS) and cardiovascular (CV) systems. In the *in vivo* functional studies, ABT-378 was co-administered with ritonavir (ABT-538) to enhance ABT-378 exposure and to model the combined effects that might be expected via co-administration clinically. A summary of the tests performed and results obtained are listed in the Table starting on page 7.

ABT-378, in the absence of serum proteins, was evaluated in various receptor and ion channel binding assays at concentrations of 0.3, 1, 3, and 10 μM. The most notable effects of ABT-378 occurred at the 10 μM concentration which reduced the binding of reference radioligands 47% to 54% of control at the L-type calcium channel, 62% of control at the sodium channel site 2 and 47% of control at the chloride ionophore. Inhibitions of less than 15% occurred in all potassium channels assays and at the muscarinic M_1 receptor site.[1]

ABT-378 was evaluated for interactions at certain classes of potassium (K^+) channels by membrane potential, cation efflux and radioligand binding assays. ABT-378 (0.1 nM to 1 μM) did not alter the membrane potential in a smooth muscle cell line (A10) indicating that it had no effect on the K^+ channels expressed in this cell line. ABT-378 (10 pM to 10 μM) also failed to displace the binding of [^{125}I]charybdotoxin to rat brain-derived membranes indicating that it had no interaction with the voltage-gated K^+ channels or calcium-activated maxi-K^+ channels labeled by [^{125}I]charybdotoxin. In a preliminary study to assess effects on calcium-activated maxi-K^+ channel function, ABT-378 (1 nM to 10 μM) did not inhibit ionomycin-stimulated $^{86}Rb^+$ influx into C6 glioma cells and alone did not alter in the basal levels of $^{86}Rb^+$ influx into C6 glioma cells.[2]

The combined administration of ABT-378 and ABT-538 was evaluated for potential central nervous system (CNS) effects in tests of locomotor stimulation/depression, motor coordination, hypnotic potentiation, pro-convulsant and anticonvulsant activity, and nociception. Effects on rectal temperature were measured and the gross physiological, behavioral and toxic effects were assessed in a preliminary observation test. ABT-378 and ABT-538 were administered orally in combination as follows: 10 and 5 mg/kg, 30 and 15 mg/kg, 50 and 100 mg/kg, and 300 and 150 mg/kg of ABT-378 and ABT-538, respectively.[3]

The co-administration of ABT-378 (10 mg/kg, p.o.) and ABT-538 (5 mg/kg, p.o.) had no effect in the preliminary observation test in mice. The dose combinations of 30 mg/kg, p.o. and 15 mg/kg, p.o. as well as that of 100 mg/kg, p.o. and 50 mg/kg, p.o. of ABT-378 and ABT-538, respectively, produced loss of traction, stereotypies (chewing) and ptosis in one of four mice examined. The dose combination of 300 mg/kg, p.o. and 150 mg/kg, p.o. of ABT-378 and ABT-538, respectively, produced loss of traction, stereotypies

(chewing) and ptosis in two of four mice examined as well as slight sedation and hypothermia.

In the dose range tested, the co-administration of ABT-378 and ABT-538 had no effects on locomotor activity of rats or motor coordination of mice, and had no clear effects in the hot plate test in mice, tail-flick test in rats or on rectal temperature in mice. The co-administration of ABT-378 and ABT-538 at 300 and 150 mg/kg, p.o., respectively, significantly increased barbital-induced sleep in mice. The dose combinations of 10 and 5 mg/kg, p.o., as well as of 30 and 15 mg/kg, p.o., significantly increased the effects of ethanol in mice, but no clear effects were observed at the larger dose combinations.

The co-administration of ABT-378 and ABT-538 significantly decreased electroshock threshold in mice in a non-dose related manner at the dosing combinations of 10 and 5 mg/kg, p.o., 100 and 50 mg/kg, p.o., and 300 and 150 mg/kg, p.o. The dose combination of 30 and 15 mg/kg, p.o. did not have a significant effect on electroshock threshold. In contrast, the latency and number of convulsions induced by pentylenetetrazol in mice were unaffected by the combined administration of ABT-378 and ABT-538, with the exception that the dose combination of 300 and 150 mg/kg, p.o. tended to increase the number of tonic convulsions.

The cardiovascular profile of ABT-378 co-administered with ABT-538 was evaluated in rats instrumented with telemetry transmitters.[4] Conscious male rats were dosed by oral gavage with either vehicle or a fixed combination (2:1) of ABT-378 and ABT-538 of 10:5, 30:15, or 100:50 mg/kg, respectively. The dose combination of 10:5 mg/kg had no effect on heart rate or blood pressure, but dose combinations of 30:15 and/or 100:50 mg/kg produced mild, sustained decreases in heart rate. In the vehicle treated animals, heart rate was elevated by 6-7% at 6 hours after dosing whereas heart rate was reduced by 8% in animals receiving the 100:50 mg/kg dose combination. These negative

chronotropic effects were observed at peak plasma concentrations of 6.08 ± 0.73 μg/mL of ABT-378 and 2.34 ± 0.75 μg/mL of ABT-538 (6 hour time point).

In a second cardiovascular study, pentobarbital-anesthetized male beagle dogs were instrumented to measure both myocardial function and hemodynamic parameters.[4] ABT-378 (2, 6 and 20 mg/kg) and ABT-538 (1, 3 and 10 mg/kg) were intravenously infused at a fixed 2:1 ratio in one group while vehicle was infused in a second group. Each of the 3 dosing combinations was administered over a 30 minute time period for a total infusion protocol time of 90 minutes. Intravenous infusion of the low dose combination (2:1 mg/kg, respectively) produced little or no cardiovascular response. After administration of the 6:3 mg/kg dose combination, there were statistically significant reductions in blood pressure and heart rate. However, these changes (which occurred at plasma concentrations of 11.95 ± 0 .92 and 3.68 ± 0.38 μg/mL, respectively) were modest and are not considered to be physiologically relevant. Administration of the 20:10 mg/kg dose combination produced marked and sustained decreases in blood pressure (-12% systolic and -32% diastolic), heart rate (-43%), and left ventricular contractility (dP/dt max, -30%). The decrease in contractility was accompanied by increases in central venous and left ventricular end-diastolic pressures. These negative chronotropic and inotropic responses were observed at peak plasma concentrations of 33.37 ± 1.15 and 17.45 ± 0.64 μg/ml, respectively. The responses were largely maintained for a 60-minute post-infusion period (final plasma concentrations of 21.75 ± 1.33 and 8.33 ± 0.82 μg/mL). It should be noted that plasma levels of sodium pentobarbital were rising in parallel with administration of ABT-378:ABT-538, possibly due to inhibition of CYP3A4 mediated metabolism by ABT-538. The plasma concentrations of sodium pentobarbital were elevated from a control value of 35.6 ± 2.1 to 49.1 ± 4.0 μg/mL at the end of the final infusion period. Therefore, elevated plasma levels of pentobarbital could have been a factor in the etiology of these responses.

To address the possible contribution of anesthesia and/or increased pentobarbital levels to the cardiovascular responses noted above, a third cardiovascular study was performed in conscious beagle dogs chronically instrumented with telemetry transmitters for measurement of systemic arterial pressure and heart rate.[4] The experimental design was a randomized, 7-way crossover using oral administration of 2:1 fixed dose combinations of ABT-378 and ABT-538. The seven treatment groups were 3:1.5, 10:5, 30:15, 100:50, 0:50, 100:0, and 0:0 mg/kg, p.o. of ABT-378 and ABT-538, respectively. In this study, neither mean arterial pressure or heart rate was altered at doses up to and including 100:50 mg/kg, p.o. Following administration of the highest dose combination, plasma concentrations of ABT-378 and ABT-538 reached a C_{max} of 23.47 ± 1.62 and 23.00 ± 2.93 μg/mL at a T_{max} of 4.0 ± 0.7 and 3.7 ± 0.6 hours, respectively. Thus, the combined administration of ABT-378 and ABT-538 produced no cardiovascular effects in conscious beagles at plasma concentrations in excess of those achieved by the end of the protocol in the anesthetized dog study.

Finally, as a follow-up due-diligence investigation of one incident of premature ventricular contractions observed in an early clinical trial, a fourth cardiovascular study was performed in pentobarbital-anesthetized beagle dogs instrumented to measure blood pressure, heart rate and electrocardiographic indices.[5] Intravenous infusion of ABT-378 and ABT-538 at 10:1.5 mg/kg over 60 minutes produced modest decreases of 6 ± 2% and 16 ± 3% in mean arterial pressure and heart rate, respectively. The PR interval was increased by 13 ± 4% at the end of the infusion, while QTc (Bazett's formula) was unaffected. These modest hemodynamic and electrocardiographic effects were associated with plasma concentrations of 10.17 ± 0.64 mg/mL and 0.93 ± 0.15 μg/mL of ABT-378 and ABT-538, respectively. When the infusion rate was increased to deliver additional ABT-378 and ABT-538 at a dosing ratio of 20:1.5 mg/kg over a subsequent 60 minutes, mean arterial pressure and heart rate were significantly decreased by -14 ± 2% and -37 ±

2%, respectively. These effects were accompanied by a significant increase in PR interval of 37 ± 6%, while QTc interval was unchanged. Plasma concentrations of 22.41 ± 2.91 μg/mL and 1.30 ± 0.28 μg/mL of ABT-378 and ABT-538, respectively, were achieved by the end of this 20:1.5 mg/kg infusion. The prolonged PR interval may be related, in whole or in part, to the decrease in heart rate.[6] In addition, the electrocardiogram was monitored for ventricular ectopic beats and none were seen.

In summary, ABT-378 was found to increase barbital and ethanol sleep times as well as decrease electroconvulsive shock threshold in mice when administered with ABT-538 at doses (10 and 5 mg/kg, p.o., respectively) which likely yielded plasma concentrations (low μM) near therapeutic. Only modest, if any, effects on other CNS, CV, receptor or ion channel functions were found at therapeutic to super therapeutic doses/plasma concentrations. These results indicate a minimal risk for marked adverse effects of this compound in clinical studies.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
Receptor Binding and Ion Transport Assays	Muscarinic M3, Ca channel (L and N types), K channel (ATP-sensitive, voltage-dependent, Ca-dependent), Na channel (sites 1 and 2) and Cl ionophore and ion transport [basal activity: Ca channel L-type, Na channal] [stimulated activity: Ca-ATPase, L-type Ca channel, Na channel, Na-K-ATPase, Na/Ca antiport, Na/H antiport, Na/K/Cl cotransport	0.3, 1, 3,10 µM (n = 2)	At 10 µM, binding of reference radioligands reduced 47% to 54% of control at the L-type Ca channel, 62% of control at the Na channel site 2 and 47% of control at the Cl ionophore. Inhibitions less than 15% in all K channel assays.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
Receptor Binding and Ion Transport Assays (cont.):			
Membrane Potential Studies of Potassium Channel Function	Changes in membrane potential assessed using the bis-oxonol dye $DiBAC(4)_3$ in Rat smooth muscle A10 cell line that express ATP-sensitive K^+ channels and calcium-activated K^+ channels.	0.1 nM to 1 μM (n=3)	No effect on membrane potential in the smooth muscle cell line (A10) within the concentration range tested.
$[^{125}I]$Charybdotoxin binding	$[^{125}I]$Charybdotoxin high affinity (pM) binding to calcium-activated K^+ channels in rodent brain membrane protein including the maxi-K^+ subtype and various K^+ channels including the Shaker family of voltage-gated channels (K_V 1.2, K_V 1.3).	10 pM to 10 μM (n=2)	No displacement of $[^{125}I]$charybdotoxin binding within the concentration range tested.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
Receptor Binding and Ion Transport Assays (cont.):			
Calcium-activated Potassium Channel Function	$^{86}Rb+$ influx stimulated by the calcium ionophore ionomycin in rat C6 glioma cells.	1 nM to 10 μM (n=1)	No inhibition of ionomycin-stimulated cation influx within the concentration range tested.
CNS Profile:			
Preliminary Observation (Irwin)	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 4)	No changes from control at 10:5 mg/kg dose combination. Loss of traction, stereotypies and ptosis in 1 of 4 mice at dose combinations of 30:15 and 100:50 mg/kg up to 30 and 60 min. after dosing, respectively. Similar effects plus slight sedation and slight hypothermia at the highest dose combination of 300:150 mg/kg up to180 min. after dosing.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Activity Motor (Locomotor) Function)	Rat	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100: 50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on locomotor activity as compared with the propyleneglycol-ethanol vehicle. Caffeine (16 mg/kg, p.o.) increased locomotor activity by 22% and chlorpromazine (16 mg/kg, p.o.) decreased activity by 71%.
Rotarod (Motor Coordination)	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100: 50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on motor coordination as compared with the propyleneglycol-ethanol vehicle. Diazepam (8 mg/kg, p.o.) decreased drop-off time 97%.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Barbital Interaction	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg; p.o., respectively (n = 10)	Sleep duration was significantly increased at the dose combination of 300:150 mg/kg, p.o., as compared with the propyleneglycol-ethanol vehicle. Diazepam (4 mg/kg, p.o) increased sleep duration 233%, and caffeine (16 mg/kg, p.o.) completely abolished sleep.
Ethanol Interaction	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	The dose combinations of 10:5 and 30:15 mg/kg, significantly increased sleep time as compared with the propyleneglycol-ethanol vehicle. No significant effects were observed at the higher dose combinations, although a similar tendency was apparent at the 100:50 mg/kg, p.o., dose combination. Diazepam (8 mg/kg, p.o.) induced sleep in all animals.

Table 1 (Continued)

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Electroconvulsive Shock (ECS) Threshold	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 15)	The dose combinations of 10:5, 100:50, and 300:150 mg/kg, p.o., significantly decreased ECS threshold (50%, 25% and 47%, respectively) as compared with the propyleneglycol-ethanol vehicle. The dose combination of 30:15 mg/kg had no effect on seizure threshold. RO 15-4513 (64 mg/kg, p.o.) decreased seizure threshold (18%) and diazepam (8 mg/kg, p.o.) increased ECS intensity from control 154%.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Pentylenetetrazol (PTZ) Induced Seizure	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	The dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg did not clearly affect the number of convulsion and death as compared with the propyleneglycol-ethanol vehicle. The dose combination of 300:150 mg/kg tended to increase the number of convulsions. RO 15-4513 (64 mg/kg, p.o.) significantly decreased clonic convulsion and death latencies, and diazepam (8 mg/kg, p.o.,) completely antagonized the PTZ-induced convulsions and death.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Hot Plate (Nociception)	Rat	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on foot-lick or jump latencies as compared with the propyleneglycol-ethanol vehicle.. Morphine (64 mg/kg, p.o.) increased latency to foot-lick by 175%.
Tail-Flick (Nociception)	Mouse	ABT-378:ABT-538 dose combinations. of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effect on tail-flick withdrawal latency as compared with the propyleneglycol-ethanol vehicle.. Morphine (128 mg/kg, p.o.) increased latency to tail-flick 388%.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
CNS Profile (Continued):			
Rectal Temperature	Mouse	ABT-378:ABT-538 dose combinations of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., respectively (n = 10)	No effects rectal temperature as compared with the propleneglycol-ethanol vehicle. Chlorpromazine (16 mg/kg, p.o.) significantly decreased body temperature.
Cardiovascular Profile:			
Hemodynamic Evaluation	Conscious Rats	ABT-378:ABT-538 dose combinations of 10:5, 30:15, and 100:50 mg/kg, p.o. (n=6/group, 4 groups)	Modest (15% max) reduction in heart rate with higher two doses. Plasma concentrations at peak effect were 6.08 ± 0.73 µg/ml of ABT-378 and 2.34 ± 0.75 µg/ml of ABT-538.

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
Cardiovascular Profile (cont.):			
Myocardial Function and Hemodynamic Evaluation	Anesthetized Dogs	ABT-378:ABT-538 dose combinations of 2:1, 6:3, and 20:10 mg/kg, i.v. 30 min. infusion/ dose, 3 doses/ animal (n=6/group, 2 groups)	Low dose produced no effect. Very modest depressor and bradycardic responses after middle dose (plasma concentration of 11.95 ± 0.92:3.68 ± 0.38 µg/ml). High dose produced marked decreases in blood pressure, heart rate, and contractility, which were sustained for ≥60 min. after end of infusions. Peak plasma concentrations were 33.37 ± 1.15: 17.45 ± 0.64 µg/ml, with levels of 21.75 ± 1.33: 8.33 ± 0.82 µg/ml at 60 min. after end of infusions. Plasma levels of pentobarbital were elevated by the end of the protocol (ABT-538 inhibits CYP3A4 metabolism).

Table 1

Ancillary Pharmacology of Abbott-157378 (ABT-378):

Summary of Tests and Results

TEST	SPECIES	DOSE, ROUTE	RESULTS
Cardiovascular Profile (cont.):			
Hemodynamic Evaluation	Conscious dog	ABT-378:ABT-538 dose combinations of 3:1.5, 10:5, 30: 15, 100:50, 0:50, 100:0, 0:0 mg/kg, p.o. (n=7, crossover)	No effect on mean arterial pressure or heart rate at doses up to and including 100:50 mg/kg (peak plasma concentration of 23.47 ± 1.62:23.00 ± 2.93 µg/ml).
Electrocardiographic Evaluation	Anesthetized Dog	ABT-378:ABT-538 dose combinations of 10:1.5 and 20:1.5 mg/kg, i.v. (n=5/ group, 2 groups)	Modest decrease in mean arterial pressure and heart rate at the low dose, moderate decreases at the high dose. PR interval lengthened, but no effect on QTc. No ventricular ectopy.

3.0 Description of Studies

3.1 Receptor Binding and Ion Transport Assays

ABT-378 was evaluated in various binding [muscarinic M_3, Ca channel (L and N types), K channel (ATP sensitive, voltage dependent, Ca dependent), Na channel (sites 1 and 2) and Cl ionophore] and ion transport [basal activity: Ca channel L-type, Na channel] [stimulated activity: Ca-ATPase, L-type Ca channel, Na channel, Na-K-ATPase, Na/Ca antiport, Na/H antiport, Na/K/Cl cotransport] assays. ABT-378 was evaluated in these assays at concentrations of 0.3, 1, 3, and 10 μM. The most notable effects of ABT-378 occurred at the 10 μM concentration. The 10 μM concentration of ABT-378 reduced the binding of reference radioligands 47% to 54% of control at the L-type Ca channel, 62% of control at the Na channel site 2 and 47% of control at the Cl ionophore. Inhibitions of less than 15% occurred in all K channels assays and at the muscarinic M_3 receptor site.[1]

The effects of ABT-378 on the functional activity of K^+ channels was studied by evaluating changes in membrane potential in a rat smooth muscle A10 cell line. A10 cells are known to express ATP-sensitive K^+ channels and calcium-activated K^+ channels. Changes in membrane potential were assessed using the bis-oxonol dye DiBAC(4)3. ABT-378 did not alter membrane potential in a smooth muscle cell line (A10) within the concentration range of 0.1 nM to 1 μM (n=3).[2]

Interaction of ABT-378 with [^{125}I]charybdotoxin binding sites was evaluated using rat brain-derived membranes. [^{125}I]Charybdotoxin binds with high affinity (pM) to calcium-activated K^+ channels including the maxi-K^+ subtype and various K^+ channels including the Shaker family of voltage-gated channels (K_V 1.2, K_V 1.3). ABT-378 failed to displace the binding of [^{125}I]charybdotoxin within the concentration range of 10 pM

to 10 μM (n=2). These results indicate that ABT-378 had no interaction with the voltage-gated K+ channels or calcium-activated K+ channels labeled by [^{125}I]charybdotoxin.[2]

^{86}Rb+ influx stimulated by the calcium ionophore ionomycin in rat C6 glioma cells was used in a preliminary study to measure the interaction of ABT-378 with the calcium-activated maxi-K+ channels. ABT-378 did not inhibit ionomycin-stimulated cation influx into C6 glioma cells within the concentration range of 1 nM to 10 μM (n=1). In contrast, charybdotoxin inhibited cation influx with an IC_{50} value of 11 nM. ABT-378 (1 μM) alone did not alter in the basal levels of ^{86}Rb+ influx into C6 glioma cells. These results indicate that ABT-378 does not modulate calcium-activated maxi-K+ channel function.[2]

3.2 Central Nervous System

The combined administration of ABT-378 and ABT-538 was evaluated for potential central nervous system (CNS) effects in tests of locomotor stimulation/depression, motor coordination, hypnotic potentiation, proconvulsant and anticonvulsant activity, and nociception. Effects on rectal temperature were measured and the gross physiological, behavioral and toxic effects were assessed in a preliminary observation test (Irwin).[3]

3.2.1 Preliminary Observation Test (Irwin) in the Mouse

Behavioral modifications, neurotoxicity symptoms, pupil diameter and rectal temperature were recorded according to standardized observation grid adapted from that described by Irwin (*Psychopharmacology* 13:222-257, 1968). Four mice were used per dose group and observed in comparison with a vehicle control group. Observations were performed at 15, 30, 60, 120 and 180 min. after dosing and also at 24, 48 and 72 hrs. after dosing. A

propyleneglycol-ethanol (95%-5%) vehicle was used for p.o. administration. ABT-378 was administered at doses of 10, 30, 100 and 300 mg/kg, p.o., immediately after ABT-538 at doses of 5, 15, 50 and 150 mg/kg, p.o., respectively. No changes from control were observed at 10:5 mg/kg dose combination. Loss of traction, stereotypies and ptosis were observed in 1 of 4 mice at dose combinations of 30:15 and 100:50 mg/kg, p.o., for up to 30 and 60 min. after dosing, respectively. Similar effects along with slight sedation and slight hypothermia were observed at the highest dose combination of 300:150 mg/kg, p.o.; these effects were apparent up to180 min. after dosing.

3.2.2 Activity Meter (Locomotor Activity) Test in the Rat

Rats were placed in covered, darkened, activity meter cages (40 x 25 x 25 cm), each equipped with two photo cell assemblies placed at the ends of the cage. The number of crossings by each animal (one per cage) from one photo cell to another was recorded by computer at 10 min. intervals for 30 min. beginning 60 min. after dosing. A propyleneglycol-ethanol (95%-5%) vehicle was used for p.o. administration. ABT-378 was administered at doses of 10, 30, 100 and 300 mg/kg, p.o., immediately after ABT-538 at doses of 5, 15, 50 and 150 mg/kg, p.o., respectively (n = 10 per group). The propyleneglycol-ethanol vehicle slightly, but significantly decreased locomotor activity. The four dose combinations of ABT-378 and ABT-538 did not clearly affect locomotor activity as compared with the vehicle. Caffeine (16 mg/kg, p.o.), a reference stimulant, non-significantly increased locomotor activity by 22% and chlorpromazine (16 mg/kg, p.o.), a reference sedative, significantly decreased activity by 71% ($p<0.001$); they were administered in a 0.2% hydroxypropylmethylcellulose (HPMC) vehicle.

3.2.3 Rotarod (Motor Coordination) Test in the Mouse

Mice were placed on a rod (diameter: 3 cm) rotating at a speed of 18 turns per min. The number of animals that dropped off the rod within three minutes was counted and the drop-off time recorded. The dose combinations of ABT-378 and ABT-538 of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., (n = 10 per group) administered 60 min. before the test did not affect rotarod performance as compared with the propyleneglycol-ethanol vehicle. The reference standard diazepam (8 mg/kg, p.o., in 0.2% HPMC) significantly decreased drop-off time 97% ($p < 0.001$).

3.2.4 Barbital Interaction (Sleep Duration) Test in the Mouse

Mice were injected with barbital sodium (200 mg/kg, i.p.). The latency and duration of sleep, indicted by a loss of righting reflex, were recorded during a 6 hour period thereafter. The dose combinations of ABT-378 and ABT-538 of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., (n = 10 per group) were administered 60 min. before barbital sodium. Sleep duration was significantly increased (73%, $p < 0.05$) at the dose combination of 300:150 mg/kg, p.o., as compared with the propyleneglycol-ethanol vehicle. The reference standards caffeine (16 mg/kg, p.o., in 0.2% HPMC) completely abolished sleep whereas diazepam (4 mg/kg, p.o., in 0.2% HPMC) significantly increased sleep duration 233% ($p < 0.001$).

3.2.5 Ethanol Interaction (Sleep Induction) Test in the Mouse

Mice were injected with a non-sleep-inducing dose of ethanol (3000 mg/kg, i.p.). The latency and duration of sleep, indicted by a loss of righting reflex, were recorded during a

3 hour period beginning 60 min. after dosing with the ABT-378 and ABT-538 combination (n = 10 per group). The propyleneglycol-ethanol vehicle induced sleep when administered with ethanol. The dose combinations of 10:5 and 30:15 mg/kg, p.o., of ABT-378 and ABT-538 significantly ($p < 0.01$ and $p < 0.05$, respectively) increased sleep time as compared with the propyleneglycol-ethanol vehicle. No significant effects were observed at the higher dose combinations, although a similar tendency was apparent at the 100:50 mg/kg, p.o., dose combination. The reference standard diazepam (8 mg/kg, p.o., in 0.2% HPMC) induced sleep ($p < 0.01$).

3.2.6 Electroconvulsive Shock (ECS) Threshold

To test for proconvulsant and anticonvulsant activity, mice were administered ECS via temporal electrodes. In treatment groups of 15 mice, animal number one was exposed to 20 mA of ECS and observed for the occurrence of tonic convulsions. If he did not convulse, animal number two was exposed to 30 mA of ECS, etc. Once the first tonic convulsion was observed, the intensity of the ECS was decreased by 5 mA for the next animal. The ECS intensity was then decreased or increased by 5 mA from animal to animal depending on whether the animal convulsed or not. The maximum intensity given was 95 mA. The results were represented as the mean ECS intensity administered and as percent change from control. A positive percent change indicated an anticonvulsant effect and a negative percent change indicated a proconvulsant effect. The number of deaths after ECS was also recorded. The propyleneglycol-ethanol vehicle significantly (135%, $p < 0.001$) increased the ECS threshold administered 60 min. before the test. The dose combinations of ABT-378 and ABT-538 of 10:5, 100:50, and 300:150 mg/kg, p.o., administered 60 min. before the test significantly decreased ECS threshold (50%, $p < 0.001$, 25%, $p < 0.01$ and 47% $p < 0.001$, respectively) as compared with the propyleneglycol-ethanol vehicle. The dose combination of 30:15 mg/kg, p.o., had no effect on seizure threshold. The reference standard RO 15-4513 (64 mg/kg, p.o., in 0.2%

HPMC) decreased seizure threshold (18%, $p < 0.05$) and the reference standard diazepam (8 mg/kg, p.o., in 0.2% HPMC) increased ECS intensity from control 154% ($p < 0.001$).

3.2.7 Pentylenetetrazol (PTZ) Induced Seizure

Mice were administered PTZ (120 mg/kg, s.c.). The occurrence and latency of clonic convulsions, tonic convulsions and deaths were noted during a 30 min. observation period. The number of convulsions and deaths were expressed as either a positive percent antagonism (anticonvulsant) or negative percent antagonism (proconvulsant) effect (n = 10 per group). The propyleneglycol-ethanol vehicle administered 60 min. before the test antagonized PTZ induced tonic convulsions and death in all mice and clonic convulsions in 80% of mice. The dose combinations of ABT-378 and ABT-538 of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., administered 60 min. before PTZ did not clearly affect the number of convulsions and deaths as compared with the propyleneglycol-ethanol vehicle, however, the dose combination of 300:150 mg/kg, p.o., tended to increase the number of convulsions. The reference proconvulsant standard RO 15-4513 (64 mg/kg, p.o., in 0.2% HPMC) significantly decreased clonic convulsion and death latencies ($p < 0.05$ and 0.01, respectively). In contrast, diazepam (8 mg/kg, p.o., in 0.2% HPMC) completely antagonized the PTZ-induced convulsions and death.

3.2.8 Hot Plate (Nociception) Test in the Mouse

Mice were place on a hot metal plate (54°C) surrounded by a Plexiglas cylinder (height: 13 cm; diameter: 19 cm). Latency to the first foot-lick and to the first jump were measured. If no response was made within 120 seconds, the test was terminated. The maximum score for foot licking was 30 seconds (n = 10 per group). The propyleneglycol-ethanol vehicle administered 60 min. before the test did not affect the foot-licking or jump latencies. The dose combinations of ABT-378 and ABT-538 of

10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., administered 60 min. before the test were without significant effect on foot-licking or jump latencies as compared with the propyleneglycol-ethanol vehicle. The reference standard morphine (64 mg/kg, p.o., in 0.2% HPMC) significantly increased foot-licking latency 175% ($p < 0.001$).

3.2.9 Tail-Flick (Nociception) Test in the Rat

The tail of the rat was heated by means of a thermal light source. The latency to tail withdrawal was measured (n = 10 per group). If no response was made within 30 seconds, the test was terminated. The propyleneglycol-ethanol vehicle administered 60 min. before the test did not affect tail-flick latency. The dose combinations ABT-378 and ABT-538 of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o., administered 60 min. before the test were without significant effect tail-flick latency as compared with the propyleneglycol-ethanol vehicle. The reference standard morphine (128 mg/kg, p.o., in 0.2% HPMC) increased tail-flick latency 388% ($p < 0.001$).

3.2.10 Rectal Temperature Test in the Mouse

Mice were measured for rectal temperature before treatment and assigned to temperature matched groups (n = 10 per group). Rectal temperatures were measured again at 30, 60 and 120 minutes after the dose combinations of ABT-378 and ABT-538 of 10:5, 30:15, 100:50, and 300:150 mg/kg, p.o. The propyleneglycol-ethanol vehicle slightly, but significantly ($p<0.001$) decreased rectal temperature at all time periods. The dose combinations of ABT-378 and ABT-538 did not affect rectal temperature as compared with the propyleneglycol-ethanol vehicle. The reference standard chlorpromazine (16 mg/kg, p.o., in 0.2% HPMC) significantly ($p <0.001$) decreased rectal temperature by a mean of 3.0 to 4.3°C from 30 to 120 min. after dosing.

3.3 Cardiovascular Profile

3.3.1 Hemodynamic Evaluation by the Oral Route in Conscious Rats[4]

Conscious male rats (220 - 270 gms, n=6/group, 4 groups) were dosed by oral gavage with either vehicle or a fixed combination (2:1) of ABT-378:ABT-538 at doses of 10:5, 30:15, or 100:50 mg/kg, respectively. In addition to monitoring mean arterial pressure and heart rate, blood samples were taken at 3, 4.5 and 6 hours post dosing for the determination of plasma drug levels. ABT-378:ABT-538 had no effect on heart rate or blood pressure at the low dose, but produced mild, sustained decreases in heart rate after the 30:15 mg/kg dose. A similar response was observed after the dose combination of 100:50 mg/kg. In the vehicle treated animals, heart rate was elevated by 6-7% at 6 hours after dosing whereas heart rate was reduced by 8% in animals receiving the 100:50 mg/kg dose combination. These negative chronotropic effects were observed at peak plasma concentrations of 6.08 ± 0.73 mg/mL of ABT-378 and 2.34 ± 0.75 mg/mL of ABT-538 (6 hour time point).

3.3.2 Myocardial Function and Hemodynamic Parameters After Intravenous Dosing in Anesthetized Dogs[4]

Pentobarbital-anesthetized male beagle dogs (9.4 to 11.1 kg, n=6/group, 2 groups) were instrumented to measure both myocardial function and hemodynamic parameters. ABT-378 (2, 6 and 20 mg/kg) and ABT-538 (1, 3 and 10 mg/kg) were intravenously infused at a fixed 2:1 ratio in one group while vehicle was infused in a second group. Each of the 3 dosing combinations was administered over a 30 minute time period, for a total infusion time of 90 minutes, which was followed by a 60-minute recovery period. Blood samples were taken at 15-minute intervals throughout the protocol for correlation of plasma drug

levels with functional effects. The low dose combination of 2:1 mg/kg produced little or no cardiovascular response. After administration of 6:3 mg/kg there were statistically significant reductions in blood pressure and heart rate. However, these changes (which occurred at plasma concentrations of 11.95 ± 0.92 and 3.68 ±0.38 μg/mL, respectively) were modest and are not considered to be physiologically relevant. Administration of the 20:10 mg/kg combination produced marked and sustained decreases in blood pressure (-12% systolic and -32% diastolic), heart rate (-43%), and left ventricular contractility (dP/dt max, -30%). The decrease in contractility was accompanied by increases in central venous and left ventricular end-diastolic pressures. These negative chronotropic and inotropic responses were observed at peak plasma concentrations of 33.37 ± 1.15 and 17.45 ± 0.64 μg/mL, respectively. The responses were largely maintained for the 60-minute post-infusion period (final plasma concentrations of 21.75 ± 1.33 and 8.33 ± 0.82 μg/mL). It should be noted that plasma levels of sodium pentobarbital were rising in parallel with administration of ABT-378:ABT-538, possibly due to inhibition of CYP3A4 mediated metabolism by ABT-538. For example, the plasma concentrations of sodium pentobarbital were elevated from a control value of 35.6 ± 2.1 μg/mL to 49.1 ± 4.0 μg/mL at the end of the final infusion period. Therefore, elevated plasma levels of pentobarbital could have been a factor in the etiology of these responses.

3.3.3 Hemodynamic Evaluation by the Oral Route in Conscious Dogs[4]

Beagle dogs (9.6 to 11.7 kg, n=7/group) were chronically instrumented with telemetry transmitters for the measurement of systemic arterial pressure and heart rate. The experimental design was a randomized, 7-way crossover using oral administration of 2:1 fixed combinations of ABT-378:ABT-538. The seven treatment groups were 3:1.5, 10:5, 30:15, 100:50, 0:50, 100:0, and 0:0 mg/kg, p.o. of ABT-378:ABT-538, respectively. Blood samples were taken at 1, 2, 3, 6 and 23 hours post dosing for correlation of plasma drug levels with any hemodynamic effects. .ABT-378:ABT-538 did

not alter either mean arterial pressure or heart rate at doses up to and including 100:50 mg/kg, p.o. It should be noted that emesis was routinely seen following administration of 50 mg/kg, p.o. of ABT-538 (alone or in combination), but not with 100 mg/kg, p.o. of ABT-378 by itself. Following administration of the highest combination dose, plasma concentrations of ABT-378 and ABT-538 reached a C_{max} of 23.47 ± 1.62 and 23.00 ± 2.93 μg/mL at a T_{max} of 4.0 ± 0.7 and 3.7 ± 0.6 hours, respectively.

3.3.4 Effect on Electrocardiographic End-Points in Pentobarbital-Anesthetized Dogs[5]

Pentobarbital-anesthetized beagle dogs (6.9 to 9.0 kg, n=5/group, 2 groups) were instrumented to measure blood pressure, heart rate and Lead II electrocardiogram (ECG). PR and QTc (QT corrected for heart rate, Bazett's formula) intervals were obtained from the ECG. Blood samples were taken at 15-minute intervals to correlation drug plasma levels with any observed effects. Intravenous infusion of ABT-378:ABT-538 at 10:1.5 mg/kg over 60 minutes produced modest decreases of 6 ± 2% and 16 ± 3% in mean arterial blood pressure and heart rate, respectively. The PR interval was increased by 13 ± 4% at the end of the infusion, while QTc was unaffected. These modest hemodynamic and EGC effects were associated with plasma concentrations of 10.17 ± 0.64 μg/mL and 0.93 ± 0.15 μg/mL of ABT-378 and ABT-538, respectively. When the infusion rate was increased to deliver ABT-378:ABT-538 at the dose ratio of 20:1.5 mg/kg over a subsequent 60 minutes, mean arterial blood pressure and heart rate were significantly decreased by -14 ± 2% and -37 ± 2%, respectively. These effects were accompanied by a significant increase in PR interval of 37 ± 6%, while QTc was essentially unchanged. Plasma concentrations of 22.41 ± 2.91 μg/mL and 1.30 ± 0.28 μg/mL of ABT-378 and ABT-538, respectively, were achieved by the end of this 20:1.5 mg/kg infusion. These results indicate that plasma levels of approximately 10:1 μg/mL of ABT-378:ABT-538

were associated with minor hemodynamic and ECG effects in the anesthetized dog. Higher plasma concentrations of 22:1 μg/mL produced modest reductions in mean arterial pressure. Marked, significant reductions in heart rate, as well as increases in PR interval were also observed at these plasma levels. The prolonged PR interval may be related, in whole or in part, to the decrease in heart rate.[6] No premature ventricular contractions were noted during a careful review of all experimental tracings. In addition, ventricular repolarization, as indicated by the QTc interval, was not lengthened by either dose tested. Therefore, it was concluded that infusion of ABT-378:ABT-538 was not associated with any ECG changes consistent with the development of ventricular ectopy at the plasma concentrations achieved in this study.

3.3.5 Summary of the Cardiovascular Effects

Plasma concentrations of 5 to 10 μg/mL of ABT-378 in combination with 2 to 3 μg/mL of ABT-538 resulted in either modest or no cardiovascular effect in the four cardiovascular models employed. Higher plasma concentrations did produce cardiovascular effects in the pentobarbital-anesthetized dog, but were without effect in the conscious dog. Administration of ABT-378 with ABT-538 did not affect QTc or produce ventricular ectopic beats.

4.0 References

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7. Toxicology Summary for NDA Submission (1-56)

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RE: STUDY NO. N/A /SCIENTIFIC REPORT NO. R&D/00/164

The attached document has been released by our division for your information:

ABBOTT-157378 (ABT-378) TOXICOLOGY SUMMARY FOR NDA SUBMISSION

If you do not intend to keep the attached overview after reviewing it, please return it to the Drug Safety Archives, D-421, AP13A-1.

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Abbott-157378 (ABT-378)
Toxicology Summary for NDA Submission

R&D/00/164

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2.5.2 Toxicology

Abbott-157378 (ABT-378; lopinavir), an inhibitor of the human immunodeficiency virus protease (HIV-1 protease), is being developed as an antiviral agent. ABT-378 is tenfold more active *in vitro* than Abbott-84538 (ritonavir), another HIV protease inhibitor, but ABT-378 exhibits poor oral bioavailability. However, coadministration of ABT-378 with ritonavir has produced sustained plasma concentrations in rats and dogs. The pharmacokinetic enhancement results from potent inhibition by ritonavir of the cytochrome P450-mediated metabolism of ABT-378. A 2:1 ratio of ABT-378/ritonavir was used in most studies based on preliminary work; but other ratios are also effective in maintaining ABT-378 levels. To assess the toxicity profile and safety of the 2:1 ratio of ABT-378 and ritonavir or ABT-378 alone, the following preclinical toxicity studies have been completed:

Summary of Completed Toxicity Studies

Type of Study	Study	Species/Test System
Single dose	Oral and I.V. acute toxicity studies	Rats & mice
	Oral acute toxicity study (ABT-378 alone)	Rats
Repeated dose	Two-week oral toxicity studies	Rats & dogs
	Two-week oral toxicity study	Neonatal rats
	Four-week oral toxicity study	Juvenile rats
	Three-month oral toxicity studies	Rats & dogs
	Three-month oral MTD study	Mice
	Three-month oral studies with impurities/degradants	Dogs
	Six-month oral toxicity studies	Rats & dogs
	Nine-month oral toxicity study	Dogs
Reproduction	Fertility & embryonic development (Seg I)	Rats
	Embryonic & fetal development (Seg II)	Rats & rabbits
	Pre- and postnatal development (Seg III)	Rats
Mutagenicity	Bacterial reverse mutation assay[a]	Bacteria (Ames)
	Mouse lymphoma forward mutation	Mouse lymphocytes
	In Vitro Cytogenetics[a]	Human lymphocytes
	In Vivo Mouse micronucleus	Mouse bone marrow

a. Assays were also conducted with ABT-378 containing high levels of impurities

Section 5.2.1. (Overview of Toxicology Studies) summarizes the findings of these completed studies. In addition, two-year carcinogenicity studies in rats and mice are in progress and the reports are scheduled for November, 2001.

Three different formulations (liquid, semi-solid and soft elastic capsule, SEC) were used in the preclinical toxicity studies in conjunction with the stage of the formulation development. The major excipients in the liquid formulation were propylene glycol and ethanol (95:5, v/v), while the semi-solid formulation included glycerides, ethanol and polyoxylene 35 castor oil (cremophor). The SEC formulation differs from the semi-solid formulation by substituting glycerides with oleic acid. Recently, propylene glycol was used to replace ethanol in the SEC formulation. It should be noted that the soft elastic capsule (SEC) formulation for the ABT-378/ritonavir combination gives similar AUC values as the liquid or semi-solid formulations in both rats and dogs, and toxicity evaluations are based on drug exposures (AUC values) regardless which formulation was used in the study. The SEC formulation was used in the later preclinical studies and has been used in the clinical trials. Furthermore, a twice daily dosing regimen of ritonavir and a 3:1 ratio of the combination were used in the six-month dog study in an effort to further maximize drug exposure to ABT-378.

ABT-378 alone or in combination with ritonavir was found to be non-mutagenic and non-clastogenic when tested in three *in vitro* and one *in vivo* mutagenicity assays. Available data also indicate that ABT-378/ritonavir combination is not a specific reproductive or developmental toxin in rats or rabbits. Repeated dose toxicity studies in rodents and dogs have identified liver, thyroid, blood, spleen and kidney as the target organs. Target organ toxicities along with gastrointestinal distress in dogs are discussed in the following sections. In addition, tabular summaries for individual studies are appended at the end of this section.

The ABT-378/ritonavir combination has been found to present minimal toxicology risk based on extensive toxicity testing up to nine months in dogs and six months in rats. In addition, it is nongenotoxic and is not a selective developmental or reproductive toxin. On the basis of this information, administration of ABT-378/ritonavir combination up to a recommended clinical dosage of 400/100 mg bid (mean AUC values for

ABT-378/ritonavir are approximately 160/9 µg•hr/ml in subjects) is unlikely to pose a toxicity risk to humans.

2.5.2.1 Effects of ABT-378/Ritonavir Combination on the Liver

2.5.2.1.1 Mouse

A three-month oral maximum-tolerated dosage study was conducted in mice at dosages of 0, 20/10, 60/30 and 200/100 mg/kg/day. Hepatic toxicity observed in mice given 200/100 mg/kg/day (458/62 µg•hr/ml) was characterized by increased hepatic enzymes (ALT, AST, GGT) and liver weights and histopathological changes (cytoplasmic vacuolation, necrosis, subacute inflammation and hepatocytomegaly). Elevations of cholesterol and triglyceride levels were also noted at this dosage level. Increased liver weights and cholesterol levels occurred at a dosage of 60/30 mg/kg/day. No signs of toxicity were seen in the dosage group of 20/10 mg/kg/day with corresponding mean AUC values of 43/3 µg•hr/ml.

2.5.2.1.2 Rat

ABT-378/ritonavir combination was administered to adult rats by oral gavage for two weeks to six months. Hepatic changes in rats included increased cholesterol levels and elevated liver enzyme activities (ALT, ALP, GGT) and histopathologic lesions such as multinucleated hepatocytes, hepatocytomegaly, single cell necrosis and histiocytosis. These changes were observed at 50/25 mg/kg/day (mean AUC values of 73/8 µg•hr/ml) for six months. Ultrastructural evaluation of the liver revealed lysosomal inclusions in hepatocytes and minimal increase in smooth endoplasmic reticulum. Histopathologic alterations had not resolved in rats during the one-month of recovery. Due to an increase in serum ALT levels in male rats receiving 10/5 mg/kg/day, the no-hepatotoxic-effect dosage level in rats when administered for six months was considered to be less than 10/5 mg/kg/day (ABT-378/ritonavir), resulting in mean AUC values of approximately 18/1 µg•hr/ml.

ABT-378/ritonavir combination was administered to neonatal rats (3-4 days old at the start of treatment) at dosages of 0, 10/5, 20/10 and 40/20 mg/kg/day for two weeks and to juvenile rats (16 days old at the start of treatment) at dosages of 0, 10/5, 30/15 and

100/50 mg/kg/day for four weeks. Changes in the liver (hepatocytomegaly) along with increases in liver weights, cholesterol levels and liver enzymes (ALT, GGT) of juvenile rats at dosages of 100/50 mg/kg/day which attained group mean AUC values of approximately 172/10 μg•hr/ml were generally similar to those observed in adult rats at the same dosages and similar drug exposures. However, significantly higher drug exposures (AUC values) were evident in neonates compared with adult rats at similar dosage levels. Less toxicity was seen in neonates relative to adults at similar drug exposures. A dosage level of 40/20 mg/kg/day (group mean AUC values of 140/13 μg•hr/ml) for two weeks in neonates produced only increased liver weights, but no microscopic findings in the liver or any other organs. Therefore, on the basis of drug exposures, neonates appear to be less sensitive to the toxicity produced by ABT-378/ritonavir combination when compared with adult rats.

2.5.2.1.3 Dog

ABT-378/ritonavir combination was administered by oral capsules to dogs for two weeks to nine months. Dogs appeared to be less sensitive than rats to the hepatotoxic effects of the ABT-378/ritonavir combination. Although elevated liver enzyme activities (ALT, AST, ALP) and hepatocellular changes were seen in dogs, such changes were only observed in dogs receiving dosages of 70/35 - 100/50 mg/kg/day (mean AUC values of 189/65 μg•hr/ml) for three months or dosages of 45/15 mg/kg/day or greater for six months and achieving plasma exposures of approximately 206/53 μg•hr/ml. An increase in liver enzyme activities (ALP, ALT) and cell swelling in the liver occurred in dogs receiving 25/8 mg/kg/day (mean AUC values of 76/11 μg•hr/ml) for six months. The hepatocellular changes seen in dogs appeared to be reversible after a one-month recovery period. A dosage of 10/3 mg/kg/day (mean AUC values of 25/3 μg•hr/ml) for the ABT-378/ritonavir combination did not produce liver toxicity in dogs when treated for six months. In a nine-month study, only elevations in ALP and increased relative liver weights, but without any histopathologic changes were seen in dogs receiving dosages up to 50/20 mg/kg/day (mean AUC values of 78/39 μg•hr/ml). This indicated that increased treatment duration from six to nine months did not change the toxicity profile of ABT-378/ritonavir combination in dogs.

There are sensitivity differences in liver toxicity between rats and dogs. The recommended therapeutic dosage for ABT-378/ritonavir combination is 400/100 mg bid and the mean AUCs are approximately 160/9 μg•hr/ml. Hepatotoxicity with histopathologic changes is produced in rats at lower exposures (approximately 73/8 μg•hr/ml), while exposures producing mild liver change in dogs are similar to the mean clinical exposures. Phase II clinical trials revealed that slight increases in ALT or AST occurred in 6-13% of subjects receiving dosages up to 400/200 mg bid of ABT-378/ritonavir combination (mean AUC values of 220/24 μg•hr/ml) for up to 48 weeks, and some of the subjects with ALT or AST elevations had positive baseline hepatitis serologies. Therefore, the rat appears to be an overly sensitive animal model for the evaluation of ABT-378/ritonavir combination-induced hepatotoxicity.

Elevated GGT observed in repeated dose studies was limited to rodents only, and no increases in total bilirubin or urobilinogen were noted in rodents or dogs. Although elevations of GGT (>5x ULN) without increases in either total bilirubin or urobilinogen were noted in approximately 26% of subjects given dosages up to 400/200 mg bid for up to 48 weeks, the isolated GGT elevations in some subjects may have been due to hepatic induction in the presence of nevirapine and was not considered to be clinically relevant. Furthermore, GGT testing can easily be monitored in humans during therapy.

Although no safety margin can be demonstrated in long-term toxicity studies in rats or dogs, effects in humans are generally milder and easily monitored. Therefore, it is our opinion that the proposed clinical dosage of this drug combination (400/100 mg bid) does not represent an undue risk in humans.

2.5.2.2 Effects of ABT-378/Ritonavir Combination on the Thyroid

Mild but dose-related hypertrophy of follicular cells in the thyroid gland along with decreased serum thyroxine (T_4) levels and elevated serum thyroid stimulating hormone (TSH) were observed in adult rats that received ABT-378/ritonavir combination for two to 26 weeks at dosages of 50/25 m/kg/day or greater (mean AUC values of 65/14 μg•hr/ml or more). Neonatal and juvenile rats appeared to be less sensitive to the thyroid change produced by ABT-378/ritonavir than adult rats. No thyroid change was

seen in neonatal rats receiving 40/20 mg/kg/day (mean AUC values of 140/13 μg•hr/ml) for two weeks, and thyroid change occurred in juvenile rats only when the dosage was up to 100/50 mg/kg/day (mean AUC values of 172/10 μg•hr/ml) for four weeks. All changes were reversible following a one-month recovery period. No effects on the thyroid gland were observed in any of the mouse or dog studies up to nine months of treatment. Similar results have been seen in rats with ritonavir alone and other compounds, especially those that affect the hormonal balance between the thyroid and pituitary. Typically, compounds that inhibit the synthesis of thyroid hormones (e.g., thiouracil) or those that increase the metabolism of thyroid hormones (e.g., phenobarbital) result in reduction of serum thyroid hormone followed by a compensatory increase in serum TSH and ultimately lead to hypertrophy and even hyperplasia of the thyroid gland. In some cases in which pronounced effects are observed, long-term administration to rats can lead to neoplasia of the follicular cells. However, with ritonavir administered alone for two years in rats, similar effects were observed but did not progress to thyroid neoplasia. In addition, in phase I and phase II clinical studies no effects on thyroid-related hormones have been reported in any subject/patient receiving ABT-378/ritonavir combination at dosages up to 400/200 mg bid for 48 weeks. Therefore, the effects on thyroid seen in rats would not indicate any increased risk to humans.

2.5.2.3 Effects of ABT-378/Ritonavir Combination on the Erythron

Decreases in erythrocytic variables (erythrocyte count, hematocrit, hemoglobin) along with an increased incidence and/or severity of anisocytosis (erythrocytes of variable size) and poikilocytosis (erythrocytes with abnormal shapes) were observed in adult rats treated with ABT-378/ritonavir combination at 50/25 mg/kg/day or higher (mean AUC values of approximately 65/14 μg•hr/ml or more) for three to six months. Erythrocyte morphological changes in rats persisted through the one-month recovery period. Similar erythrocytic changes also occurred in one female dog that received dosages of 45/15 - 60/20 mg/kg/day (mean AUC values of approximately 205/53 μg•hr/ml) for six months. Erythrocytic changes were not detected in mice that received the drug combination at dosages up to 200/100 mg/kg/day (mean AUCs = 458/62 μg•hr/ml) for three months or in dogs given the drug combination at dosages up to 50/25 mg/kg/day (mean AUCs = 78/39 μg•hr/ml) for nine months.

Anisocytosis reflected the presence of microcytes/spherocytes and large polychromatophilic erythrocytes in the peripheral blood. Poikilocytosis was caused by circulating acanthocytes. To determine if the effects were immune-mediated, a direct immunoglobulin assay (direct Coomb's test) on RBCs was performed. Results were negative, supporting the contention that alterations in erythrocytes were physically induced by imbalances in lipid/cholesterol in the RBC membrane. It has been reported that acanthocytosis can occur in humans or animals with liver disease due to alteration of cholesterol and phospholipid content in the erythrocyte membrane. Rats and dogs receiving the drug combination had liver changes, thus, the acanthocytosis was probably a secondary effect of the liver alteration. The lack of reversibility of the morphologic changes in erythrocytes after a one-month recovery period is not surprising considering the circulating life span of erythrocytes is approximately 100 days.

Although elevations in clotting times (APTT) were noted in rats in the three- or six-month repeated dose studies, no clinically meaningful effects on clotting times or erythrocytic parameters have been reported in phase II clinical studies in which HIV-infected subjects received the ABT-378/ritonavir combination at dosages up to 400/200 mg bid for up to 48 weeks.

2.5.2.4 Effects of ABT-378/Ritonavir Combination on the Kidney and Spleen

No kidney changes were observed in rat or dog studies with durations through six months or in dogs treated for nine months. Changes in kidney (microvesicular cytoplasmic vacuolation) occurred only in mice that received a combination dosage of 200/100 mg/kg/day (mean AUCs = 458/62 μg•hr/ml) for three months. Changes in spleen (histiocytosis and increased spleen weight) were limited to rats receiving combination dosages of 50/25 mg/kg/day or higher (mean AUCs of 73/8 μg•hr/ml or greater) for six months. Since no similar effects on the kidney were observed in any of the rat or dog studies and changes in spleen were limited to rats only, these changes were not considered relevant to humans. Furthermore, tests for renal function are routinely conducted in all phases of clinical testing. To date, no effects on kidneys or spleen have been reported in any clinical studies.

2.5.2.5 Effects of ABT-378/Ritonavir Combination on the Gastrointestinal Tract and Associated ECG Changes

The most sensitive indication of toxicity in dogs that received ABT-378/ritonavir combination was gastrointestinal (GI) distress consisting of emesis, diarrhea and/or loose stools. Dosage-related GI distress occurred generally within 1-2 hours after dosing at all dosages tested in the repeated dose studies. In the three-month toxicity study, moderate to severe GI distress occurred in dogs receiving the high dosages of 70/35-100/50 mg/kg/day (mean AUCs = 189/65 μg•hr/ml), subsequently hypokalemia, hyponatremia, hypochloridemia and variable blood acid-base imbalances were observed in the affected dogs. Subsequently, the following electrocardiographic changes were seen in six male (#3001, 3003, 3005, 3007, 3009 and 3011) and one female (#3006) dogs during the treatment period of the study.

ECG Changes in Individual Dogs

	Changes in Electrocardiograms			
Day in Study	Prominent U wave	Fusion of T &U waves	Triggered ventricular extrasystoles	First degree atrioventricular block
19/20	3003, 3005, 3007, 3009, 3011, 3006	3005,[d] 3009	3006	3005
29	-[a]	-	-	-
40/41	-	-	-	-
54/55	3005, 3011	-	-	-
82/83	3001[b], 3005, 3011	3001	-	-
117[c]	-	-	-	-

a. ECGs were measured, but no abnormalities were seen
b. Dog #3001 was found dead on Day 72, EKGs were obtained from this dog on Day 64.
c. Near the end of the 1-month recovery period
d. As a result of T and U waves merging, the Q-T interval appeared to be increased in this dog.

Among those affected, dogs #3006, 3007 and 3009 died or were euthanized between Study Days 21-26. In general, anorexia, weight loss, acid-base, electrolyte and fluid-balance alterations that included alkalosis, hypochloridemia and hypokalemia were noted prior to death.

Prominent U waves were the primary ECG changes seen in the affected dogs. The occurrence of large U waves as well as T wave flattening are typical ECG abnormalities related to hypokalemia. Although hypokalemia (≤ 3.5 mEq/L) was not observed during the treatment period for all the affected dogs, it has been reported that ECG changes associated with hypokalemia correlate poorly with serum potassium levels. Total serum potassium levels may best reflect skeletal muscle potassium, but it may not correlate well with myocardial potassium. Therefore, the factors most critical for the arrhythmogenic mechanism of hypokalemia remain uncertain and/or difficult to determine. Abnormalities on electrocardiograms seen in these dogs were interpreted by a veterinary cardiologist to be related to serum chemistry changes such as hypokalemia rather than direct cardiotoxic effects.

Since the cardiac effects seen in the three-month dog study appeared to be secondary to changes in plasma electrolyte concentrations attributed to gastrointestinal disturbances (emesis, abnormal stools and anorexia), early and aggressive dietary supplementation including oral administration of PEDIALYTE® and BIO-SERVE® liquid diet were conducted in the subsequent six-month study in dogs. The results from this study indicated that with early and aggressive dietary supplementation in dogs, ABT-378/ritonavir combination in the SEC formulation at dosages up to 60/20 mg/kg/day, producing similar plasma exposures (mean AUC values = 264/50 μg•hr/ml), did not cause any electrocardiogram (ECG) or electrolyte changes and no early deaths occurred. This further indicates that the ECG changes seen in the previous three-month study were related to hypokalemia and/or other electrolyte changes rather than direct cardiac events. Early and aggressive dietary supplementation conducted in the six-month study appeared to minimize the life-threatening electrolyte imbalances and subsequent ECG changes in the previous three-month study. Furthermore, there were no ECG changes seen in dogs that received the drug combination for nine months. On the basis of these observations, it is unlikely that humans are at risk to the ECG changes that occurred in the three-month dog study.

2.5.2.6 Effects of ABT-378/Ritonavir Combination on the Testis

Testicular degeneration, generally classified as minimal or mild, was observed in dogs that received the drug combination at dosages of 10/3 to 60/20 mg/kg/day (mean AUC values of 20/2 - 206/53 μg•hr/ml) for six months. Features of the degeneration included loss of germ cells, germ cell degeneration and tubular vacuolization. The incidence and severity of testicular degeneration did not appear to be related to dosage or to correlate with the systemic drug exposures. In addition, no testicular changes were seen in dogs receiving the drug combination for nine months at dosages up to 50/25 mg/kg/day (mean AUC values of 78/39 μg•hr/ml). Bilateral testicular degeneration was also noted in male dogs receiving ritonavir alone at dosages of 50 mg/kg/day (mean AUC =64 μg•hr/ml) or greater for six months or longer. Based on the lack of a dose response and the absence of similar findings in dogs exposed to higher plasma exposures for a longer treatment period, the toxicological significance of the changes seen in dogs in the six-month study is unknown. These changes might have been spontaneous lesions rather than produced by the drug combination.

2.5.2.7 Effects of ABT-378/Ritonavir Combination on Serum Cholesterol and Triglycerides

Elevations in serum cholesterol levels were seen in mice and rats, and an increase in triglyceride levels was limited to mice that received the drug combination. No effects on cholesterol or triglycerides occurred in dogs receiving the drug combination for up to nine months. Increases in cholesterol and triglycerides occurred in mice receiving a combination dosage of 100/50 mg/kg/day (mean AUCs = 292/29 μg•hr/ml) for two weeks or dosages of ≥ 60/30 mg/kg/day (mean AUCs = 121/12 μg•hr/ml) for three months. Increased cholesterol levels were evident in juvenile rats receiving dosages of ≥ 30/15 mg/kg/day (mean AUCs ≥ 62/3 μg•hr/ml) for four weeks and in adult rats given dosages of ≥ 50/25 mg/kg/day (mean AUCs of 65/7 - 73/8 μg•hr/ml) for three or six months. The elevations of cholesterol levels were considered possibly secondary to hepatic effects.

Elevations in serum cholesterol (≥ 300 mg/dL) and triglyceride (≥ 750 mg/dL) levels also occurred in 10 - 25% of patients receiving the drug combination at dosages up to 400/200 mg bid in the phase II clinical studies. No mechanism can be ascribed at present

to these changes seen in humans. However, triglyceride and cholesterol testing can generally be monitored in humans during therapy.

2.5.2.8 Single Dose Toxicity

ABT-378 alone or in combination with ritonavir at a 2:1 ratio has a low order of acute toxicity in rodents by the oral route but is more toxic when administered as an intravenous injection. Increased acute toxicity following intravenous administration is most likely related to considerably higher peak plasma concentrations following a bolus dose. ABT-378 alone or in combination with ritonavir at a 2:1 ratio exhibited only transient clinical signs (decreased activity, ataxia, dyspnea, increased salivation and/or squinting) when administered orally to rats and mice at moderate doses (20/10 - 1250/625 mg/kg for mice and 78/39 - 1250/625 mg/kg for rats) in acute toxicity studies. No deaths occurred in rats or mice given the highest feasible single combination dose that could be given orally (i.e., 1250/625 mg/kg), and no deaths or histopathologic changes occurred at the highest feasible single oral dose of 2500 mg/kg for ABT-378 alone (corresponding to a group mean AUC value of 45 μg•hr/ml).

A summary of single dose acute toxicity studies in rats and mice is given below.

Summary of Single Dose Acute Toxicity Studies

Species	Route	Sex	ALD (mg/kg)[a]	NOEL (mg/kg)[b]	Report No.
Mouse	Oral	Combined	>1250/625	<20/10	R&D/96/458
Rat	Oral	Combined	> 1250/625	39/20	R&D/96/456
Rat	Oral[c]	Combined	> 2500	100	R&D/96/669
Mouse	I.V.	Male	> 62.5/31.3	<1.0/0.5	R&D/96/459
		Female	>62.5/31.3	2.0/1.0	
Rat	I.V.	Male	31.3/15.6	3.9/2.0	R&D/96/457
		Female	31.3/15.6	1.0/0.5	

a. ALD = approximately lethal dose (ABT-378/ritonavir combination)
b. NOEL = no-observed effect level (ABT-378/ritonavir combination)
c. ABT-378 alone

2.5.2.9 Repeated Dose Toxicity

2.5.2.9.1 Rat

The repeated dose toxicity of ABT-378/ritonavir combination has been assessed in rats in studies ranging in duration from two weeks to six months of oral administration. Studies in neonatal and juvenile rats have also been conducted. Tabular summaries for individual studies to indicate treatment-related changes in survival, body weight gain, clinical signs, hematology, clinical chemistries, organ weights and anatomic pathology are appended at the end of this section. In addition, a summary of repeated oral dose toxicity studies in rats is given in the following table.

Summary of Repeated Toxicity Studies in Rats

Species/ Strain	Group Size	Duration (weeks)	Dosages (mg/kg/day)[a]	NTED[b] (mg/kg/day)	Target Organs	Report No.
Rat/ Crl:CD®BR	10M, 10F	2	0, 10/5, 30/15, 100/50	30/15	Liver, thyroid, spleen	R&D/96/300
Rat/ Crl:CD®BR	15M, 15F	13 (4-wk recovery)	0, 10/5, 50/25, 150/75	10/5	Liver, thyroid, RBC	R&D/96/574
Rat/ Crl:CD®BR	20M, 20F	26	0, 10/5, 50/25,100/50-150/75[c]	<10/5	Liver, thyroid, spleen, RBC	R&D/97/720
Neonatal Rat/ Crl:CD®BR	10M, 10F	2	0, 10/5, 20/10, 40/20	40/20	Liver (↑ liver weight only)	R&D/98/307
Juvenile Rat/ Crl:CD®BR	10M, 10F	4	0, 10/5, 30/15, 100/50	30/15	Liver, thyroid	R&D/98/375

a. ABT-378/ritonavir combination
b. NTED = No-toxic-effect dosage
c. The dosage for the high dose group was lowered from 150/75 mg/kg/day to 100/50 mg/kg/day on Day 11 for females and on Day 99 for males because of toxicity.

Repeated dose toxicity studies in rats have identified liver, thyroid, blood, and spleen as the target organs. Target organ toxicities and the minimum average plasma exposure (AUC) for ABT-378/ritonavir at which each occurs are shown in the table below.

Summary of Target Organ Toxicities in Rats

Organ	Toxicity	Treatment Duration	AUC (µg•hr/ml), ABT-378/ritonavir[a]
Liver	Hepatocellular changes	2 weeks[b]	121/10
	Hepatocellular changes, ↑ Chol, GGT, ALT	4 weeks[c]	172/10
	Hepatocellular changes, ↑ Chol	13 weeks	65/7
	Hepatocellular changes, ↑ ALP, AST, ALT, GGT, Chol	26 weeks	73/8
Thyroid	Hypertrophy of follicular cells, ↓ T_4, ↑ TSH	2 weeks[b]	121/10
		4 weeks[c]	172/10
		13 weeks	65/14
		26 weeks	73/8
Erythron	Morphologic changes in RBC, ↓ RBC, Hct, Hb	13 weeks	65/14
		26 weeks	73/8
Spleen	Histiocytosis, ↑ spleen weight	26 weeks	73/8

a. Values represented the average values of mean AUC values obtained on different sampling days.
b. No hepatocellular or thyroid changes were seen in neonatal rats at mean AUC values of 140/13 µg•hr/ml.
c. Juvenile rat study.

2.5.2.9.2 Dog

The repeated dose toxicity of ABT-378/ritonavir combination has been assessed in dogs in studies ranging in duration from two weeks to nine months of oral administration. Tabular summaries for individual studies to indicate treatment-related changes in survival, body weight gain, clinical signs, hematology, clinical chemistries, organ weights and anatomic pathology are appended at the end of this section. In addition, a summary of repeated oral dose toxicity studies in dogs is given in the following table.

Summary of Repeated Toxicity Studies in Dogs

Species/ Strain	Group Size	Duration (weeks)	Dosages (mg/kg/day)[a]	NTED[b] (mg/kg/day)	Target Organs	Report No.
Dog/Beagle	3M, 3F	2	0, 5/2.5,15/7.5, 50/25	50/25	None	R&D/96/243
Dog/Beagle	4-6M,4-6F	13 (4-wk recovery)	0, 10/5, 30/15, 70/35-100/50[d]	10/5	Liver	R&D/96/675
Dog/Beagle	4M, 4F	26	0,10/3, 25/8, 45/15-60/20[c]	10/3	Liver, testis	R&D/97/752
Dog/Beagle	4M, 4F	39	0, 10/5, 25/12.5, 50/25	50/25	Liver (↑ liver wt., ALP)	R&D/99/124

a. ABT-378/ritonavir combination
b. NTED = No-toxic-effect dosage
c. The high dosage was lowered from 100/50 mg/kg/day to 70/35 mg/kg/day on Day 30 due to toxicity.
d. The high dosage was lowered from 60/20 mg/kg/day to 45/15 mg/kg/day on Day 91 due to toxicity.

No toxicity was noted in dogs receiving dosages up to 50/25 mg/kg/day (mean AUCs = 155/66 μg•hr/ml) for two weeks. Repeated dose toxicity studies with a treatment duration of three to nine months in dogs have identified liver, blood and testis as the target organs. Target organ toxicities and the minimum average plasma exposure (AUC) for ABT-378/ritonavir at which each occurs are shown in the table below.

Summary of Target Organ Toxicities in Dogs

Organ	Toxicity	Treatment Duration	AUC (μg•hr/ml), ABT-378/ritonavir[a]
Liver	Hepatocellular changes, ↑ ALP, AST, ALT	13 weeks	189/65
	Hepatocellular changes, ↑ ALP, AST, ALT	26 weeks	206/53
	↑ liver wt, ALP[b]	39 weeks	68/19
Erythron	Morphologic changes in RBC, ↓ RBC, Hct, Hb	26 weeks	206/53
Testis	Loss of germ cells, germ cell degeneration & tubular vacuolization	26 weeks	20/2

a. Values represented the average values of mean AUC values obtained on different sampling days.
b. No histopathology changes were seen in dogs receiving the high-dosage (50/25 mg/kg/day; mean AUCs = 78/39 μg•hr/ml) for nine months.

2.5.2.10 Special Toxicity Studies

Tabular summaries for individual special toxicity studies to indicate treatment-related changes in survival, body weight gain, clinical signs, hematology, clinical chemistries,

organ weights and anatomic pathology are appended at the end of this section. In addition, a summary of these special studies in mice and dogs is given in the following table.

Summary of Special Studies

Species/ Strain	Group Size	Duration (weeks)	Dosages (mg/kg/day)[a]	NTED[b] (mg/kg/day)	Target Organs	Report No.
Mouse/ Crl:CD-1	10M, 10F	13[c]	0, 20/10, 60/30, 200/100	20/10	Liver, kidney	R&D/97/501
Dog/Beagle	4M, 4F	13[d]	0, 30/15	-	Liver (↑ liver wt.)	R&D/98/371
Dog/Beagle	4M, 4F	13[d]	0, 30/15	-	Liver (↑ liver wt., ALP)	R&D/99/093
Dog/Beagle	4M, 4F	13[d]	0, 50/25	-	Liver (↑ ALP, ALT)	R&D/00/030

a. ABT-378/ritonavir combination
b. NTED = No-toxic-effect dosage
c. An oral maximum-tolerated dosage study to establish dosage selection for a chronic bioassay.
d. The studies were designed to assess the toxicity of increased levels of impurities and degradation products in the drug formulations.

A three-month oral maximum-tolerated dosage study in mice has identified liver and kidney as the target organs. The toxicity seen in the three-month dog studies of the drug formulations with increased levels of impurities and degradation products was limited to increased liver weights and/or increased ALT and ALP values. Target organ toxicity and the minimum average plasma exposure (AUC) for ABT-378/ritonavir at which each occurs are shown in the table below.

Summary of Target Organ Toxicities

Organ	Toxicity	Treatment Duration	AUC (μg•hr/ml), ABT-378/ritonavir[a]
Liver	Hepatocellular changes, ↑ ALP, AST, GGT, Chol, Trig	13 weeks	Mice: 458/62
	↑ liver wt. , ↑ ALP	13 weeks	Dogs: 82/30
	↑ ALP, ALT	13 weeks	Dogs: 87/15
Kidney	Microvesicular cytoplasmic vacuolation	13 weeks	Mice: 458/62

a. Values represented the mean AUC values of males and females.

2.5.2.10.1 Justification of Drug Impurities and Degradation Products

Table 4 and Table 5 in Section 5.2.1. (Overview of Toxicology Studies) list the various impurities and degradation products which could occur in bulk drug or formulations of ABT-378 and ritonavir at levels ≥0.2 %, their proposed specification levels in bulk drug or

formulations and the potential maximum amount which could be exposed assuming ABT-378/ritonavir use at the proposed clinical dosage of 400/100 mg bid. All of these related substances have been included as impurities in bulk lots of drugs or as degradation products in formulations tested in separate repeated dose toxicology studies in dogs.

There was no evidence that the presence of these impurities and degradation products increased the level of toxicity in any of these studies. Furthermore, target organs have generally been the same in all studies indicating that these related substances did not change the toxicity profile of the parent compounds. In addition to testing in subchronic toxicity studies in dogs, two *in vitro* mutagenicity tests (Ames and cytogenetic assay) have also been conducted with several different lots of ABT-378 and ritonavir containing varying amounts of related substances. No mutagenicity was detected in any of these studies.

All of the identified impurities and degradation products are closely related to the parent compounds and generally differ by only slight modifications to the structure. Available data have revealed that these related substances do not alter the toxicity profile of the parent compounds, and these related substances will occur only at low concentrations resulting in a dosage of no more than 0.08 mg/kg/day in humans. Therefore, the levels of these related substances are considered to represent no undue risk to humans.

Loss of propylene glycol due to migration of propylene glycol from the capsule fill to the capsule shell and formation of propylene glycol-fatty acid esters on storage due to a chemical reaction of propylene glycol with oleic acid in the solvent system have been noted in ABT-378/ritonavir combination soft gelatin capsules (SGC Formulation B2). Approximately 74% of propylene glycol-esters formed in the formulation has been confirmed to be propylene glycol-oleate. The rate of propylene glycol loss is approximately 0.18 mg/g per month. Propylene glycol-fatty acid esters are predicted to go to approximately 45 mg/g (10 mg/g as propylene glycol equivalent) after two years at 5° C. Propylene glycol-fatty acid esters are predicted to go to approximately 27 mg/g (6 mg/g as propylene glycol equivalents) after three months at 25° C.

The 21 CFR, 172.856 states that propylene glycol mono- and di-esters of fats and fatty acids may be safely used in food providing they are produced from edible fats/fatty acids and/or oleic acid derived from total oil fatty acids, as well as they are used in amounts not

in excess of that reasonably required to produce their intended effect. In addition, an ABT-378/ritonavir SGC formulation (lot 61003-16) containing total estimated propylene glycol-esters of up to 23 mg/g including approximately 17 mg/g propylene glycol-oleate has been tested in a nine-month dog toxicity study.[21] Furthermore, a placebo formulation (lot E900825) containing approximately 107 mg/g propylene glycol-oleate (estimated level of total propylene glycol-esters is approximately 145 mg/g) and ABT-378/ritonavir SGC formulation (lot 61003-16) were tested in a recently completed two week monkey study.[48] These formulations are also being used in an ongoing two-year carcinogenicity study in rats. Based on the data generated from the nine-month dog study and two-week monkey study, as well as the in-life data from the two-year rat study, no toxicity associated with the levels of propylene glycol-esters or propylene glycol-oleate has been noted in the tested animals. Therefore, the safety risks to humans associated with the presence of propylene glycol-esters at levels up to approximately 135 mg/g including 100 mg/g propylene glycol-oleate in the SGC formulation are considered to be negligible.

2.5.2.11 Reproduction Studies

ABT-378/ritonavir combination was tested for potential reproductive hazards in separate studies covering all phases of the reproductive process. Tabular summaries for the individual segment I, II and III studies are appended at the end of this section. In addition, the summaries of reproduction studies in rats or rabbits are given in the following table.

Summary of Reproduction Toxicity Studies

Species/ Strain	Group Size	Oral Dosage (mg/kg/day)	Study Type	Endpoint	NTED (mg/kg/day)	Report No:
Rat/ Crl:CD®BR	24M, 24F	0, 10/5, 30/15, 100/50	Seg I	Fertility & embryonic development	100/50 (AUCs=114/8 µg•hr/ml)	R&D/97/382
Rat/ Crl:CD®BR	24F	0, 20/10, 50/25, 100/50	Seg II	Embryonic & fetal development	50/25 (AUCs = 64/9 µg•hr/ml)	R&D/97/335
Rat/ Crl:CD®BR	25F	0, 20/10, 40/20, 80/40	Seg III	Pre- & postnatal development	40/20 (estimated AUC for ABT-378 = 55 µg•hr/ml)	R&D/98/315
Rabbit/ New Zealand White	19-20F	0, 30/15, 50/25, 80/40	Seg II	Embryonic & fetal development	80/40 (AUCs= 90/9 µg•hr/ml)	R&D/97/365

NTED = No-toxic-effect dosage for reproductive or developmental toxicity (ABT-378/ritonavir)

There were no effects on male or female rat reproductive capabilities at dosages up to 100/50 mg/kg/day (mean AUCs = 114/8 µg•hr/ml which is approximately 71% of that achieved with the recommended therapeutic dose of 400/100 mg bid, AUCs = 160/9 µg•hr/ml), the highest dosage level tested in the segment I study. In the segment II study in rats, the drug combination was given during the period of organogenesis. Fetal toxicity in rats was observed at a maternally toxic dosage (100/50 mg/kg/day, AUC 116/16 µg•hr/ml) and was characterized by reduced fetal viability, reduced fetal weight, delayed skeletal ossification and an increased incidence in skeletal variations (14^{th} ribs and 27 presacral vertebrae). The fetal changes were considered as developmental variations. In a segment II study in rabbits, maternal toxicity including reductions in food consumption, decreased body weight gain and emaciation was observed only at a dosage of 80/40 mg/kg/day (mean AUCs = 90/9 µg•hr/ml which is approximately 56% of that achieved with the recommended therapeutic dose), the highest dosage tested in the study. However, no developmental toxicity, including teratogenicity was observed in this study. In a segment III study, the drug combination was given during late gestation through weaning. Findings were limited to a reduction in pup survival during lactation, which was noted at the high dosage of 80/40 mg/kg/day (estimated AUC value for ABT-378 = 95 µg•hr/ml which is approximately 59% of that achieved with the recommended therapeutic dose) that also produced a slight degree of transient maternal toxicity (diminished body weight gain and food consumption).

Although some developmental toxicity was observed at maternally toxic dosages, no drug-induced malformations were observed and ABT-378/ritonavir combination exhibited no evidence of being a specific reproductive or developmental toxin.

2.5.2.12 Mutagenicity Studies

ABT-378 alone or in combination with ritonavir has been tested in three *in vitro* and one *in vivo* mutagenicity assays. Tabular summaries for individual studies are appended at the end of this section. In addition, the summaries of these studies are given in the following table.

Summary of Short-Term Mutagenicity Studies

Test System	Endpoint	Concentration or Dosage	Result	Report No.
Bacteria - Ames	Reverse mutation	100-10,000 µg/plate (+/- S9)	Negative	R&D/96/439
Bacteria - Ames	Reverse mutation	100-5,000 µg/plate (+/- S9), with high impurity levels	Negative	R&D/98/303
Bacteria - Ames	Reverse mutation	100-5,000 µg/plate (+/- S9), with high new impurity levels	Negative	R&D/98/589
Bacteria - Ames	Reverse mutation	30-5,000 µg/plate (+/- S9), with related substances	Negative	R&D/99/447
Mammalian cell human lymphocytes (*in vitro*)	Chromosome aberration	1-10 µg/ml (- S9) 3-50 µg/ml (+ S9)	Negative	R&D/96/440
Mammalian cell human lymphocytes (*in vitro*)	Chromosome aberration	3-30 µg/ml (- S9), 20-50 µg/ml (+ S9) with high impurity levels	Negative	R&D/98/304
Mammalian cell human lymphocytes (*in vitro*)	Chromosome aberration	10-30 µg/ml (- S9), 10-50 µg/ml (+ S9) with high new impurity levels	Negative	R&D/98/645
Mammalian cell human lymphocytes (*in vitro*)	Chromosome aberration	10-50 µg/ml (- S9, 4-hr), 2-5 µg/ml (- S9, 24-hr), 5-30 µg/ml (+ S9) with related substances	Negative	R&D/99/448
Mouse bone marrow (*in vivo*)	Micronuclei	625, 1250, 2500 mg/kg/day (ABT-378 alone); 39/78, 78/156, 156/313 mg/kg/day (ABT-378/ritonavir)	Negative	R&D/96/520
Mammalian cells L5178Y mouse lymphocytes (*in vitro*)	Forward mutation	1-20µg/ml (- S9) 25-120 µg/ml (+ S9)	Negative	R&D/96/773

No evidence for mutagenic or clastogenic effects was detected in any of the above listed tests. Ames tests and *in vitro* cytogenetics in human lymphocytes on a lot of ABT-378 or ABT-378/ritonavir combination formulation containing high levels of impurities and degradation products were also negative.

2.5.2.13 Conclusion

ABT-378 alone or in combination with ritonavir at a 2:1 ratio has a low order of acute toxicity in rodents by the oral route but is more toxic when administered as an intravenous injection. ABT-378 alone or in combination with ritonavir was found to be non-mutagenic and non-clastogenic when tested in three *in vitro* and one *in vivo* mutagenicity

assays. Available data also indicate that ABT-378/ritonavir combination is not a specific reproductive or developmental toxin in rats or rabbits.

The most significant target organ for toxicity in the preclinical toxicity studies has been the liver. Hepatic changes, including hepatocytomegaly, multinucleated hepatocytes, single cell necrosis and/or cytoplasmic vacuolation were observed in rodents. Cytoplasmic vacuolation, single cell necrosis, bile accumulation and cell swelling were the hepatic changes seen in dogs. These changes were generally accompanied by increases in ALT, AST, ALP or GGT, but also by increases in cholesterol and triglycerides on occasion. Increases in cholesterol levels were limited in rats and mice, and an increase in triglycerides occurred in mice only. The hepatocellular changes seen in dogs appeared to be reversible after a one-month recovery period, but the occurrence of multinucleated hepatocytes and hepatocyte lysosomal inclusions in rats persisted through the one-month recovery period. Based on drug exposures, there are differences in liver toxicity between rats and dogs. Although no safety margin can be demonstrated in long-term toxicity studies in rats or dogs, effects in humans such as slight increases in ALT or AST values are generally milder and easily monitored. Therefore, it is our opinion that the proposed clinical dosage of this drug combination (400/100 mg bid) does not represent an undue risk in humans. The lower incidence of hepatic complications in the clinical studies than in the preclinical toxicity studies is consistent with humans being at reduced risk relative to rodents and dogs

Rats are generally more sensitive to endocrine effects than humans and this has been confirmed with drugs such as phenobarbital, with respect to the thyroid gland. The ABT-378/ritonavir combination has not produced any thyroid-related effects in dogs and no effects on thyroid-related hormones have been observed in human studies. Kidney changes reported in the three-month mouse study appear to be an exacerbation of a species-specific spontaneous change and has no relevance to man. Changes in erythrocytes appear to be a secondary effect subsequent to liver alterations. These changes have been monitored in routine clinical workups and have not been reported in the clinical trials. Mild testicular degeneration in dogs receiving ABT-378/ritonavir combination for six months did not appear to be dose-related or correlated with systemic exposure, and no testicular effects were seen in dogs given the combination for nine months. Based on the lack of a dose response and the absence of similar findings in dogs

exposed to higher plasma exposures for a longer treatment period, the changes are judged to have been spontaneous rather than drug-related.

Studies conducted in neonatal (3-4 days old at the start of treatment) or juvenile (16 days old at the start of treatment) rats indicated that the toxicity profile of ABT-378/ritonavir combination is similar in neonatal, juvenile and adult rats. On the basis of drug exposures, neonates appear to be less sensitive to the toxicity produced by ABT-378/ritonavir combination when compared with adult rats.

Clinical trials have demonstrated increased triglyceride ($\geq$ 750 mg/dL) and cholesterol ($\geq$ 300 mg/dL) levels in subjects administered the ABT-378/ritonavir combination. No effects on triglyceride or cholesterol levels were reported in any of the preclinical studies in dogs. Elevations in triglycerides occurred in mice only while cholesterol elevations in mice and rats were considered to be possibly secondary to hepatic effects. No mechanism can be ascribed at present to cholesterol or triglyceride changes seen in humans. However, triglyceride and cholesterol levels are routinely monitored in humans during therapy and the degree of elevations observed to date do not represent an unacceptable safety risk.

The pharmacokinetics of ABT-378 and ritonavir after multiple oral doses of the combination appeared to be nonlinear in rats or dogs. In general, C_{max} and AUC values increased less than proportionately with increasing dose. Female rats generally exhibited higher AUC values of ABT-378 and ritonavir than male rats. However, in dogs there were no consistent sex-related differences in mean plasma drug exposures. AUC values of both ABT-378 and ritonavir were quite variable between dogs of the same sex and dosage group. Due to the possibility of non-linear pharmacokinetics, consideration needs to be given to both dosage and plasma exposure when projecting the safety, in humans, of ABT-378 in combination with ritonavir.

The identified impurities and degradation products in the bulk drugs or the combination formulations have been tested in three-month toxicity studies in dogs and two *in vitro* mutagenicity studies. None of these related substances was found to alter the toxicity profile of the parent compounds.

It is our conclusion that considering the patient population to be treated with the ABT-378/ritonavir combination, an appropriate battery of tests and studies have been conducted and none of the findings preclude the approval for this drug combination for the treatment of AIDS patients at a recommended dosage of 400/100 mg bid.

Tabulated Summary

Acute Rodent Toxicity Studies of Abbott-157378/ritonavir Combination or Abbott-157378 Alone (Abbott Study Nos. TA96-218, TA96-219, TD96-220, TD96-221 and TA96-315)

Species	Route	Sex	LD_{50}[a]	ALD[b] (mg/kg)	ANOEL[c] (mg/kg)
Mouse	Oral	Combined	-	>1250/625	< 20/10
Rat	Oral	Combined	-	>1250/625	39/20
Rat	Oral (A-157378 alone)	Combined	-	>2500	100
Mouse	I.V.	Male Female	- -	>62.5/31.3 >62.5/31.3	<1.0/0.5 2.0/1.0
Rat	I.V.	Male Female	- -	31.3/15.6 31.3/15.6	3.9/2.0 1.0/0.5

a. LD_{50} = median lethal dose
b. ALD = approximately lethal dose
c. ANOEL = no-observed effect level

Tabulated Summary

Two-Week Oral Toxicity Study of Abbott-157378 in Combination with Abbott-84538 in Rats (Abbott Study No. TA96-079)

Dosage (mg/kg/day) A-157378/A-84538	0/0	10/5	30/15	100/50
Rats/Sex/Group	10 (5)[a]	10 (5)	10 (5)	10 (5)
Deaths	1[b]	0	0	0
Body Weight Gain	-	No treatment-related differences (NTD)		
Food Consumption	-			↓ (F, transient)
Clinical Signs	-			
Mean Plasma Drug AUC (µg•hr/ml) Day 9				
A-157378				
Males	-	16.7	31.8	107.2
Females	-	24.5	57.2	134.2
A-84538				
Males	-	0.7	1.4	6.9
Females	-	1.7	5.2	13.3
Ophthalmoscopy	-	NTD		
Plasma Thyroxine (T_4)	-			↓
Plasma TSH	-			↑
Urinalysis	-			
Hematology	-		↑ Retic (F)	↑ Retic, Plt (M), APTT (M),
	-	↑ Poikilocytosis		
Clinical Chemistry	-			↓ Na, Cl, ↑ TP, Alb (M), Glob,
Organ Weights	-		↑ Liver, ↑ Spleen (F)	↑ Liver, Thyroid, ↑ Spleen (F)
Anatomic Pathology	-	NTD	Spleen: Extra-medullary erythropoiesis (F)	Liver - Hepatocytomegaly Thyroid - Hypertrophy of follicular epithelium, reduced follicular diameter Spleen - Extra-medullary erythropoiesis (F)
Conclusion:	Drug-related tissue changes were observed in blood (reticulocytosis), liver (hepatocytomegaly), spleen (extra-medullary erythropoiesis) and thyroid (hypertrophy) at 100/50 mg/kg/day. Similar, although milder or less frequent changes, were observed at 30/15 mg/kg/day in the blood, spleen and liver, however, at this dosage the findings were not judged to be toxicologically meaningful. Based on these findings the no-toxic-effect dosage for two weeks of exposure was 30/15 mg/kg/day (A-157378/A-84538).			

[a] Five rats/sex constituted a satellite group for plasma drug level determinations.
[b] Morphologic findings were compatible with a dosing accident.

Tabulated Summary

Two-Week Oral Toxicity Study of Abbott-157378/Abbott-84538 (Ritonavir) Combination in Neonatal Rats (Abbott Study No. TA98-069)

Abbott-157378/Abbott-84538 Dosage (mg/kg/day)[a]		0/0	10/5	20/10	40/20
Abbott-157378/Abbott-84538 Dosage (mg/m^2/day)[b]		0/0	35/17	70/35	140/70
Neonatal Rats/Sex/Group[c,d]		10	10	10	10
Deaths		0	1M[e]	0	2M[e]
Body Weight Gain		-	no treatment-related differences (NTD)		
Clinical Signs		-			
Mean Plasma Drug AUC (µg•hr/ml)					
Abbott-157378	Males	-	59.5	77.3	129.7
	Females	-	56.0	64.2	149.6
Abbott-84538	Males	-	5.3	5.9	12.5
	Females	-	4.6	7.3	13.9
Hematology		-	NTD		
Clinical Chemistry		-			
Organ Weights		-	↑ absolute and relative liver		
Anatomic Pathology		-	NTD		
CONCLUSION:		Although mean liver weights were increased for all drug-treated groups, there were no microscopic alterations to explain the weight changes. Therefore, the highest dosage, 40/20 mg/kg/day, was considered the no-observable-adverse effect dosage in this study.			

a. A pediatric formulation containing Abbott-157378 and Abbott-84538 in a 2:1 ratio was administered to the drug-treated rat pups. The pups in the control group (0/0 mg/kg/day) received placebo formulation.
b. Estimated surface area for a 30 g rat pup is 0.0086 m^2.
c. Rats were 3-4 days old at the start of treatment.
d. Each group contained an additional 5 or 15 satellite pups per sex for determination of plasma drug concentrations.
e. None of the deaths were considered drug-related.

Tabulated Summary

Four-Week Oral Toxicity Study of Abbott-157378 in Combination with Ritonavir (Abbott-84538) in Immature (Juvenile) Rats (Abbott Study No. TA98-022)

A-157378/A-84538 Dosage (mg/kg/day)[a]	0/0	10/5	30/15	100/50
A-157378/A-84538 Dosage (mg/m^2/day)[b]	0/0	50/25	150/75	500/250
Rats/Sex/Group[c]	10 (3)	10 (15)	10 (15)	10 (15)
Deaths	0	0	0	2 M[d]
Body Weight Gain	-	No treatment-related differences (NTD)		↓
Food Consumption	-			↓
Clinical Signs	-	Noisy respiration		
Mean Plasma Drug AUC (µg•hr/ml) Abbott-157378				
Day 26 Males	-	12.04	42.29	135.96
Females	-	23.92	61.65	206.92
Abbott-84538				
Day 26 Males	-	0.06	1.59	8.59
Females	-	0.27	3.26	11.03
Ophthalmoscopy	-	NTD		
Urinalysis	-			
Hematology	-			
Clinical Chemistry	-		↑ cholesterol (F)	↑ cholesterol, GGT, ALT (F), TSH (F); ↓ thyroxine
Organ Weights	-		↑ relative liver	↑ absolute and relative liver
Anatomic Pathology Gross Findings	-		NTD	
Microscopic Findings	-		Liver: hepatocytomegaly Thyroid: follicular cell hypertrophy/hyperplasia	
CONCLUSION:	Target organ changes in the liver (hepatocytomegaly) and thyroid (follicular cell hypertrophy/hyperplasia) of immature rats in this study were generally similar to those previously observed in adult rats administered this drug combination at the same dosages. Since the mild liver and thyroid changes observed at 30/15 mg/kg/day Abbott-157378/ritonavir were not accompanied by relevant alterations in clinical chemistry parameters or decrements in body weight and food consumption, this dosage was considered to approximate a no-toxic-effect level.			

a. Pediatric formulations containing 96.8 mg/ml Abbott-157378 and 59.5 mg/ml Abbott-84538 were combined and diluted with placebo as needed for administration to the drug-treated groups. The pups in the control group received placebo formulation.
b. Based on the estimated surface area of 0.0194 m^2 for a 100 g rat.
c. Rats were 16 days old at the start of treatment. Numbers within parentheses indicate the number of satellite rats used for plasma drug level determinations.
d. The deaths of the two males in the high dosage group were attributed to gavage dosing errors.

Tabulated Summary

Three-Month Oral Toxicity Study of Abbott-157378 in Combination with Ritonavir (Abbott-84538) in Rats (with a One-Month Recovery Period) (Abbott Study No. TA96-156)

Dosage[a] (mg Abbott-157378/kg/day)	0[b]	10	50	150
Dosage[c] (mg Abbott-84538/kg/day)	0[d]	5	25	75
Rats/Sex/Group	15[e] (5)[f]	15[e] (5)[f]	15[e] (5)[f]	15[e] (5)[f]
Deaths[g]	0	0	0	0
Body Weight	-	No treatment-related differences (NTD)		↓
Food Consumption	-			↓
Clinical Signs	-			rough hair, dehydration, emaciation, weakness and hunched posture
			urine-stained hair, salivation	
			matted hair	
Mean Plasma Drug AUC (µg•hr/ml) ± SD				
Abbott-157378				
Day 28 Males	-	6.40 ± 1.90	40.23 ± 12.40	112.35 ± 6.40
Females	-	18.66 ± 3.55	73.71 ± 6.45	187.34 ± 23.21
Day 84 Males	-	8.30 ± 3.32	52.96 ± 27.48	142. 09 ± 44.10
Females	-	20.48 ± 3.37	91.36 ± 21.70	203.45 ± 26.86
Abbott-84538				
Day 28 Males	-	nc[h]	2.69 ± 1.55	7.55 ± 2.42
Females	-	0.83 ± 0.27	8.95 ± 3.62	20.74 ± 18.65
Day 84 Males	-	0.53 ± 0.34	5.33 ± 3.26	12.81 ± 5.03
Females	-	1.27 ± 0.84	10.73 ± 3.79	12.19 ± 6.33
Ophthalmoscopy	-	NTD		

a. Abbott-157378 was administered in combination with ritonavir in a vehicle of propylene glycol:ethanol (95:5, v/v) once daily by oral gavage (1 ml/kg) at dosages of 0, 10, 50 and 150 mg/kg/day.
b. Vehicle, for Abbott-157378 was propylene glycol:ethanol (95:5, v.v).
c. Abbott-84538 was administered in propylene glycol:ethanol (95:5, v/v) with two molar equivalents of p-toluene sulfonic acid monohydrate once daily by oral gavage (1 ml/kg) at dosages of 0, 5, 25 and 75 mg/kg/day.
d. Vehicle, for Abbott-84538 was propylene glycol:ethanol (95:5, v/v) with two molar equivalents of p-toluene sulfonic acid monohydrate.
e. Five additional rats/sex/group were designated as satellite rats for the determination of Abbott-157378 and Abbott-84538 plasma drug levels.
f. Five rats/sex/group were designated for recovery.
g. No drug-related deaths occurred during the study. However gavage-related deaths included two vehicle control rats and one satellite rat from the 50/25 mg/kg/day dosage group. One male rat in the 150/75 mg/kg/day dosage group was found moribund related to hematology sampling and was euthanized.
h. Not calculated (nc) due to insufficient data points.

Tabulated Summary (Cont.)

Dosage[a] (mg Abbott-157378/kg/day)	0[b]	10	50	150
Dosage[c] (mg Abbott-84538/kg/day)	0[d]	5	25	75
Rats/Sex/Group	15[e] (5)[f]	15[e] (5)[f]	15[e] (5)[f]	15[e] (5)[f]
Urinalysis	-	No treatment-related differences (NTD)		
Hematology[g] 12-week analysis	-		↑ reticulocytes & ↑ RDW, ↓ Hb, Hct, (females), ↓ Hb (males)	↑ reticulocytes, ↑ APTT; ↑ RDW , ↓ MCH & MCV (females); ↓ RBC, Hb (males)
		poikilocytosis and other RBC morphological changes		
Clinical Chemistry[g] 12-week analysis	-	NTD	↑TP, ↑Chol; ↓ Trig &↑Alb (males); ↑ Glob (females)	
				↑ GGT;↓ Glu (males)
Biochemical Assay[g] 12-week analysis TSH	-		↑ (females)	↑
T4			↓ (males)	↓
Organ Weights	-		↑ liver, thyroid	
Anatomic Pathology Gross	-			
Microscopic	-		hepatocytomegaly	
	multinucleated hepatocytes (1 female)		multinucleated hepatocytes (males)	multinucleated hepatocytes
	-	↑ thyroid hypertrophy and hyperplasia		
Ultrastructural Pathology	-	minimal increase in hepatic smooth endoplasmic reticulum and hepatocyte lysosomal inclusions (males)		
		NTD		hepatocyte lysosomal inclusions, minimal increase in smooth endoplasmic reticulum, aggregates of tubules within peroxisomes (females)
Recovery:	Erythrocyte morphological changes, the occurrence of multinucleated hepatocytes and hepatocyte lysosomal inclusions persisted through the recovery period. The cholesterol levels also remained elevated in high-dosage group females. All other toxicologic and pathologic effects were reversed or returned toward normal during this period.			
Conclusion:	Given the mild thyroid microscopic changes 10 mg Abbott-157378 in combination with 5 mg ritonavir/kg/day approximates the no-toxic-effect level.			

a. Abbott-157378 was administered in combination with ritonavir in a vehicle of propylene glycol:ethanol (95:5, v/v) once daily by oral gavage (1 ml/kg) at dosages of 0, 10, 50 and 150 mg/kg/day.
b. Vehicle, for Abbott-157378 was propylene glycol:ethanol (95:5, v.v).
c. Abbott-84538 was administered in propylene glycol:ethanol (95:5, v/v) with two molar equivalents of p-toluene sulfonic acid monohydrate once daily by oral gavage (1 ml/kg) at dosages of 0, 5, 25 and 75 mg/kg/day.
d. Vehicle, for Abbott-84538 was propylene glycol:ethanol (95:5, v/v) with two molar equivalents of p-toluene sulfonic acid monohydrate.
e. Five rats/sex/group were designated for recovery.
f. Five additional rats/sex/group were designated as satellite rats for the determination of Abbott-157378 and Abbott-84538 plasma drug levels.
g. Only the twelve-week findings have been tabulated for the sake of clarity and brevity. Four-week and eight-week data are discussed in the Results section.

Tabulated Summary

Six-Month Oral Toxicity Study with Abbott-157378 in Combination with Abbott-84538 (Ritonavir) in Rats (Abbott Study No. TA97-002)

Dosage (mg/kg/day)[a]					
A-157378		0	10	50	150/100[b]
A-84538		0	5	25	75/50[b]
Rats/Sex/Group		20 (5)	20 (5)	20 (5)	20 (5)
Deaths		1M[c], 1F[c]	2M[c], 1F[c]	1M	8M[c], 1F
Body Weight Gain		-	↑ F	NTD	↓
Food Consumption		-		↓ transient	↓ transient
Clinical Signs		-	NTD (No treatment-related differences)		emaciation, hunched posture, salivation, decreased activity, noisy respirations and poor grooming.
Mean Plasma Drug AUC (µg•hr/ml)					
A-157374					
Day 28	Males	-	14.4 ± 2.8	53.6 ± 8.3	93.7 ± 29.3
	Females	-	19.7 ± 4.0	83.5 ± 18.9	120.3 ± 31.9
Day 98	Males	-	16.6 ± 1.8	50.6 ± 5.7	126.7 ± 41.0
	Females	-	19.1 ± 5.0	90.5 ± 15.9	124.2 ± 35.2
Day 174	Males	-	18.4 ± 5.0	63.3 ± 20.8	139.2 ± 35.2
	Females	-	19.3 ± 3.2	97.9 ± 18.6	113.7 ± 43.6
A-84538					
Day 28	Males	-	0.0 ± 0.0	4.6 ± 2.0	9.8 ± 2.3
	Females	-	0.9 ± 0.6	7.5 ± 3.3	9.0 ± 0.9
Day 98	Males	-	0.1 ± 0.1	5.9 ± 1.5	8.6 ± 3.1
	Females	-	0.8 ± 0.5	9.0 ± 2.5	10.8 ± 3.0
Day 174	Males	-	0.0 ± 0.0	5.2 ± 3.1	9.1 ± 2.9
	Females	-	1.2 ± 1.0	14.1 ± 3.6	8.6 ± 1.4
Ophthalmoscopy		-	NTD		

a. Abbott-157378 and Abbott-84538 were prepared using the SEC formulation (oleic acid, ethanol, Cremophor, and BHT). The two drugs were prepared separately but combined prior to dosing; dose volume was 2 ml/kg.

b. The dosage for the high-dose treatment group was reduced to 100 mg/kg/day Abbott-157378 and 50 mg/kg/day Abbott-84538 on Day 11 for females and Day 99 for males because of toxicity.

c. Deaths were not drug-related in the control and low dosage groups. Two deaths in the high dosage males were not drug-related

Tabulated Summary (Cont.)

Dosage (mg/kg/day)[a] A-157378 A-84538	0 0	10 5	50 25	150/100[b] 75/50[b]
Rats/Sex/Group	20 (5)	20 (5)	20 (5)	20 (5)
Urinalysis			NTD	
Hematology, Erythron	-	NTD	anisocytosis, poikilocytosis, (spherocytosis, acanthocytosis)	
			↓ Hb, MCV, MCH; ↑ RDW (M), Retics (M)	
				↓ Hct(M); ↑ RDW (F), Retics (F)
Leukocytes	-		↑ Plt; ↓ MPV (M); ↑ Lymphocytes (M)	↑ Plt ; ↑ Lymphocytes
Clinical Chemistry Enzymes/clotting factors	-	NTD	↑ ALT (M)	
			↑ GGT; ↓ AST (F); ↑ APTT (M), ↑ PT (M)	
				↑ ALP
Proteins	-		↑ Chol, TP, Glob; ↑ BUN (M), ↑ Alb (M), ↓ Trig (M)	
				↑ BUN (F)
Electrolytes	-		↓ Cl	↓ Na, Cl
Thyroid Hormone	-	↑ TSH (F)	↓ T_4, ↑ TSH (F)	↓ T_4, ↑ TSH (F)
Organ Weights	-	NTD	↑ liver, ↑ spleen; ↑ thyroid (F)	
				↑ thyroid (M); ↓ pituitary (F), ↓ thymus
Anatomic Pathology Liver	-		multinucleated hepatocytes, single-cell necrosis, karyomegaly	
			histiocytosis (M), hematopoiesis (M); hepatocytomegaly (F)	histiocytosis, hematopoiesis; hepatocytomegaly (M), centrilobular necrosis (M)
Spleen	-		histiocytosis	
Thyroid	-		hypertrophy/hyperplasia	
CONCLUSION:	Based on these findings the primary target organs were liver and thyroid with changes in blood and spleen likely to be secondary events. Based on the increase in serum ALT levels in males at the low dosage, the no-toxic-effect dosage was less than 10 mg/kg/day Abbott-157378 and 5 mg/kg/day Abbott-84538.			

a. Abbott-157378 and Abbott-84538 were prepared using the SEC formulation (oleic acid, ethanol, Cremophor, and BHT). The two drugs were prepared separately but combined prior to dosing; dose volume was 2 ml/kg.

b. The dosage for the high-dose treatment group was reduced to 100 mg/kg/day Abbott-157378 and 50 mg/kg/day Abbott-84538 on Day 11 for females and Day 99 for males because of toxicity.

Tabulated Summary

Two-Week Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs

(Abbott Study No. TB96-067)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	5/2.5	15/7.5	50/25
Dogs/Sex/Group	3	3	3	3
Deaths	0	0	0	0
Clinical Signs	emesis (F)	salivation, diarrhea/abnormal stools (F)	emesis, diarrhea, salivation (M); diarrhea/abnormal stools, salivation (F)	emesis, salivation, diarrhea/abnormal stools (M&F)
Body Weight Gain	-	No treatment-related differences (NTD)		
Food Consumption	-			
Ophthalmoscopy	-			
Electrocardiograms	-			
Mean Plasma Drug Levels (Abbott-157378) C_{max} (µg/ml)				
Day 1 Males	-	4.3 ± 3.1	8.6 ± 3.6	10.8 ± 3.1
Females	-	6.8 ± 1.3	10.5 ± 4.2	13.4 ± 0.7
Day 13 Males	-	3.8 ± 1.1	10.3 ± 3.8	14.8 ± 4.0
Females	-	4.8 ± 0.9	13.0 ± 5.6	18.5 ± 4.6
AUC (µg•hr/ml)				
Day 1 Males	-	23.6 ± 18.8	78.7 ± 40.4	108.5 ± 32.9
Females	-	35.0 ± 8.3	92.7 ± 44.9	118.7 ± 33.0
Day 13 Males	-	21.8 ± 7.0	79.1 ± 25.2	172.3 ± 68.7
Females	-	19.8 ± 7.4	114.6 ± 48.3	220.6 ± 30.5
(Abbott-84538) C_{max} (µg/ml)				
Day 1 Males	-	1.9 ± 1.8	6.9 ± 6.7	11.0 ± 4.6
Females	-	2.2 ± 0.8	10.6 ± 3.6	10.0 ± 6.1
Day 13 Males	-	0.9 ± 1.1	5.5 ± 4.1	20.1 ± 2.1
Females	-	0.3 ± 0.3	5.8 ± 1.5	17.2 ± 2.9
AUC (µg•hr/ml)				
Day 1 Males	-	4.8 ± 4.7	23.6 ± 19.3	39.2 ± 12.5
Females	-	4.8 ± 1.5	33.1 ± 5.7	38.0 ± 32.0
Day 13 Males	-	2.5 ± 3.2	22.6 ± 15.8	98.1 ± 25.9
Females	-	0.6 ± 0.7	27.8 ± 6.7	90.0 ± 26.5
Hematology	-	NTD		
Clinical Chemistry	-			
Urinalysis	-			
Organ Weights	-			
Gross Findings	-			
Microscopic Findings	-			
CONCLUSION:	Two-week treatment with Abbott-157378 and Abbott-84538 combination in beagle dogs at dosage levels of 5/2.5, 15/7.5 and 50/25 mg/kg/day did not produce overt toxicity. When compared to placebo controls, increased incidence of adverse clinical signs that included emesis, salivation and diarrhea/abnormal stools were evident in dogs receiving mid or high dosage. The no-toxic-effect level in this study was considered to be 50/25 mg/kg/day for a combination of Abbott-157378 and Abbott-84538.			

a. Semi-solid formulations were used.

[b] Control dogs received placebo formulations.

Tabulated Summary

Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (with a One-Month Recovery Period) (Abbott Study No. TB96-157)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	10/5	30/15	100/50 (Days 0 - 29)[c] 70/35 (Days 30 - 92)
Dogs/sex/group	6[d]	4	4	6[d]
Deaths	0	0	0	3 M & 2 F
Clinical Signs[e]	emesis (+), diarrhea/loose stools (+)	emesis (+), diarrhea/loose stools (++)	emesis, diarrhea/loose stools (+++); ↑ salivation (++), ↓ activity (1F)	emesis, diarrhea/loose stools (++++); ↑ salivation (+++), ataxia, tremors, jerks, prostration, dehydration, emaciation, weakness, pale/dilated eyes, ↓ activity, circling and/or labored/deep respiration
Body Weight Gain	-	No treatment-related differences (NTD)		↓
Food Consumption[f]	-			
Ophthalmoscopy	-			
Electrocardiograms[g]	-			prominent U waves (6 M&1 F); fusion of QT & U waves (3 M); triggered ventricular extrasystoles (1F); first degree atrioventricular block (1M)

a. Semi-solid formulations were used.
b. Control dogs received placebo formulations.
c. The high dosage was lowered to 70/35 mg/kg/day on Day 30.
d. Two males and two females were held for a one-month recovery period.
e. + = mild, ++ = moderate, +++ = severe, ++++ = most severe (based on a total number of occurrences).
f. Qualitative assessments of food consumption were conducted.
g. Abnormalities were considered to be related to observed hypokalemia and liver damage rather than to direct cardiac effects.

Tabulated Summary (Cont.)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	10/5	30/15	100/50 (Days 0 - 29)[c] 70/35 (Days 30 - 92)
Dogs/sex/group	6[d]	4	4	6[d]
Mean Plasma Drug Levels[e]				
Abbott-157378				
C_{max} (µg/ml)				
Males		7.7	12.5	14.7
Females		8.2	10.3	22.4
AUC (µg·hr/ml)				
Males		58.6	102.6	162.2
Females		59.8	89.0	216.0
Abbott-84538				
C_{max} (µg/ml)				
Males		2.8	8.4	14.3
Females		3.2	8.5	12.9
AUC (µg·hr/ml)				
Males		10.3	29.9	69.2
Females		11.3	33.6	60.8
Hematology	-	-	-	↓ RBC, Hb, Hct, MPV
Clinical Chemistry		↑ ALP (F)	↑ ALT (F), AST (F), ALP	↓ K, Na, Cl, acid-base imbalances, ↑ ALT, AST, ALP
Urinalysis	-	NTD		
Organ Weights	-			↑ liver (rel) (F)
		↓ testis (rel)	↓ testis (abs, rel)	
Gross Findings	-	-	-	yellowed aorta
Microscopic Findings	-	-	-	Hepatocellular vacuolization, hepatic inflammation, canalicular bile casts
Ultrastructure	-	-	-	canalicular bile plugs
Recovery Period	-	Normalization of prior abnormalities		
CONCLUSION:	Morbidity and early deaths during treatment were attributed to gastrointestinal disturbances resulting from oral dosage. No significant signs of toxicity were observed in the low-dose group (10/5 mg/kg/day of Abbott-157378/Abbott-84538, with corresponding mean AUC values of 59.2/10.8 µg·hr/ml).			

a. Semi-solid formulations were used.
b. Control dogs received placebo formulations.
c. The high dosage was lowered to 70/35 mg/kg/day on Day 30.
d. Two males and two females were held for a one-month recovery period.
e. Average values of the mean values obtained on Days 1, 29 and 82.

Tabulated Summary

Six-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs

(Abbott Study No. TB97-003)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	10/3	25/8	60/20 (Days 0 - 90)[c] 45/15 (Days 91 - 183)
Dogs/Sex/Group	4	4	4	4
Deaths	0	0	0	0
Body Weight Gain	-	No treatment-related differences (NTD)		
Food Consumption[d]	-			↓.
Clinical Signs[e]	emesis (+), loose stools (++)		emesis (++), loose stools (++)	emesis (+++), loose stools (++++), diarrhea (++), ↑ salivation, ↓ activity, dehydration, emaciation, weakness
Mean Plasma Drug AUC (µg•hr/ml) + SD				
A-157378				
Day 1: Males	-	32.5 ± 31.9	83.4 ± 15.1	219.0 ± 51.3
Females	-	41.2 ± 28.7	72.3 ± 53.5	217.1 ± 46.6
Day 90: Males	-	17.4 ± 3.3	62.1 ± 18.3	242.0 ± 99.7
Females	-	22.0 ± 15.7	45.6 ± 8.4	286.4 ± 135.7
Day 167: Males	-	11.0 ± 10.3	88.7 ± 26.5	154.1 ± 88.5
Females	-	25.4 ± 17.5	104.9 ± 61.2	116.5 ± 65.0
A-84538				
Day 1: Males	-	5.0 ± 4.6	15.1 ± 7.8	58.8 ± 13.7
Females	-	6.8 ± 2.3	18.9 ± 2.6	76.8 ± 21.9
Day 90: Males	-	0.9 ± 0.3	7.7 ± 2.9	40.7 ± 39.2
Females	-	2.6 ± 3.3	4.7 ± 0.4	58.9 ± 26.9
Day 167: Males	-	0.5 ± 0.6	8.8 ± 4.5	35.8 ± 23.7
Females	-	1.5 ± 1.0	13.2 ± 8.0	44.7 ± 29.0

a. The SEC formulation containing approximately 20% (wt/wt) of each active drug was administered in capsules. The daily dosages of Abbott-84538 was given in divided dosages twice daily (bid).
b. Control dogs received placebo formulation
c. The high dosage was lowered to 45/15 mg/kg/day on Day 91.
d. Semi-quantitative assessments of food consumption were conducted.
e. + = mild, ++ = moderate, +++ = severe, ++++ = most severe (based on a total number of occurrences).

Tabulated Summary (Cont.)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	10/3	25/8	60/20 (Days 0 - 90)[c] 45/15 (Days 91 - 183)
Dogs/Sex/Group	4	4	4	4
Protein Binding	-	The protein binding of both Abbott-157378 and Abbott-84538 at all dosage levels was greater than 98%.		
Electrocardiograms	-	NTD		
Ophthalmoscopy	-			
Urinalysis	-			
Hematology	-	NTD		↓ Plt, Neut; spherocytes (F, Day 84); ↓ Hb, Hct, RBC, Retic (Days 84 & 175)
Clinical Chemistry	-		↑ ALP, ALT (F)	↑ ALP, ALT, AST; ↓ Alb (Day 84) ↑ ALP, ALT, AST (Day 175)
Organ Weights	-	NTD	↑ liver	↑ liver, ↓ prostate
Anatomic Pathology	-	-		Liver - cytoplasmic vacuolation, single cell necrosis and bile accumulation Prostate - atrophy/hypoplasia (1 M)
	-		Liver - increased incidence of cell swelling	
	-	Testis - loss of germ cells, germ cell degeneration and tubular vacuolization (2 at low dosage, 3 at mid dosage and 2 at high dosage).		
Ultrastructural Pathology	-	Focal crystalline inclusions in hepatocytes (no enlarged mitochondria)	Not evaluated	Crystalline inclusions in enlarged mitochondria of hepatocytes
CONCLUSION:	Oral dosage levels of 45/15 - 60/20 mg/kg/day (Abbott-157378/ritonavir) for six months produced overt toxicity including gastrointestinal disturbance, but no changes in ECG were seen. The target organs were identified as the liver and testis. Hepatic lesions accompanying elevations in hepatic enzyme activities occurred only in dogs that had systemic drug exposures (AUCs) for Abbott-157378 of approximately 170 µg•hr/ml or greater. Testicular changes did not appear to be correlated with the AUC values. No significant signs of toxicity were observed in the low dosage group (10/3 mg/kg/day of Abbott-157378/ritonavir, with corresponding averaged group mean AUC values of 25/3 µg•hr/ml).			

a. The SEC formulation containing approximately 20% (wt/wt) of each active drug was administered in capsules. The daily dosages of Abbott-84538 was given in divided dosages twice daily (bid).
b. Control dogs received placebo formulation
c. The high dosage was lowered to 45/15 mg/kg/day on Day 91.

Tabulated Summary

Nine-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination in Beagle Dogs (Abbott Study No. TB98-020)

Dosage (mg/kg/day) (Abbott-157378/Abbott-84538)[a]		0[b]	10/5	25/12.5	50/25
Dogs/Sex/Group		4	4	4	4
Deaths		0	0	0	0
Body Weight Gain		-	↓		
Food Consumption		-	No treatment-related differences (NTD)		↓
Clinical Signs		-	emesis, abnormal stools/diarrhea	emesis, abnormal stools/diarrhea, salivation	
Plasma AUC (μg•hr/ml)[c]					
Abbott-157378	Males	-	35.9	67.3	85.6
	Females	-	21.5	69.2	69.9
Abbott-84538	Males	-	7.8	20.1	48.5
	Females	-	5.9	17.4	29.1
Plasma C_{max} (μg/ml)[c]					
Abbott-157378	Males	-	5.3	7.0	7.8
	Females	-	3.3	9.5	8.2
Abbott-84538	Males	-	2.4	5.7	10.4
	Females	-	1.7	5.5	8.2
Ophthalmoscopy		-	NTD		
Electrocardiography		-			
Hematology		-			
Clinical Chemistry		-	↑ ALP (M)		
Urinalysis		-	NTD		↑ bilirubin (1M)
Organ Weights		-	NTD	↑ relative liver (F)	↑ relative liver
Anatomic Pathology		-	NTD		
CONCLUSION:		Drug-related changes were observed in dogs treated with the Abbott-157378/Abbott-84538 combination, but none was considered toxicologically meaningful. Therefore, 50/25 mg/kg/day was considered the no-toxic-effect dosage.			

a. A soft elastic capsule formulation containing Abbott-157378 and Abbott-84538 in a ratio of approximately 2:1 was placed in gelatin capsules just prior to oral administration to the dogs.
b. Control dogs received placebo formulation (same composition as drug formulation but without either drug) comprised of oleic acid, propylene glycol, polyoxyl 35 castor oil and butylated hydroxytoluene.
c. Average of Day 1, Day 182 and Day 267 mean values.

Tabulated Summary

Three-Month Oral Maximum-Tolerated Dosage Study with Abbott-157378 in Combination with Abbott-84538 (Ritonavir) in Mice (Abbott Study No. TD97-029)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	20/10	60/30	200/100
Mice/Sex/Group	10 (5)[c]	10 (25)	10 (25)	10 (25)
Deaths (main study)[d]	2	2	2	6
Body Weight	-		↑ (M)	↑
Food Consumption	-	No treatment-related differences NTD		
Clinical Signs[e]	Matted hair/rough coat	↑ incidence of matted hair/rough coat and urine stained hair; distended abdomen (+)	↑ incidence of matted hair/rough coat and urine stained hair; distended abdomen (++)	↑ incidence of matted hair/rough coat and urine stained hair; distended abdomen (+++); ↓ activity, dehydration, emaciation, hunched posture, tremors and/or cold to touch
Mean Plasma Drug Abbott-157378				
C_{max} (µg/ml) + SD				
Day 62 Males	-	10.7 ± 2.6	16.5 ± 1.4	40.7 ± 7.6
Females	-	8.2 ± 1.9	17.2 ± 3.0	36.9 ± 8.0
AUC (µg•hr/ml)				
Day 62 Males	-	50.8 ± 7.1	108.3 ± 10.5	520.5 ± 4.8
Females	-	35.0 ± 6.7	133.6 ± 41.7	395.5 ± 72.7
Abbott-84538				
C_{max} (µg/ml) + SD	-	0.6 ± 0.4	3.0 ± 1.3	11.1 ± 10.1
Day 62 Males	-	1.2 ± 0.3	3.2 ± 1.0	14.2 ± 7.2
Females				
AUC (µg•hr/ml)				
Day 62 Males	-	2.8 ± 0.9	9.3 ± 4.0	52.7 ± 16.0
Females	-	3.4 ± 0.8	15.2 ± 4.8	71.5 ± 37.1

a. The soft-elastic capsule (SEC) formulation for Abbott-157378 and Abbott-84538 was used.
b. Control mice received the placebo formulation.
c. Animal numbers within parentheses constituted satellite subgroups for plasma drug level determinations.
d. Dosing injuries were most likely responsible for deaths in the control, low and mid dosage groups as well as some mice from the higher dosage groups. Three deaths from the high dosage groups were considered drug-related.
e. + = mild, ++ = moderate, +++ = severe (based on a total number of occurrences).

Tabulated Summary (Cont.)

Dosage (mg/kg/day) (A-157378/A-84538)[a]	0[b]	20/10	60/30	200/100
Mice/Sex/Group	10 (5)[c]	10 (25)	10 (25)	10 (25)
Hematology	-	NTD		↓Hct, Hb, RBC (F)
Clinical Chemistry	-	NTD	↑ Chol	↑ Chol, Trig, ALT, AST, ↑ GGT
Organ Weights	-	NTD	↑ liver	
Anatomic Pathology Gross Findings	- -	NTD	Gas-filled small intestines and cecum (one female)	Gas-filled small intestines and cecum/stomach, accentuated lobular pattern in the liver, hepatic discoloration
Microscopic Findings	-	NTD	-	Liver - cytoplasmic vacuolation, necrosis, subcute inflammation and hepatocytomegaly; Kidney - moderate to marked microvesicular cytoplasmic vacuolation (M).
Electron Microscopy	-	NTD	NTD	Liver - lipid accumulation and electron dense inclusions; Kidney - myeloid bodies in renal cortex (M)
CONCLUSION:	An oral dosage level of 200/100 mg/kg/day (Abbott-157378/Abbott-84538) for three month produced overt toxicity including deaths in mice. Target organs were identified as the liver and kidney. Minimal toxicity that included elevation of serum cholesterol values and increased liver weights were seen at 60/30 mg/kg/day. No significant signs of toxicity were observed in the dosage group of 20/10 mg/kg/day with corresponding mean AUC values of 43/3 µg•hr/ml.			

a. The soft-elastic capsule (SEC) formulation for Abbott-157378 and Abbott-84538 was used.

b. Control mice received the placebo formulation.

c. Animal numbers within parenthesis constituted satellite subgroups for plasma drug level determinations.

Tabulated Summary

Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with Impurities in Beagle Dogs (Abbott Study No. TB98-013)

Abbott-157378/Abbott-84538 Dosage (mg/kg/day)[a]	0/0	30/15 (normal impurities/degradants)	30/15 (high impurities/degradants)
Dogs/Sex/Group	4	4	4
Deaths	0	0	0
Body Weight Gain	-	↓ (M), slightly ↓ (F)[b]	slightly ↓ (M & F)[b]
Food Consumption	-	No treatment-related differences (NTD)	
Ophthalmoscopy	-		
Electrocardiograms	-		
Clinical Signs[c]	diarrhea/loose stool (+)	diarrhea/loose stool (++), emesis (+)	diarrhea/loose stool (+), emesis (+++), ↑ salivation (+,F)
Mean Plasma Drug AUC (µg•hr/ml) ± SD			
Abbott-157378 Males	-	75.4 ± 42.6	50.5 ± 17.0
Females	-	78.3 ± 20.4	77.1 ± 54.0
Abbott-84538 Males	-	27.2 ± 21.2	23.9 ± 14.4
Females	-	24.6 ± 12.2	18.3 ± 13.6
Urinalysis	-	NTD	
Hematology	-		
Clinical Chemistry	-		
Organ Weights	-	↑ liver[d]	
Anatomic Pathology	-	NTD	

CONCLUSION: The data generated in this three-month study indicated that increased levels of total impurities/degradants up to approximately 7% and 10% in Abbott-157378 and Abbott-84538 SEC formulations, respectively, did not change the toxicity profile of the Abbott-157378/Abbott-84538 combination at a dosage level of 30/15 mg/kg/day.

a. The SEC formulation containing approximately 20% (wt/wt) of each active drug was administered in capsules. The dogs in the control group (0/0 mg/kg/day) received placebo formulation in capsules.
b. The differences from controls were not statistically significant except for males receiving normal impurities/degradants.
c. + = mild, ++ = moderate, +++ = severe (based on a total number of occurrences)
d. Statistical significance was limited to the relative weights in females.

Tabulated Summary

Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with New Impurities in Beagle Dogs

(Abbott Study No. TB98-150)

Abbott-157378/Abbott-84538 Dosage (mg/kg/day)[a]	0/0[b]	30/15 (normal impurities)	30/15 (high impurities)[c]
Dogs/Sex/Group	4	4	4
Deaths	0	0	0
Body Weight Gain	-	↓ (M & F)	
Food Consumption	-	No treatment-related differences (NTD)	
Ophthalmoscopy	-		
Electrocardiograms	-		
Clinical Signs[d]	diarrhea/loose stool (++), emesis (+)	diarrhea/loose stool (++), emesis (+++), ↑ salivation (+++)	diarrhea/loose stool (++), emesis (++), ↑ salivation (+)
Mean Plasma Drug AUC (μg•hr/ml) ± SD			
Abbott-157378 Males	-	107.0 ± 87.0	83.6 ± 42.5
Females	-	60.9 ± 42.3	75.6 ± 36.9
Abbott-84538 Males	-	41.6 ± 27.7	22.1 ± 12.6
Females	-	20.4 ± 13.7	36.1 ± 23.3
Urinalysis	-	NTD	
Hematology	-		
Clinical Chemistry	-	↑ ALP (M,[e] F)	↑ ALP (F)[e]
Organ Weights	-	↑ relative liver[e]	
Anatomic Pathology	-	NTD	

CONCLUSION: The data generated in this three-month study indicated that increased levels of total impurities up to approximately 9%, including 2-3% each of new impurities (core-wing-A-urea-dimer, N-formyl-core-wing-B and N-acetyl-core-wing-A) in Abbott-157378 SEC formulation, did not change the toxicity profile of the Abbott-157378/Abbott-84538 combination at a dosage of 30/15 mg/kg/day.

a. Abbott-157378 SEC formulation containing 20% (wt/wt) of Abbott-157378.0 and Abbott-84538 SEC formulation containing 10% (wt/wt) of Abbott-84538.0 were administered in capsules. The dogs in the control group (0/0 mg/kg/day) received placebo formulation in capsules.
b. Placebo (74% oleic acid, 13% ethanol, 12% cremophor EL, 1.1% water, 0.01% BHT)
c. Major impurities (N-formyl-core-wing-A, N-formyl-core-wing-B, core-wing-A-urea-dimer and N-acetyl-core-wing-A).
d. + = mild, ++ = moderate, +++ = severe (based on a total number of occurrences)
e. The differences from controls were not statistically significant.

Tabulated Summary

Three-Month Oral Toxicity Study of Abbott-157378 and Abbott-84538 Combination with Related Substances in Beagle Dogs (Abbott Study No. TB99-127)

ABT-378/ABT-538 Dosage (mg/kg/day)[a]	0/0[b]	50/25 (normal related substances)	50/25 (high related substances)[c]
Dogs/Sex/Group	4	4	4
Deaths	0	0	0
Body Weight Gain	-	No treatment-related differences (NTD)	
Food Consumption	-		
Ophthalmoscopy	-		
Electrocardiograms	-		
Clinical Signs[d]	emesis (+), diarrhea/loose stool (+)	emesis (++), diarrhea/loose stool (+)	emesis (++), diarrhea/loose stool (+)
Day 89 Mean Plasma Drug AUC (µg•hr/ml) ± SD			
ABT-378: Males	-	47.1 ± 11.9	102.6 ± 68.6
Females	-	47.2 ± 21.3	150.6 ± 107.4
ABT-538 Males	-	5.7 ± 3.1	21.5 ± 26.9
Females	-	7.0 ± 3.3	27.0 ± 28.2
Urinalysis	-	NTD	
Hematology	-		
Clinical Chemistry	-	↑ ALP, ALT (F)	
Organ Weights	-	↓ prostate	
Anatomic Pathology	-	NTD	

CONCLUSION: The data generated in this three-month study indicated that increased levels of related substances (0.4% glycerin adduct, 2.0% propylene glycol adduct, 3.3% ethanol adduct, 1.3% core-wing-B, 1.1% B-wing-diacyl and 1.1% regioisomer) in ABT-378/ABT-538 liquid formulation did not change the toxicity profile of the ABT-378/ABT-538 combination at a dosage of 50/25 mg/kg/day.

a. ABT-378/ABT-538 combination liquid formulation containing approximately 80 mg ABT-378 and 40 mg ABT-538 per ml was administered in capsules.
b. Placebo formulation containing ethanol, corn syrup, propylene glycol, distilled water, glycerin, povidone and polyoxyl 40 hydrogenated castor oil.
c. Major related substances (core-wing-B, B-wing-diacyl, regioisomer, propylene glycol adduct, ethanol adduct and glycerol adduct).
d. + = mild , ++ = moderate (based on a total number of occurrences).

Tabulated Summary

Evaluation of the Effects of Orally Administered Abbott-157378 in Combination with Ritonavir (Abbott-84538) on the Reproductive Function of Male and Female Rats (Seg. I DART) (Abbott Study No. TA97-047)

A-157378/A-84538[a] Dosage (mg/kg/day)	0[b]	10/5	30/15	100/50
Rats/Sex/Group[c]	24	24	24	24
Deaths	0	1 M (intubation error) 1M (cause unknown)	1 F (intubation error)	0
Body Weight Gain	-	No treatment-related differences (NTD)		↓ (M)
Food Consumption	-		↓ (F)	↓
Clinical Signs	-	Rales (F), rough hair (M), matted hair (F)		
		Increased salivation (M)	Rales (M), increased salivation (F)	
				Increased salivation (M)
Mean Plasma Drug AUC (µg•hr/ml) ± SD Abbott-157378				
Males	-	12.4 ± 1.2	34.4 ± 1.2	94.4 ± 18.3
Females	-	13.3 ± 1.5	40.5 ± 8.0	134.2 ± 32.1
Abbott-84538				
Males	-	nc[d]	1.0 ± 0.2	7.3 ± 3.2
Females	-	nc	3.2 ± 2.7	8.7 ± 3.7
Estrous Cycle	-	NTD		
Reproductive Indices	-			
Uterine Findings	-			
Necropsy Findings	-			Liver: enlarged (F)
CONCLUSION:	The systemic no-toxic-effect level in the present study was considered to be 30/15 mg/kg/day (Abbott-157378/Abbott-84538). The no-toxic-effect level for male and female reproductive toxicity was 100/50 mg/kg/day (Abbott-157378/Abbott-84538), the highest dosage tested.			

a. Abbott-157378 SEC formulation containing approximately 20% (wt/wt) Abbott-157378.0
Abbott-84538 SEC formulation containing approximately 20% (wt/wt) Abbott-84538.0.
b. Vehicle comprised of 75% oleic acid, 12.5% ethanol, 12.5% Cremophor and 0.013% BHT (wt/wt).
c. An additional 4 rats/sex/group constituted a satellite subgroup for plasma drug level determinations.
d. nc = not calculated due to insufficient data.

Tabulated Summary

Evaluation of the Effects of Orally Administered Abbott-157378 in Combination with Ritonavir (Abbott-84538) on the Embryonic and Fetal Development of the Rat (Seg. II DART) (Abbott Study No. TA96-162)

Dosage (mg Abbott-157378/ ritonavir/ kg/day)[a]	0[b]	20/10[c]	50/25[c]	100/50[c]
Mated Rats/Group	24 (4)[d]	24 (4)	24 (4)	24 (4)
Gravid Rats/Group	20	22	22	19
Maternal Deaths	0	1[e]	0	0
Total Litter Resorptions	0	0	0	6
Maternal Clinical Signs	-	No treatment-related-difference		emaciation, hunched posture, decreased activity, discharge from eyes and nose, tinted hair
Maternal Body Weight	-	(NTD)		↓ GD[f] 9 through GD 18
Maternal Body Weight Gain	-			↑ GD18-20 interval
Maternal Food Consumption	-		↑ GD18-20 interval	↓ GD 6-9 & 9-12 intervals; ↑ GD18-20 interval
Maternal Mean Plasma Drug AUC (µg•hr/ml) ± SD **Abbott-157378** Treatment Day 12	-	28.12 ± 5.84	64.12 ± 13.33	116.44 ± 43.75
Ritonavir (Abbott-84538) Treatment Day 12	-	4.92 ± 0.63	8.83 ± 1.24	16.09 ± 5.41
Maternal Morphological Examination	-	NTD		
Fetal Viability	-			↓
Fetal Body Weights	-			
Fetal External Examination	-			
Variations Visceral Skeletal	- -			↓ ossification, increased 14th ribs and 27 presacral vertebrae
Malformations Visceral Skeletal	- -			
CONCLUSION	Administration of 100 mg/kg/day Abbott-157378 in combination with 50 mg/kg/day ritonavir resulted in maternal toxicity (reduced body weights). Drug-related decreases in fetal body weights were observed in litters from dams receiving 100/50 mg/kg/day. The no-effect-level for maternal toxicity and developmental toxicity was 50/25 mg/kg/day.			

a. Abbott-157378 was administered daily as the SEC formulation containing approximately 20% (wt/wt) Abbott-157378 in combination with ritonavir by oral gavage (2 ml total volume/kg).
b. Vehicle, 75% oleic acid, 12.5% ethanol, 12.5% Cremophor and 0.013% BHT.
c. Ritonavir (Abbott-84538) was administered daily as the SEC formulation containing approximately 20% (wt/wt) ritonavir in combination with Abbott-157378 by oral gavage (2 ml total volume/kg).
d. Four additional rats/group were designated as satellite rats for measurement of plasma drug levels.
e. This animal (#1008) was euthanized in a moribund condition on treatment Day 0 likely related to a

gavage accident.
f. GD=gestation day

Tabulated Summary

Evaluation of the Effects of Orally Administered Abbott-157378 and Abbott-84538 Combination on the Embryonic and Fetal Development of the Rabbit (Seg. II DART)

(Abbott Study No. TE96-152)

A-157378/A-84538 Dosage (mg/kg/day)	0[a]/0[b]	30/15	50/25	80/40
Pregnant rabbits/Group[c]	20	19	20	20
Maternal Mortality	1	0	2	1
Aborted	0	0	0	1[d]
Maternal Body Weight Gain	-	NTD (no treatment-related differences)		↓
Maternal Food Consumption	-			
Maternal Clinical Signs	-			Emaciation, loose stool, absent stool
Mean Plasma Drug Abbott-157378 AUC (µg•hr/ml) ± SD Gestation Day 19	-	11.2 ± 3.5	39.4 ± 10.8	89.6 ± 27.4
Abbott-84538 AUC (µg•hr/ml) ± SD Gestation Day 19	-	0.3 ± 0.1	3.9 ± 2.7	9.1 ± 3.6
Fetal Viability	-	NTD		
Fetal Body Weight	-			
Fetal Anomalies[e]	-			
Maternal Morphological Exam	-			
Conclusion	Oral administration of Abbott-157378 in combination with Abbott-84538 to pregnant rabbits during the period of major organogenesis resulted in significant reductions in food consumption and decrease maternal weight gain at 80/40 mg/kg/day. No developmental toxicity, including teratogenicity was observed in this study. The no-observed-adverse effect level for maternal and developmental toxicity was considered to be 50/25 mg/kg/day and 80/40 mg/kg/day, respectively, under the conditions of this study.			

a. Vehicle for Abbott-157378: propylene glycol:ethanol (95:5, v/v).
b. Vehicle for Abbott-84538: propylene glycol:ethanol (95:5, v/v) with two molar equivalents of p-toluene sulfonic acid monohydrate.
c. Twenty rabbits/ group were tested for developmental toxicity; four rabbits/group constituted a satellite subgroups for plasma drug level determinations.
d. Not considered test article related.
e. Skeletal examination in the control and high dose only.

Tabulated Summary

Study of the Effects of Abbott-157378 in Combination with Ritonavir on Pre- and Postnatal Development, Including Maternal Function in the Rat

(Wil Laboratories, Inc., Ashland, OH, Report No. WIL-57014)

(Abbott Study No. TA97-109)

Dosage (mg/kg/day)[a]	0	20/10	40/20	80/40
Vehicle	75% oleic acid, 12.5% ethanol, 12.5% Cremaphor, 0.013% BHT			
Number Females/Group	25	25	25	25
Number Delivering Litters	25	24	25	24
Clinical Signs/Death[b]	-	-		
Body Weight Gain		No treatment-related differences		
F_0 Dams	-	(NTD)		↓ transient
F_1 Generation	-			
Food Consumption				
F_0 Dams	-			↓ transient
F_1 Generation	-	Not Applicable		
Gestation Length	-			
Litter Size	-			
Pup Survival (F_1)	-			↓
Pup Growth (F_1)	-	NTD		
Developmental Landmarks (F_1)	-			
Reflex Ontogeny (F_1)	-			
Activity (F_1)	-			
Biel Maze	-			
Reproductive Indices (F_1)	-			
Pre/Postimplantation Losses (F_1)	-			
CONCLUSION:	A slight degree of transient maternal toxicity (diminished body weight gain and food consumption) and a reduction in pup survival during lactation was noted at the 80/40 mg/kg/day dosage. The no-observed-effect level for maternal and developmental toxicity was considered to be 40/20 mg/kg/day.			

a. Mg Abbott-157378/mg ritonavir
b. One dam in the 80/40 mg/kg/day group was euthanized in moribund condition on Lactation Day 8; not judged test article related.

Tabulated Summary

Bacterial Reverse Mutation Assay

(Ames Test Plus *E. Coli*) of Abbott-157378

(Abbott Study No. TX96-114)

Bacterial Strain	Micrograms of Abbott-157378 Tested per plate	S-9 Activation	Results
Salmonella typhimurium			
TA-1535	100 - 10,000 100 - 10,000	- +	negative negative
TA-1537	100 - 10,000 100 - 10,000	- +	negative negative
TA-98	100 - 10,000 100 - 10,000	- +	negative negative
TA-100	100 - 10,000 100 - 10,000	- +	negative negative
Escherichia coli			
WP2uvrA-	100 -10,000 100 - 10,000	- +	negative negative
Conclusion: Abbott-157378 was non-mutagenic in this assay. Toxicity was seen in only one strain at 10,000 µg/plate.			

Tabulated Summary

Bacterial Reverse Mutation Assay

(Ames Test Plus *E. Coli*) of Abbott-157378 with High Impurities

(Abbott Study No. TX98-072)

Bacterial Strain	Micrograms of Abbott-157378 with High Impurity levels Tested per plate	S-9 Activation	Results
Salmonella typhimurium			
TA-1535	100 - 5,000 100 - 5,000	- +	negative negative
TA-1537	100 - 5,000 100 - 5,000	- +	negative negative
TA-98	100 - 5,000 100 - 5,000	- +	negative negative
TA-100	100 - 5,000 100 - 5,000	- +	negative negative
Escherichia coli			
WP2uvrA-	100 - 5,000 100 - 5,000	- +	negative negative
Conclusion: Abbott-157378 was non-mutagenic in this assay. No toxicity was seen in any strains.			

Tabulated Summary

Bacterial Reverse Mutation Assay

(Ames Test Plus *E. Coli*) of Abbott-157378 with New Impurities

(Abbott Study No. TX98-185)

Bacterial Strain	Micrograms of Abbott-157378 with New Impurities Tested per plate	S-9 Activation	Results
Salmonella typhimurium			
TA-1535	100 - 5,000 100 - 5,000	- +	negative negative
TA-1537	100 - 5,000 100 - 5,000	- +	negative negative
TA-98	100 - 5,000 100 - 5,000	- +	negative negative
TA-100	100 - 5,000 100 - 5,000	- +	negative negative
Escherichia coli			
WP2uvrA-	100 - 5,000 100 - 5,000	- +	negative negative
Conclusion: Abbott-157378 was non-mutagenic in this assay. No toxicity was seen in any strains.			

Tabulated Summary
Bacterial Reverse Mutation Assay
(Ames Test Plus *E. Coli*) of a Liquid Combination Formulation of Abbott-157378 and Abbott-84538 with Related Substances
(Abbott Study No. TX99-137)

Bacterial Strain	Micrograms of Abbott-157378 with Related Substances Tested per plate	S-9 Activation	Results
Salmonella typhimurium			
TA-1535	30 - 5,000	-	negative
	30 - 5,000	+	negative
TA-1537	30 - 5,000	-	negative
	30 - 5,000	+	negative
TA-98	30 - 5,000	-	negative
	30 - 5,000	+	negative
TA-100	30 - 5,000	-	negative
	30 - 5,000	+	negative
Escherichia coli			
WP2uvrA-	30 - 5,000	-	negative
	30 - 5,000	+	negative
Conclusion: A liquid combination formulation of Abbott-157378 and Abbott-84538 with related substances was non-mutagenic in this assay. No toxicity was seen in any strains.			

Tabulated Summary

In Vitro Cytogenetics

Human Lymphocyte Culture Assay of Abbott-157378

(Abbott Study No. TX96-185)

Micrograms of A-157378 per ml of Culture Medium	S-9 Activation	Mutagenicity
1 - 10	-	Negative
3 - 50	+	Negative
Conclusion: Abbott-157378 was non-clastogenic in this assay. Toxicity was seen at 3 µg/ml (non-activated) and 20 µg/ml (activated) or greater concentrations		

Tabulated Summary

In Vitro Cytogenetics

Human Lymphocyte Culture Assay of Abbott-157378 with High Impurities

(Abbott Study No. TX98-073)

Micrograms of A-157378 per ml of Culture Medium	S-9 Activation	Mutagenicity
3 - 30	–	Negative
20 - 50	+	Negative
Conclusion: Abbott-157378 was non-clastogenic in this assay. Toxicity was seen at 10 and 20 µg/ml (non-activated) and 30 µg/ml (activated) or greater concentrations		

Tabulated Summary

In Vitro Cytogenetics

Human Lymphocyte Culture Assay of Abbott-157378 with New Impurities

(Abbott Study No. TX98-186)

Micrograms of A-157378 per ml of Culture Medium	S-9 Activation	Mutagenicity
10 - 30	–	Negative
10 - 50	+	Negative
Conclusion: Abbott-157378 was non-clastogenic in this assay. Toxicity was seen at 20 µg/ml (non-activated) and 30 µg/ml (activated) concentrations		

Tabulated Summary
In Vitro Cytogenetics
Human Lymphocyte Culture Assay of a Liquid Combination Formulation of Abbott-157378 and Abbott-84538 with Related Substances
(Abbott Study No. TX99-138)

Micrograms of A-157378 per ml of Culture Medium	S-9 Activation	Mutagenicity
10 - 50 (4-hour exposure)	–	Negative
2 - 5 (24-hour exposure)	–	Negative
5 - 30 (4-hour exposure)	+	Negative
Conclusion: A liquid combination formulation of Abbott-157378 and Abbott-84538 with related substances was non-clastogenic in this assay. Toxicity was seen at 30 µg/ml (4-hour non-activated), 5 µg/ml (24-hour non-activated) and 10 µg/ml (4-hour activated) concentrations.		

Tabulated Summary

Mouse Micronucleus Assay of Abbott-157378 Alone and in Combination with Abbott-84538 (Abbott Study No. TD96-211)

	Dosage (mg/kg/day)			
Abbott-157378 Alone	0	625	1250	2500
Abbott-157378/ Abbott-84538	0	39/78	78/156	156/313
Conclusion:	There was no dose-related statistically significant increase in the number of micronucleated bone marrow polychromatic (PCEs) erythrocytes compared to vehicle-treated control mice. Therefore, Abbott-157378 alone or in combination with Abbott-84538 was non-mutagenic in this assay.			

Tabulated Summary
L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay
of Abbott-157378
(Microbiological Associates, Inc., Rockville, MD,
Study No. G96BC78.702)
(Abbott Study No. TX96-230)

Concentrations Tested	Activation	Result
25-100 µg /ml (Initial Assay)	Yes	Negative
1-20 µg /ml (Initial Assay)	No	Negative
40 to 120 µg /ml (Supplemental Assay)	Yes	Negative
CONCLUSIONS:	Not Mutagenic	

8. NDA Summary of Absorption, Distribution, Metabolism and Excretion of ABT-378 (Abbott-157378) in Animals (1-20)

ABBOTT
Interoffice Correspondence

FROM: Experimental Sciences
DEPT: D-4EK Bldg: AP9 Ext: 88630

TO: Research Information Center D421, J28 **DATE:**

CC:

Dr. D. Hickman	D46V, AP9
Dr. K. Marsh	D4EK, AP9A
Dr. S. Roberts	D46V, AP9
Dr. R. Bertz	D4PK, AP13A
Dr. A. Hsu	D4PK, AP13A
Dr. R. Granneman	D4PK, AP13A
Dr. R. Patterson	D46G, AP13A
Mr. N. Ballinger	D4EK, AP9A
Regulatory Affairs	D491, AP6B

RE: **Abbott-157378 Drug Metabolism Report No. 72 - Division 46 (R&D/00/231)**

NDA Summary of Absorption, Distribution, Metabolism and Excretion of ABT-378 (Abbott-157378) in Animals

If you do not intend to keep the attached report, please return it to the Research Information Center (D421, J28). Additional copies must be requested through the Research Information Center.

Kennan C. Marsh, Ph.D.,
Director, Experimental Sciences

KCM/cs

Attachment: Drug Metabolism Report No. 72

Abbott-157378

Drug Metabolism Report No. 72

NDA Summary of Absorption, Distribution, Metabolism and Excretion of ABT-378 (Abbott-157378) in Animals

R&D/00/231

Author:

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Abbott Laboratories

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2.5.3 Absorption, Distribution, Metabolism and Excretion

The absorption, distribution, metabolism and excretion of [^{14}C]ABT-378 administered alone have been investigated in rats, whereas these parameters were examined for [^{14}C]ABT-378 coadministered with ritonavir in a 2:1 ratio in rats and dogs, and in a 4:1 ratio in humans. Pharmacokinetic and/or toxicokinetic parameters for ABT-378 coadministered with ritonavir have been estimated in mice, rats, dogs, monkeys and humans. Single dose animal studies involved administration of single intravenous doses of ABT-378 alone (rat and dog) and single and/or rising oral doses of ABT-378 administered with or without ritonavir (rat, dog and monkey).[1] Multiple dose pharmacokinetic studies involved oral administration of ABT-378 and ritonavir in a fixed ratio of 2:1 (rat and dog).[1] Toxicokinetic studies involved multiple oral administrations of ABT-378:ritonavir in a 2:1 (mouse, rat and dog) and/or 3:1 ratio (dog) with ritonavir for up to 9 months.[2-12] Absorption, metabolism and excretion studies in animals also included a single 10 mg/kg (rat – with and without 5 mg/kg ritonavir)[13,14] or 5 mg/kg (dog – with 2.5 mg ritonavir)[15] intravenous dose to provide a basis for evaluating oral dose data. A tissue distribution study was conducted in male rats after an single 10 mg/kg (with 5 mg/kg ritonavir) oral dose.[16] A maternal fetal tissue distribution study was conducted in pregnant rats and a lacteal excretion study was conducted in lactating rats, each after an single 10 mg/kg (with 5 mg/kg ritonavir) oral dose.[17] Additionally, *in vitro* studies have been conducted with mouse, rat, dog, monkey and human liver microsomes.[18] *In vitro* models of rat and human liver metabolism have also been utilized to help understand the beneficial pharmacokinetic interaction that ritonavir exerts on the disposition of ABT-378 in humans.[19-23] The *in vitro* plasma protein binding was determined for mouse, rat, dog, monkey and human at concentrations of drug chosen to encompass the broad range of ABT-378 plasma concentrations anticipated in clinical and toxicology studies.[24-30]

All doses refer to milligrams of ABT-378 and ritonavir free base equivalents per kilogram of body weight (mg/kg). Concentrations are reported as micrograms per unit of volume (µg/mL) except *in vitro* metabolism data that are reported in micromolar (µM) and converted back to µg/mL when appropriate for comparison with *in vivo* concentration data. The molecular weight of ABT-378 base is 629 g/mole; thus, 1 µM is equivalent to 0.629 µg/mL.

Concentrations of ABT-378 and ritonavir in plasma were determined with high-performance liquid chromatography (HPLC) using ultraviolet detection.[31] ABT-378 bearing a carbon-14 radiolabel in the dimethylphenoxyacetyl moiety of the molecule was used in all of the metabolism, distribution and protein binding studies.[32] Metabolic patterns in plasma, bile, feces, urine and *in vitro* microsomal incubations were determined by HPLC with radioactivity flow detection.

2.5.3.1 Absorption and Pharmacokinetics

Administered without ritonavir, ABT-378 was poorly bioavailable in rats, dogs, monkeys and humans. Single oral doses of 5-10 mg/kg did not result in detectable plasma concentrations in dogs and monkeys.[1] Single oral doses of approximately 5-10 mg/kg ABT-378 to rats and humans resulted in peak plasma concentrations (C_{max}) less than 1 μg/mL and AUC values of 1.92 and 0.67 μg·h/mL in rats (10 mg/kg; 0-8 h) and humans (5.7 mg/kg; 0-12 h), respectively.[1,33]

Coadministration with ritonavir resulted in a substantial increase in both C_{max} and AUC in all species investigated (rats, dogs, monkeys and humans- see summary table below).[1,33] For example when ABT-378:ritonavir was dosed (single oral dose) in a 2:1 ratio to rats (10 mg/kg ABT-378) and humans (5.7 mg/kg ABT-378), the ritonavir resulted in a 2-fold increase in C_{max} and a 6-fold increase in AUC for the rat and a 44-fold increase in C_{max} and a 182-fold increase in AUC in humans.[1,33] Mean peak plasma concentrations (C_{max}) of ABT-378 in rat, dog, monkey and human were recorded at 1.5-4.6 hours after a single oral administration of 10 mg/kg ABT-378 (with 5 mg/kg ritonavir in rats), ~5 mg/kg ABT-378 (with ~2.5 mg/kg ritonavir in dog and human) or 10 mg/kg ABT-378 (with 10 mg/kg ritonavir in monkey). C_{max} values for ABT-378 averaged 2.11 μg/mL in male rats (10 mg/kg ABT-378:5 mg/kg ritonavir), 2.61 μg/mL in dogs (5 mg/kg ABT-378:2.5 mg/kg ritonavir), 3.06 μg/mL in monkeys (10 mg/kg ABT-378:10 mg/kg ritonavir) and 8.3 μg/mL in humans (~5.7 mg/kg ABT-378: ~2.9 mg/kg ritonavir). The C_{max} and AUC values for ritonavir were at least 2-fold less than the C_{max} and AUC values for ABT-378 when these two drugs were given in a 2:1 ratio (ABT-378:ritonavir). Furthermore, in humans at the anticipated clinical dose and dose ratio of 400 mg ABT-378:100 mg ritonavir (~5.7 mg/kg ABT-378: ~1.4 mg/kg ritonavir), the ritonavir C_{max} and AUC were 14- and 22-fold less than those observed for ABT-378 after a single dose, indicating that low circulating concentrations of ritonavir are sufficient to result in

sustained plasma concentrations of ABT-378. Bioavailability values were not estimated due to the absence of intravenous pharmacokinetic data for the ABT-378:ritonavir combination.

Summary of Single Dose Pharmacokinetics of ABT-378 for Rat, Dog, Monkey and Human When Coadministered With or Without Ritonavir

	Dose (mg/kg)[a,b]		ABT-378			RTV (Ritonavir)		
Species	ABT-378	RTV	C_{max} (μg/mL)	AUC_{0-t} (μg·h/mL)	CL/F [c] (L/h/kg)	C_{max} (μg/mL)	AUC_{0-t} (μg·h/mL)	CL/F [c] (L/h/kg)
Rat, Male	10	0	0.96	1.92	5.21	-.-	-.-	-.-
	10	1	1.19	4.37	2.29	<0.00	<0.00	-.-
	10	5	2.11	12.29	0.81	0.15	0.38	13.15
	10	10	3.68	23.41	0.43	1.13	4.83	2.07
Dog	5	0	<0.00	<0.00	-.-	-.-	-.-	-.-
	5	1	1.17	2.86	1.75	0.12	0.19	5.26
	5	2.5	2.61	10.07	0.50	1.16	1.67	1.50
	5	5	2.50	11.26	0.44	1.32	2.19	2.28
Monkey	10	0	<0.00	<0.00	-.-	-.-	-.-	-.-
	10	10	3.06	14.72	0.68	1.48	5.01	2.00
Human	5.7	0	0.19	0.67	8.51	-.-	-.-	-.-
	5.7	0.7	5.7	50.5	0.11	0.2	1.2	0.58
	5.7	1.4	8.5	105.3	0.05	0.6	4.7	0.30
	5.7	2.9	8.3	121.9	0.05	2.4	14.8	0.39

This table was abstracted from Table 2 of Section 5.3 (Overview of Absorption, Distribution, Metabolism and Excretion of ABT-378 (Abbott-157378) in Animals.[34]

t. Last sampling time point in each pharmacokinetic study: rat - 8 hours, dog, monkey and human - 12 hours

a. Vehicle for rat, dog and monkey was ethanol: propylene glycol: dextrose 5% in water (20:30:50, by volume) containing two molar equivalents of methane sulfonic acid[1]

b. Human doses correspond to 200, 400 and 800 mg ABT-378 dose and 0, 50, 100 and 200 mg ritonavir normalized to an assumed 70 kg body weight for the purposes of comparison. Administered in a soft elastic capsule formulation under non-fasting conditions.[33]

c. Apparent oral clearance CL/F = Dose/AUC.

Comparison of mean apparent oral clearance (Dose/AUC or CL/F) values for rat (0.81-0.95 L/h/kg), dog (0.19-0.50 L/h/kg), monkey (0.68 L/h/kg) and human (~0.05 L/h/kg) showed that humans exhibit lower apparent oral clearances of ABT-378 compared with the animal models at single doses of 5-10 mg/kg ABT-378 in a 2:1(rat, dog and human) a 1:1 (monkey) and a 4:1 (human) ratio with ritonavir.[1,33]

In multiple dose studies, the exposure (as measured by plasma AUC) of male and female rats and dogs to ABT-378 was significantly less than that in humans at comparable doses (see summary table below). In male and female rats, a 150 mg/kg/day dose of ABT-378 (with 75 mg/kg/day ritonavir) is required to achieve a mean plasma AUC_{0-24} (127.2 or 195.4 μg·h/mL for males or females) comparable to that obtained in humans given the anticipated clinical regimen of ~11.4 mg/kg/day (400 mg BID) dose of ABT-378 with ~2.8 mg/kg (100 mg BID) ritonavir to steady state (188 μg·h/mL). Doses of 70-100 mg/kg/day ABT-378 (35-50 mg/kg/day ritonavir) in male and female dogs are required to achieve AUC_{0-24} values (163.9-239.4 μg·h/mL) comparable to the human clinical dose AUC.

Summary of Estimated Oral Clearance of ABT-378 for Mouse, Rat, Dog and Human After Multiple Dosing When Coadministered With Ritonavir

Species	ABT-378 Dose (mg/kg/day)[a]	AUC_{0-24} (μg·h/mL)	Dose/AUC (CL/F)[b] (L/h/kg)
Mouse, Male (Female)[c,d]	20	50.8 (35.0)	0.39 (0.57)
	60	108.3 (133.6)	0.55 (0.45)
	200	520.5 (395.5)	0.38 (0.51)
Rat, Male (Female) [c,d]	10	7.35 (19.6)	1.36 (0.51)
	50	46.6 (82.5)	1.07 (0.61)
	150	127.2 (195.4)	1.18 (0.77)
Dog[d,e]	10	59.2	0.17
	30	95.8	0.31
	100/70[f]	163.9/239.4	0.61/0.29
Human	11.4[g]	188[h]	0.06

a. ABT-378 was dosed in a 2:1 ratio with ritonavir in mouse, rat and dog but 4:1 in human.
b. Apparent oral clearance CL/F = Dose/AUC.
c. Values in parentheses are for female mice and rats.
d. AUC values at each dose from the 3 month toxicology study. If more than one sampling interval then the average was taken (Abstracted from Tables 5, 6 and 12 of Section 5.3 (Overview of Absorption, Distribution, Metabolism and Excretion of ABT-378 (Abbott-157378) in Animals).[34]
e. Average of male and female data.
f. Dose reduced from 100:50 mg/kg/day ABT:378:ritonavir to 70:35 mg/kg/day ABT-378:ritonavir on Day 30.
g. Equivalent to a single 400 mg dose of ABT-378 given twice daily (800 mg) normalized to body weight (mg/kg) assuming a 70 kg body weight.
h. The estimate of steady-state ABT-378 pharmacokinetics for the 400:100 mg/kg ABT:378:ritonavir q12h anticipated clinical regimen.

The lower calculated oral clearance in humans reflects the need to administer larger mg/kg doses of ABT-378:ritonavir to rats and dogs to achieve plasma AUC values comparable to

those in humans. Since the liver is the major organ of elimination of ABT-378 and ritonavir, this may suggest the potential for greater exposure of the liver to ABT-378:ritonavir in rats and dogs compared to humans, assuming comparable absorption of the drug.

A consistent sex difference in plasma drug levels was apparent in rats, but not in mice or dogs. In rats, plasma levels were generally higher in females than in males, very likely due to the gender specific expression of CYP isozymes, presumably CYP3A2, responsible for ABT-378 and ritonavir metabolism.[35]

The C_{max} and AUC of both ritonavir and ABT-378 in mice, rats, dogs and humans generally increased with increasing dose of the ABT-378:ritonavir combination.[34] These parameters increased in an approximately proportional manner in mouse and a less than dose-proportional manner in rats and dogs.[34] A less than proportional increase, where it occurred, was generally attributed to solubility-limited aqueous dissolution of these lipophilic compounds in the GI tract, resulting in a lower fraction of the dose being absorbed at higher doses.

There was no apparent effect on T_{max} in rats or dogs with increasing single doses of ABT-378:ritonavir in a fixed ratio despite an apparent prolongation of the T_{max} for ABT-378 in rats given ritonavir compared to rats given ABT-378 alone.[1] As the ritonavir dose rises at a fixed dose of ABT-378 in rats, the T_{max} of ritonavir increases with dose as does the T_{max} of ABT-378, indicating a dependence of ABT-378 pharmacokinetics upon ritonavir.[1] No dose effect on T_{max} was observed in humans.[33]

The effect of multiple dosing (the day effect) on C_{max} and AUC of ABT-378 was not assessed in mice and was not apparent in rats (Day 1 *vs.* Day 9) or dogs (Day 1 *vs.* Day 13).[33] However a moderate, but statistically significant, effect was observed in humans (≤30% decrease in C_{max} and AUC from Day 5 to Day 16).[36] In the 3 month toxicology study in dogs at the high dose, AUC values actually increased after 1 to 3 months of dosing, relative to the Day 7 values.[8]

After a 5 mg/kg intravenous dose of ABT-378 alone, the ABT-378 plasma clearance in rat and dog was rapid and essentially the same (≈1.3 L/h/kg).[1] Mean apparent elimination half-lives after intravenous dosing were also similar in rat and dog (0.6-1.1 h). The plasma half-lives of ABT-378, when coadministered with ritonavir were difficult to evaluate due to the dependence of ABT-378 clearance on ritonavir plasma concentrations.

After oral administration of a 10 mg/kg dose of [^{14}C]ABT-378 to rats, the C_{max} for total plasma radioactivity of 0.18-0.26 μg equivalents/mL (μg eq/mL) was not attained until 4-6 hours after dosing.[13] An intravenous dose of [^{14}C]ABT-378 indicated that plasma levels declined with an apparent elimination half-life of 1.06-1.35 hours, consistent with the rapid elimination of ABT-378 observed in pharmacokinetic experiments. The mean total radioactivity AUC value for female rats (1.46 μg eq/mL) was similar to that of male rats (1.25 μg eq/mL).[13] Coadministration of [^{14}C]ABT-378 with 5 mg/kg ritonavir by the oral route produced a substantial increase in both the C_{max} (~7-fold) and AUC (~13-fold) of total radioactivity compared to dosing ABT-378 alone. A smaller effect of ritonavir on the C_{max} of ABT-378 (~2-fold) and AUC (~5-fold) was apparent after intravenous dosing, suggesting that ritonavir had both pre-systemic and systemic (i.e. post-absorptive) effects on ABT-378 exposure.[13] In dogs, an average plasma C_{max} for total radioactivity of 3.34 μg eq/mL was observed at 2 hours after a single oral dose of 5 mg/kg [^{14}C]ABT-378 coadministered with 2.5 mg/kg ritonavir.[15] The average plasma C_{max} after intravenous dosing was 10.69 μg eq/mL. Comparison of oral and intravenous AUC values for total radioactivity in rats (18.6 *vs.* 32.5 μg eq·h/mL) and dogs (19.2 *vs.* 40.3 μg eq·h/mL) afforded estimates of the percentage of radiolabeled dose absorbed of 57% for rats and 48% for dogs.[13,15]

Unchanged parent drug was the major circulating radioactive component in both rat and dog plasma collected 0-4 hours after intravenous and oral (dog only) dosing, accounting for greater than 80% and 95% of plasma AUC_{0-4} in rats and dogs, respectively.[13,15] Plasma radioactivity from rats after oral dosing was not analyzed by radio-HPLC due to insufficient plasma radioactivity. The 4-oxo derivative of ABT-378 (M-1) was present in rat plasma, the epimeric pair of 4-hydroxylated ABT-378 metabolites (M-3/4) were detected in rat and dog plasma and the dimethylphenoxy-hydroxylation product of ABT-378 (M-5) was detected in dog plasma.[13,15] These data were similar to those obtained from the human [^{14}C]ABT-378 study where unchanged ABT-378 was also the major component of human plasma.[37]

After a single 5.7 mg/kg dose of [^{14}C]ABT-378 was coadministered with 1.4 mg/kg ritonavir to five male human volunteers, peak plasma concentrations of total radioactivity ranging from 8.6-10.6 μg/mL were achieved between 6 and 12 hours after dosing.[37] The mean AUC for total radioactivity (136.6 μg eq·h/mL) and the mean AUC for ABT-378 determined by a UV-HPLC assay (121.2 μg·h/mL) were similar to that obtained from

single dose pharmacokinetic data for ABT-378 (105.2 μg·h/mL). Of some 28 human plasma samples that were profiled for metabolites, 3 to 6 samples contained minor amounts of the rat plasma metabolite M-1 and the dog and rat plasma metabolites M-3/4.[37] In addition, 3-4 out of the 28 human plasma samples contained minor amounts of the diphenyl core moiety hydroxylation products of 4-oxo-ABT-378 (M-9/10) and/or the diphenyl core moiety hydroxylation products of 4-hydroxy-ABT-378 (M-11/12/13/14/15). There were insufficient data for a meaningful determination of AUC for these metabolites. Other unidentified polar metabolites, presumed to be tertiary or quaternary oxidative metabolites of ABT-378 were also detected in several plasma samples accounting for much of the non-ABT-378 radioactivity. It should be noted that although unidentified polar metabolites were not a substantial portion of animal plasma radioactivity, perhaps due to detection limitations, the presence of other polar metabolites in rat and dog urine indicate that these metabolites may be present in their plasma during transit from the liver. The *in vitro* antiviral activity for these metabolites is unknown except for M-1 and M-3/4, which have potency comparable to that of the parent drug.

2.5.3.2 Protein Binding

The *in vitro* protein binding of [^{14}C]ABT-378 was extensive in mouse, rat, dog, monkey and human plasma.[24] At ABT-378 concentrations from 0.1 to 100 μg/mL, mean plasma protein binding percentages ranged from 98.90-97.56% in mouse, 99.76-95.89% in rat, 99.45-96.29% in dog, 98.30-95.36% in monkey and 99.72-97.37% in human plasma. The extent of protein binding generally decreased with increasing drug concentration in all five species. In human plasma, binding did not change appreciably between 0.1 and 10 μg/mL of [^{14}C]ABT-378, averaging ≥ 99.4%, but did decrease at 30 and 100 μg/mL. Between 10 and 100 μg/mL, the free fraction of [^{14}C]ABT-378 increased approximately 5-fold in human plasma. There was no sex difference in plasma protein binding of ABT-378.[24] The effect of ritonavir (1 and 15 μg/mL) on the binding of [^{14}C]ABT-378 (concentration range 0.1-10 μg/mL) suggested that ritonavir may have the potential to decrease the plasma protein binding of ABT-378 in human plasma.[25] However, for this to occur, ritonavir concentrations needed to be in excess of ABT-378, a situation that does not occur with regimens examined toxicologically or in the clinic.

At concentrations below ~2-4 μg/mL, [^{14}C]ABT-378 was more extensively bound to physiological concentrations of human α1-acid glycoprotein (AAG; >99%) than to human

serum albumin (HSA; ~96%).[27] Binding to AAG and HSA appears to diminish at drug concentrations > 4 μg/mL, raising the possibility that ABT-378 may bind to other plasma components. Due to the apparent affinity of ABT-378 for AAG and the ability of the drug to saturate AAG binding at therapeutic plasma concentrations, a clinically relevant effect involving displacement of drugs with an affinity for AAG from plasma protein by ABT-378 is theoretically possible. However, displacement interactions do not affect free drug clearance and concentrations, therefore interpretations based on total plasma drug levels in this case would be misleading. It is important to note that the combination of ABT-378 and ritonavir, at clinically relevant concentrations, did not displace a selection of drugs known to be bound to AAG and/or HSA (e.g. warfarin, digoxin and imipramine) to a degree that was considered to be clinically significant (< 2-fold).[38] Furthermore ibuprofen, saquinavir, nelfinavir or amprenavir, drugs that are highly bound to AAG and/or HSA, did not displace ABT-378 from its protein binding sites in human plasma.[39]

2.5.3.3 Tissue Distribution

[^{14}C]ABT-378 was widely distributed in the tissues of male rats after oral co-administration of a 10 mg/kg dose with 5 mg/kg ritonavir.[16] At 4 hours after dosing (peak plasma concentration), the highest tissue radioactivity concentrations were found in the liver (52.3 μg eq/g), adrenals and thyroid, affording respective tissue to plasma (T/P) ratios of 22.5, 2.07 and 1.90, respectively. The brain contained radioactivity levels (0.046 μg eq/g) that were approximately equal to unbound plasma concentration, or more than 30-fold less than the corresponding total plasma concentration at 4 hours. The observation of 0.127 and 0.816 μg eq/g of dose radioactivity in the lumbar and submaxillary lymph nodes at 1 hour (T/P ratio of 0.06 and 0.37 at 1 hour and 0.37-0.63 at 7 hours) indicates that ABT-378 distributes into lymphatic tissue despite high plasma protein binding. By 48 hours, the liver was the only organ containing appreciable radioactivity (0.26 μg eq/mL), while all other organs contained less than 0.1 μg eq/mL. The tissue distribution of radioactivity into maternal tissues after oral co-administration of a 10 mg/kg dose with 5 mg/kg ritonavir was consistent with the distribution observed in male rats.[17] Fetal tissues and amniotic fluid contained low concentrations of radioactivity (≤ 0.14 μg eq/g tissue) which, except for the liver (0.007 μg eq/g tissue), were undetectable after 72 hours.

2.5.3.4 Enzyme Induction

An *ex vivo* examination of liver microsomal enzyme induction after multiple dosing with ABT-378 and ritonavir in combination has not been conducted in any species. A previous study with ritonavir dosed alone to rats indicated an increase in microsomal protein with no significant increase in functional cytochrome P450 (CYP) activity with multiple dosing.[40] However this may have been due to the presence of ritonavir carried over in the microsome preparations inhibiting the CYP activities since a time dependent increase in oral clearance of ritonavir of approximately 2-fold was also noted in both rats and humans when ritonavir was dosed alone.[40] A similar, but more moderate, increase in oral clearance of ABT-378 has been noted after multiple dosing of ABT-378:ritonavir in the clinic.[36] Induction of the CYP3A subfamily of microsomal monooxygenases *in vitro* was observed as an increase in immunoreactive CYP3A protein in human hepatocyte cultures incubated with ritonavir but not in cultures incubated with ABT-378.[23] Since the CYP3A subfamily are responsible for the metabolic clearance of both ritonavir and ABT-378, these data taken together suggest that the increase in clearance observed during multiple dosing of ABT-378:ritonavir may be largely due to the ritonavir component of the combination.

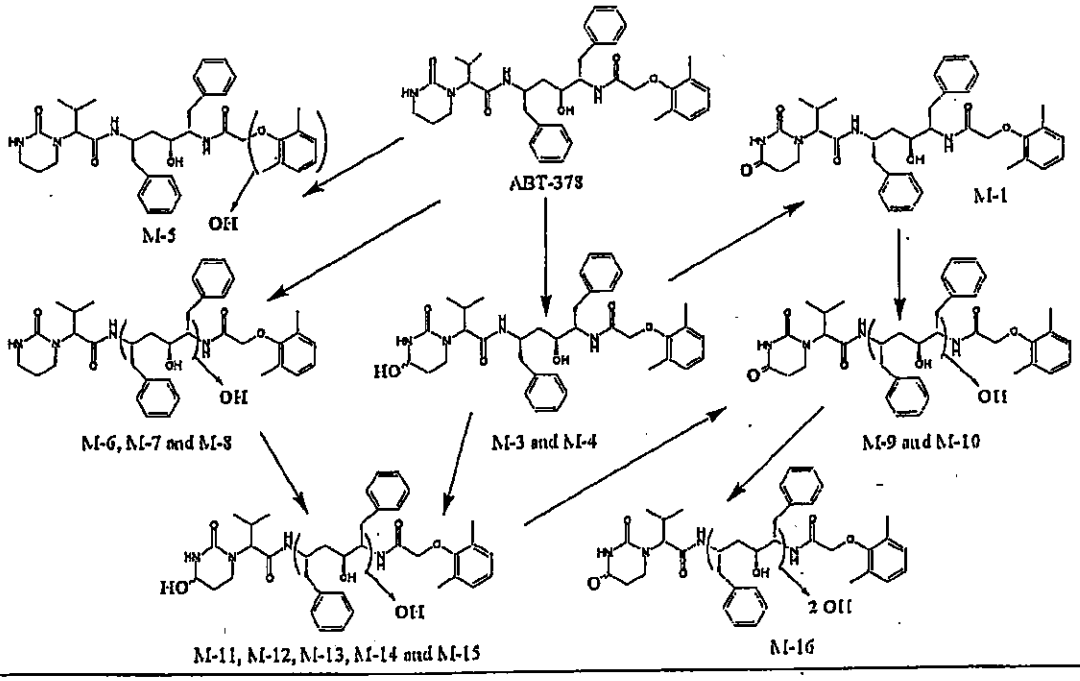
2.5.3.5 Metabolic Pathways

ABT-378 is metabolized exclusively by oxidative pathways in rat, dog and human resulting in similar metabolic profiles (see pathway below).[13-15,18,37] M-1 and M-3 through M-12 have been identified in rat, dog and human excreta whilst M-13, M-14 and M-15 have been identified in dogs and humans. M-16 would appear to be a dog specific metabolite. There are five identified primary metabolites: an epimeric pair of 4-hydroxylated derivatives (M-3 and M-4), and three mono-hydroxylated ABT-378 derivatives with the hydroxyl group located on the diphenyl core moiety (M-6, M-7 and M-8).† Several secondary metabolites are then produced by further biotransformation of the primary metabolites. M-1 is the 4-oxo derivative of ABT-378 which is the result of oxidation of M-3 and/or M-4 whilst M-11 through M-15 are dihydroxylated metabolites of ABT-378 (4-hydroxy and another hydroxyl group located on the diphenyl core moiety),

† The locations of the oxidations were originally labeled incorrectly according to an arbitrary numbering scheme.[13-15,18] The correct numbering for the metabolites M-3/4 are 4-hydroxylated metabolites and the sequential metabolites M-1, M-9/10, M-11/12/13/14/15 are also 4-substituted compared to ABT-378 and not 3-substituted as indicated by the old nomenclature. The metabolite M-2 is 6-hydroxy-ABT-378 and not 5-hydroxy-ABT-378 as indicated by the old nomenclature.[18]

presumably the products of hydroxylation of M-2, M-3 and/or M-4 in the diphenyl core. Tertiary metabolites that were identified included M-9 and M-10, which are the hydroxy-4-oxo-ABT-378 with the hydroxyl group located on the diphenyl core moiety. These metabolites are the products of hydroxylation of M-1 in the central diphenyl core of ABT-378 and/or the oxidative product of M-11/12/13/14 and/or M-15, which would yield the same metabolites *via* a different route. M-16 is the diphenyl core moiety dihydroxylation product of 4-oxo-ABT-378 and thus represents an identified quaternary metabolite. Given the extensive metabolism of ABT-378 *in vivo*, several other unidentified polar metabolites present in rats, dogs and humans have been attributed to tertiary or quaternary metabolic products of ABT-378. For simplicity, the identified metabolites are grouped by their structural characteristics (see figure below).

Proposed Metabolic Pathway for [^{14}C]ABT-378 in Rat, Dog and Human *In Vivo*



M-13/14/15 and M-16 have not been formally identified in rat excreta.[13,14] M-2 has been identified from rat, dog and human liver microsomes but has not been formally identified in rat, dog or human excreta.[13-15,18,37] M-5 was not identified in human excreta but is a minor metabolite identified *in vitro*.[18,37] M-6/7/8 have not been formally identified in rat excreta but are minor *in vitro* metabolites and by retrospective examination possible minor metabolites in rat bile and feces that were combined with polar unidentified metabolites.[13,14,18] M-16 is apparently a metabolite specific to the dog.[15] Other metabolites not identified are presumed to be the result of further oxidative metabolism of the structures indicated above. Modifications of ABT-378 are indicated by a larger font size.

Radioactivity in rat, dog and human feces consisted largely of unchanged parent drug (19.8-52.85% of total dose-^{14}C) after oral administration of [^{14}C]ABT-378 with ritonavir (see table below).[13,15,37] Unchanged [^{14}C]ABT-378 was also a major component of rat feces (28.07% of dose) but not bile (<2% of dose), after intravenous dosing with ritonavir, implying elimination of unchanged ABT-378 *via* some other mechanism such as exsorption/transport into the intestinal lumen in the rat.[13,14] Unchanged [^{14}C]ABT-378 was not a major component of dog feces (<5% of dose) after intravenous dosing.[15] Therefore, in the absence of intravenous data in humans, no firm conclusion regarding the origin on the unchanged ABT-378 in the feces in humans (mean 19.8% of the dose, range 12.0-23.2%) can be made. However, it is likely that a substantial proportion of the ABT-378 in human feces is the result of unabsorbed drug.

Summary of the Metabolism of ABT-378 for Rat, Dog and Human after a Single Oral Dose Coadministered With Ritonavir

Parameter	Rats[a] Oral	Dogs[b] Oral	Human[c] Oral
Route of Excretion as % of Total Dose			
Feces	107.15	94.32	82.6
Urine (Days 0-3)	1.10	0.79	10.4
Cage Wash (Days 0-3)	0.23	0.68	-.-
Total Recovered	108.48	95.81	93.0
Metabolic Profile[d] as % of Total Dose Radioactivity			
Feces (*Urine*)[e]			
ABT-378	52.85	32.5 (<0.1)	19.8 (2.2)
M-1	4.19	1.4 (<0.1)	3.2 (0.2)
M-2	0.0	0.0	0.0
M-3/4	18.40	6.9 (<0.1)	10.1 (0.2)
M-5	0.70	1.5	0.0
M-6/7/8	0.0	4.1	-3.9 (0.1)
M-9/10	7.78	9.7 (0.1)	7.4 (0.1)
M-11/12/13/14/15	7.47	13.2	8.9 (0.1)
M-16	0.0	1.7	0.0
Other	15.76 (1.10)	23.4 (0.5)	29.3 (7.5)

a. Rats (n=2 per sex) received a single dose of 10 mg/kg [^{14}C]ABT-378 with 5 mg/kg ritonavir in a solution formulation.[13]

b. Dogs (n=2 per sex) received a single dose of 5 mg/kg [^{14}C]ABT-378 with 2.5 mg/kg ritonavir in a solution formulation.[15]

c. Human volunteers (n=5 males) received a single dose of 400 mg (≈5.7 mg/kg) [^{14}C]ABT-378 with 100 mg (≈1.4 mg/kg) ritonavir in a liquid filled capsule formulation.[37]

d. Structures of metabolites are shown in the figure above.

e. Urine data are shown in parentheses – absence of data in parentheses implies none detected.

The major metabolites present in human feces after oral dosing were M-3/4 (10.1% of mean total dose), M-9/10 (7.4%) and M-11/12/13/14/15 (8.9%).[37] This represents a distribution of radioactivity in feces that was similar to that observed in the rat and dog (see table above). Metabolites M-1 and M-6/7/8 were also identified in human feces, together accounting for 7.1% of the dose radioactivity. Again, similar to rats and dogs, a substantial portion of the dose radioactivity (29.3% of mean total dose) was present as unidentified polar metabolites. These numerous polar metabolites are attributed to tertiary and quaternary metabolites of ABT-378. The radioactivity recovered in human urine (10.4% of mean total dose) consisted mainly of early eluting polar metabolites (7.5% of total dose-^{14}C), accompanied by unchanged parent (≈2% of dose).[37] Rat and dog urine contained an insignificant amount of unchanged ABT-378 and largely consisted of early eluting polar metabolites.[13,15] The remainder of the urinary radioactivity in dogs and humans comprised lesser amounts of metabolites M-1, M-3/4, M-6/7/8 (dog and human), M-9/10 and one or more of M-11/12/13/14/15 (human only) which together accounted for ≈0.3% or ≈0.7% of dose-^{14}C in dogs and humans, respectively.[15,37]

2.5.3.6 Excretion

Rats, dogs and humans given a 5-10 mg/kg intravenous dose and a 5-10 mg/kg oral dose of [^{14}C]ABT-378 coadministered with ritonavir displayed high fecal excretion (>80% of dose) of the radiolabeled dose within 3-8 days, indicating that the drug was cleared predominantly by hepatobiliary processes in all three species (see table above).[13,15,37] This was verified in bile exteriorized male rats, where 66.8% of a 10 mg/kg intravenous dose coadministered with 5 mg/kg ritonavir was excreted in the bile within 24 hours after dosing, accompanied by minimal dose recovery in urine (<2%).[14] Intraduodenal administration of a 10 mg/kg dose resulted in the recovery of 24.7% of the dose in the 0-24 hour bile of rat.[14] Recovery of radioactivity in dog bile (0-6 hours) was 19.6% and 8.0% of the administered dose after intravenous and intraduodenal dosing, respectively.[15] The low recovery from the dog bile experiment is likely due to the short course of the experiment (6 hours) coupled with the effect of ritonavir coadministration delaying the hepatic metabolism of ABT-378 and therefore biliary elimination of metabolites. This is supported by data which demonstrates the delaying effect of ritonavir coadministration on the biliary elimination of radioactivity after administration of [^{14}C]ABT-378 to the rat.[14] The radioactivity recovered in human urine (10.4% of mean total dose) was substantially

greater than observed in rats or dogs (<2% of dose).[37] However urinary elimination was still a minor route of elimination in all species compared to fecal elimination (>80% of dose).

2.5.3.7 *In Vitro* Metabolism and Enzyme Inhibition

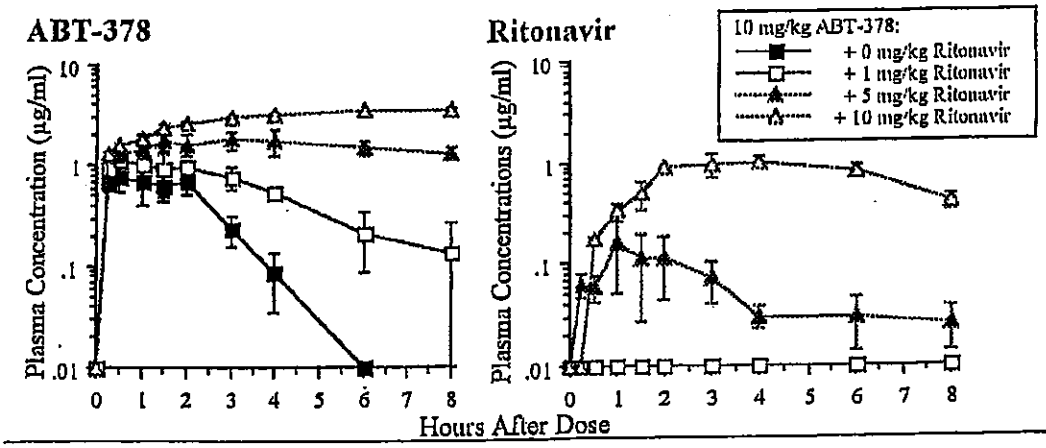
The *in vitro* metabolism of [^{14}C]ABT-378 by human liver microsomes was examined with the objective of determining the likely products of *in vivo* metabolism of the drug in humans.[18] Incubation of [^{14}C]ABT-378 with rat and dog hepatic microsomes resulted in metabolite profiles which were qualitatively similar to the corresponding metabolite profiles of the drug in rat and dog bile, thus verifying the predictive accuracy of the *in vitro* microsomal model of hepatic metabolism.[18] Human liver microsomes converted [^{14}C]ABT-378 largely to M-1, M-3 and M-4, suggesting that these metabolites would likely be major products of hepatic biotransformation of the drug in humans, just as they are in rats and dogs. Human hepatocytes also resulted in essentially the same metabolic profile observed in microsomes.[18] Rapid rates of formation of M-1, M-3 and M-4 were also observed across species. Additionally the metabolism of ABT-378 in rat liver microsomes was found to be sensitive to inhibition by ritonavir ($IC_{50} \approx 0.02$ μg/mL) agreeing with the inhibition of the metabolic clearance of ABT-378 by ritonavir observed *in vivo* in the rat.[20]

The kinetics of the metabolism of [^{14}C]ABT-378 were determined with microsomes from four human livers, affording apparent mean K_m values of 6.81±3.62 μM and mean V_{max} values of 9.38±5.53 nmoles/min/mg protein.[21] Metabolism by microsomes derived from cDNA transfected B-lymphoblastoid cells, metabolic correlation with isoform-specific CYP activities and chemical and immunoinhibition studies indicated that the members of the CYP3A subfamily (CYP3A4 and CYP3A5) are the exclusive contributors to the human liver microsomal metabolism of ABT-378.[21] Ritonavir, another potent anti-HIV agent known to inhibit CYP3A, was found to be a very potent inhibitor of the metabolism of ABT-378 with an inhibitory constant (K_i) of 0.013 μM (0.009 μg/mL).[21] Conversely, ABT-378 was found to be a very weak inhibitor of ritonavir metabolism, with a K_i of 130 μM (82 μg/mL).[21] A typical plasma concentration of ritonavir (0.2 to 0.9 μg/mL) at the anticipated clinical dose of 100 mg ritonavir twice daily is 20- to 100-fold greater than the *in vitro* determined K_i for inhibition of ABT-378 metabolism.[36] Therefore this

provides a clear rationale for the decrease in ABT-378 clearance by inhibition of the metabolism of ABT-378.

The pharmacokinetics of unlabeled ABT-378 (10 mg/kg) and ritonavir (1, 5 or 10 mg/kg) after oral dosing of the combination to rats appears to substantiate the dependence of sustained plasma ABT-378 on ritonavir plasma concentrations (see figure below).[1]

Effect of Dose and Dose Ratio on the Plasma Concentrations of ABT-378 and Ritonavir following Concomitant Oral Dosing in Rat (10 mg/kg ABT-378 + 0, 1, 5 or 10 mg/kg Ritonavir)



ABT-378 was found to be a very weak inhibitor of ritonavir metabolism, with a K_i of 130 µM (82 µg/mL).[21] However, ABT-378 inhibited CYP3A-dependent nifedipine oxidase activity with an IC_{50} of 1.3 µM.[21] The disparity between the potency of ABT-378 for inhibition of these two largely CYP3A-dependent activities (ritonavir *vs.* nifedipine), may at least in part be due to the relatively high affinity of ritonavir for the CYP3A active site (i.e. tight binding). Ritonavir exhibits significant potential for *in vivo* inhibition of the metabolism of drugs which are CYP3A4, CYP2D6 and, to a lesser extent, CYP2C9/10 and CYP2C19 substrates during coadministration.[40] By comparison with ritonavir, ABT-378 is a weaker inhibitor of CYP3A, CYP2D6, CYP2C9 and CYP2C19. Furthermore, CYP1A2, CYP2A6, CYP2B6 and CYP2E1 were not inhibited by ABT-378. However, since ABT-378 is coadministered with the potent CYP inhibitor ritonavir in the clinic, the effect of this combination on isoform-specific cytochrome P450-dependent

monooxygenase activities in human liver microsomes was examined using concentration ratios (3:1 and 29:1 ABT-378:ritonavir) that would encompass the range of ratios likely to occur in a clinical setting.[22] These data were then compared with data obtained for ritonavir alone.

Effect of ABT-378/Ritonavir Combination on Isoform-Specific Cytochrome P450 Activities

		IC_{50} (μM)		
		ABT-378:ritonavir ratio		Ritonavir
Isoform	Assay[a]	3:1	29:1	(historical data)
CYP1A2	Phenacetin *O*-deethylation	No effect	No effect	>50
CYP2A6	Coumarin 7-hydroxylation	No effect	No effect	No effect
CYP2B6	(S)-Mephenytoin *N*-demethylation	> 30	> 30	8.1
CYP2C9	Tolbutamide hydroxylation	13.7	23.0	8.0
CYP2C19	(S)-Mephenytoin 4'-hydroxylation	28.7	38.0	13.0
CYP2D6	Dextromethorphan *O*-demethylation	13.5	29.0	2.5
CYP2E1	Chlorzoxazone 6-hydroxylation	No effect	No effect	No effect
CYP3A	Terfenadine hydroxylation	1.1	4.6	0.14

[a]CYP assays were conducted at approximate K_m concentrations.

As would be predicted based on the inhibitory characteristics of ABT-378 and ritonavir alone, the combination of the two drugs resulted in a less potent inhibition of CYP3A, CYP2D6, CYP2C9 and CYP2C19 than ritonavir alone. CYP1A2, CYP2A6 and CYP2E1 isoforms were not inhibited by the ABT-378:ritonavir combination, with only a marginal effect on CYP2B6 activity.

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9. Human Pharmacokinetics / Bioavailability (1-18)

ABT-378/ritonavir

Documents for Application Summary

Section 2.6

Human Pharmacokinetics/Bioavailability

2.6 Human Pharmacokinetics/Bioavailability

2.6.1 Fundamental Pharmacokinetic Characteristics of ABT-378

ABT-378 is a peptidomimetic inhibitor (MW = 628.8) of the HIV protease which has been shown to be clinically effective when orally administered in combination with ritonavir in the suppression of HIV viremia. Both ABT-378 and ritonavir have limited solubility in aqueous systems. Capsule and liquid formulations have been developed to improve gastrointestinal solubility. Later studies with the clinical dose revealed that ABT-378 absorption was enhanced when administered with food. Except for a limited period in early Phase II, studies generally employed nonfasting administration. Key ABT-378 pharmacokinetic parameters obtained from the various Phase I and II studies are provided in the following table.

ABT-378 Pharmacokinetic Characteristics

Parameter	Units	Values (Mean ± SD)
C_{max} (400A/100R mg q12h; HIV-infected)	µg/mL	9.58 ± 4.41
T_{max} (400A/100R mg q12h; HIV-infected)	h	3 ± 2
C_0 (400A/100R mg q12h; HIV-infected)	µg/mL	5.49 ± 4.02
C_{min} (400A/100R mg q12h; HIV-infected)	µg/mL	3.83 ± 3.44
F_u	%	1 to 2
CL/F (single 400A/100R mg dose, healthy subjects)	L/h	4.1 ± 1.1
CL/F (ss, 400A/100R mg q12h; HIV-infected)	L/h	6.4 ± 4.4
C_{max}/C_{min} $t_{1/2}$ (ss, 400A/100R mg q12h; HIV-infected)	h	5 to 6
CL_r	L/h	□ 0.1
Principal clearance process		metabolism
CYP isoforms involved		CYP3A
Activity from metabolites		minimal[#]
CL/F, time- and dose-dependence		yes[$]
CL/F, age 6 months to 12 year		no difference[ϕ]
CL/F, age ≥ 65 year		not determined[Ψ]
CL/F, gender		no difference[+]
CL/F, race		Blacks have increase (14% lower AUC)[+]
CL/F, renal impairment		not determined
CL/F, hepatic impairment		not determined
CL/F, inducers		↓ C_{min} (rifampin, efavirenz & nevirapine)[δ]
Inhibitor of other drugs		CYP3A[£]
Inducer of CYP and glucuronidation		decrease in AUC of methadone and EE2[¥]

Abbreviations: A = ABT-378; R – ritonavir; C_{max} = maximum conc., T_{max} = time to C_{max}, C_{min} = minimum conc., C_0 = pre-morning dose conc., f_u = unbound fraction in plasma, CL/F = apparent oral clearance, ss = steady state, C_{max}/C_{min} $t_{1/2}$ = effective or clinically relative half-life, CL_r = renal clearance.

\# Some metabolites are active but concentrations are low.

$ CL/F of ABT-378 is dependent on both the ABT-378 and ritonavir dose size. C_{min} declines with multi-dosing, but stabilized by 10 days to 2 weeks.

ϕ From pediatric Phase I/II study in HIV-infected subjects.

Ψ Limited data available in subjects ≥ 65 yr.

\+ Formal Phase I studies not conducted; effects were estimated from analyses of seven combined bioavailability studies; lower AUC in Blacks not considered clinically relevant.

£ ABT-378/ritonavir increased AUC of ketoconazole (3.0-fold), rifabutin (3.0-fold), 25-O-deacetyl rifabutin (47.5-fold), atorvastatin (5.9-fold) and other HIV-protease inhibitors amprenavir, saquinavir, indinavir and nelfinavir.

¥ ABT-378/ritonavir decreased AUC of ethinyl estradiol (EE2) (42%), and methadone (53%).

δ ABT-378 C_{min} decreased by rifampin (99%), efavirenz (39%) and nevirapine (55% in pediatric subjects).

2.6.2 Formulations

2.6.3 Absorption

ABT-378 is a potent HIV protease inhibitor with relatively low aqueous solubility (~45 μg/mL in water, 25°C), yet reasonably good permeability, and would be categorized as a Biopharmaceutical Classification System (BCS) Class II compound. In CACO-2 culture, the apical to basolateral (AB) apparent permeability (P_{app}) was determined to be $10.65 \pm 0.58 \times 10^{-6}$ cm/sec at pH 6.8. In general, agents with CACO-2 P_{app} values above 10×10^{-6} cm/sec have high human absorption. The basolateral to apical (BA) P_{app} was $9.55 \pm 2.10 \times 10^{-6}$ cm/sec. The similarity in the permeabilities from both sides of the cells indicate that p-glycoprotein (Pgp) or other apical transporters do not appreciably polarize directional movement. However, ABT-378 is rapidly and completely metabolized by CYP3A with a very high intrinsic clearance. Accordingly, systemic bioavailability is quite low when ABT-378 is administered alone. After single dose administration of 400 mg ABT-378, the apparent oral clearance (CL/F) was 1016 L/h, which is indicative of a large and extensive first pass metabolism. For this reason, ABT-378 is co-formulated with small amounts of ritonavir as a pharmacokinetic enhancer. When co-administered with 100 mg ritonavir, CL/F is profoundly reduced to approximately 4 L/h after single dose administration of 400 mg ABT-378. The effect appears to be a composite of inhibition of both first pass and systemic clearances.

Owing to low solubility of the agents and the complexities of the metabolic interaction between ritonavir and ABT-378, intravenous administration and absolute bioavailability assessment are not possible. The extent of absorption of ABT-378 administered under nonfasting conditions with ritonavir was assessed as a part of the analyses in the human [^{14}C]study. In that study, an average of 20% of the administered dose radioactivity was recovered in the feces as unchanged ABT-378, with individual values ranging from 12 to 23%. Since this recovered unchanged drug represents the sum of unabsorbed ABT-378

plus material that was secreted in the bile or intestinally exsorbed, it is inferred that the extent of absorption was greater than or equal to 80% of the administered dose. Fecal recovery data after intravenous administration in animals as well as biliary metabolic profiling indicate that part of the unchanged ABT-378 in feces may be the result of biliary secretion or intestinal exsorption; thus, the extent of ABT-378 absorption may be greater than 80%.

Although an early assessment of bioavailability in the first single rising dose study, M96-552, at an ABT-378/ritonavir dose of 400/200 mg (separately formulated capsules) indicated no food effect, subsequent investigations with the ABT-378/ritonavir 400/100 mg dose have shown that the systemic availability of ABT-378 is reduced and is more variable under fasting conditions for both separately formulated and co-formulated SGCs and the co-formulated liquid. From definitive bioavailability study data, relative to administration with a meal of moderate fat content, administration of the co-formulated SGC Formulation B2 under fasting conditions decreased the AUC by 28 to 36% at an ABT-378/ritonavir dose of 400/100 mg. The AUC of the liquid co-formulation was decreased to a somewhat greater extent, 44%, when given under fasting conditions relative to a meal of moderate fat content. In the definitive bioequivalence Study M99-072, the separately formulated capsules (Phase II) and to-be-marketed co-formulated capsules (Phase III) provided equivalent ABT-378 exposure under both fasting and nonfasting conditions. This differential food effect between the liquid and capsule co-formulations probably contributes to the ability to demonstrate bioequivalence between the liquid and the SGCs under nonfasting conditions, but not under fasting conditions. Administration of the co-formulated SGC and liquid formulations with a meal of high fat content, further increased the bioavailability by 26% and 37%, respectively. ABT-378/ritonavir is to be taken with food due to the decreased bioavailability and increased variability noted during administration under fasting relative to nonfasting conditions.

With multiple dosing under nonfasting conditions, there is a diurnal effect in the pharmacokinetics of ABT-378 which may be related to absorption differences, with later

and lower peak concentrations and higher trough concentrations occurring after evening doses.

2.6.4 Plasma Protein Binding and Distribution

The *in vitro* protein binding of [^{14}C]ABT-378 in human plasma is high. As determined by equilibrium dialysis, binding averaged 99.7 to 97.4% over a drug concentration range of 0.1 to 100 µg/mL. In human plasma, binding did not change appreciably within the clinical range of 0.1 and 10 µg/mL of [^{14}C]ABT-378, but was lower at 30 and 100 µg/mL. Specifically, the mean protein binding was 99.7%, 99.7% and 99.5% at concentrations of 0.1, 1 and 10 µg/mL, respectively, then decreased to 98.7% at 30 µg/mL and to 97.4% at 100 µg/mL. No major differences were observed in the degree of protein binding in plasma between males and females over the entire concentration range. ABT-378 is bound to both albumin and α_1-acidglycoprotein. From *in vitro* potency determinations for HIV-1 in MT_4 cells in the absence (EC_{50} = 0.019 µM) and presence (EC_{50} = 0.102 µM) of human serum, protein binding attenuates the potency of ABT-378 (~5.4 x), but not as dramatically as would be expected based on the free fraction (> 50 x).

ABT-378 has not been administered intravenously to humans; thus, it is not possible to accurately estimate the true distribution volume. Moreover, the determination of the distribution volume is further complicated by the nonlinear dispositional kinetics of ABT-378 in the presence of ritonavir. Computations based on "effective half-life" and tissue distribution data in rats suggest that the distribution volume in humans is around 40 L, which is comparable to total body water. The tissue to plasma radioactivity ratios at 4 hours after oral dosing in rat were less than unity in bone marrow (0.13), eyes (0.16), perirenal fat (0.78), heart (0.52), kidneys (0.92), lungs (0.51), lumbar lymph nodes (0.15), submaxillary lymph nodes (0.61), pancreas (0.94), prostate gland (0.45), skeletal muscle (0.25), skin (0.29), spleen (0.41), testes (0.17), thymus (0.42) and urinary bladder (0.55). The 4-hour tissue to plasma radioactivity ratios in the gastrointestinal tract plus contents, liver (22.5), adrenal (2.07) and thyroid (1.90) glands were greater than unity. The high tissue/plasma (T/P) ratio observed in liver probably reflects large contributions from metabolites, since metabolism and biliary secretion of the metabolites represents the

major route of elimination. Based on brain to plasma ratios of 0.02 to 0.03 in rat, it would appear that central nervous system (CNS) penetration is restricted by the plasma protein binding. Determination of ABT-378 concentrations in human cerebral spinal fluid (CSF) is currently ongoing.

2.6.5 Metabolism

The The *in vitro* metabolism of ABT-378 has been determined in microsomes from mouse, rat, dog, monkey and human liver, and the metabolic profiles were globally comparable. The patterns from human excreta were comparable to those from human liver microsomes and hepatocytes. The kinetics of the metabolism of [^{14}C]ABT-378 were determined with microsomes from four human livers, affording apparent mean ± SD K_m of 6.81 ± 3.62 μM and mean ± SD V_{max} of 9.38 ± 5.53 nmoles/min/mg protein. When this is scaled to human liver size, an intrinsic clearance of nearly 7400L/h is predicted in the absence of protein binding, and $f_u \cdot CL_{int}$ is in the order of 37 L/h, assuming that binding is totally restrictive at a free fraction level of 0.005. The high V_{max} indicates that a high degree of intestinal first pass metabolism is likely.

Metabolism by microsomes derived from cDNA transfected B-lymphoblastoid cells, metabolic correlation with isoform-specific CYP activities and chemical and immunoinhibition studies indicated that the members of the CYP3A subfamily (CYP3A4 and CYP3A5) are the exclusive contributors to the human liver microsomal metabolism of ABT-378. Ritonavir, another potent anti-HIV agent known to inhibit CYP3A, was found to be a very potent inhibitor of the metabolism of ABT-378, with an inhibitory constant (K_i) of 0.013 μM (0.009 μg/mL).

ABT-378 when dosed with ritonavir is metabolized exclusively by oxidative pathways in rat, dog and human, resulting in very similar metabolic profiles. M-1 and M-3 through M-12 have been identified in rat, dog and human excreta, whereas M-13, M-14 and M-15 have been identified in dogs and humans. There are five identified primary metabolites: an epimeric pair of 4-hydroxylated derivatives (M-3 and M-4), and three mono-hydroxylated ABT-378 derivatives with the hydroxyl group located on the diphenyl core

moiety (M-6, M-7 and M-8). Several secondary metabolites are then produced by further biotransformation of the primary metabolites.

In rat, dog and man, unchanged ABT-378 was the major circulating radioactive component in plasma. After intravenous and oral (dog only) dosing, parent accounted for greater than 80% and 95% of plasma AUC_{0-t} in rats and dogs, respectively. After a single 5.7 mg/kg dose of [^{14}C]ABT-378 co-administered with 1.4 mg/kg ritonavir to five male human subjects, ABT-378 was the predominant circulating component, accounting for 89% of the AUC for total radioactivity. Trace levels of multiple metabolites were observed in the plasma, but the concentrations were too low for a meaningful determination of their AUCs. Accordingly, the clinical antiviral activity of ABT-378 would appear to be overwhelmingly associated with parent drug.

2.6.6 Elimination.

In all species investigated, ABT-378 is extensively metabolized, with elimination of the majority of the dose as metabolites in the feces. In the radiolabel study in man, 82.6% of the dose was recovered in the feces over a 7 to 8 day period, while 10.4% of the dose was recovered in the urine over the first three days after dosing. The mean overall recovery of radioactivity was 93%, and consisted mostly of a variety of metabolites. The recovery pattern for ABT-378 was very similar to that previously reported for ritonavir, for which fecal recovery was also the major route of elimination. Unchanged ABT-378, accounting for 19.8% of the dose, was found in the feces, and represents unabsorbed and/or biliary-secreted/exsorbed drug. In addition, approximately 2% of the administered dose was recovered in the urine unchanged. From the elimination, protein-binding and distribution data, the inferences may be drawn that ABT-378 pharmacokinetics are unlikely to be affected by renal impairment or hemodialysis, but may be affected by hepatic impairment. Due to the solubility characteristics and possibility of transintestinal elimination, it is proposed that management of overdose could entail gastric lavage and administration of activated charcoal.

2.6.7 Factors Affecting ABT-378 Clearance

ABT-378 apparent clearance is affected by the administration with food. In the definitive bioavailability studies, M99-072 and M99-073, administration of either the liquid or capsule formulation under fasting conditions significantly decreased the AUC of both formulations by 28 to 35% and 44%, respectively. Aside from the apparent effect of food on the extent (F) and rate of ABT-378 absorption, the major factors found to affect ABT-378 CL/F are the amount of ritonavir co-administered with ABT-378 for single and multiple dosing or dose ratio of ABT-378 and ritonavir, and the duration of treatment with multiple dosing. Based on population data, other factors, such as age, race, gender, disease status (viral load or CD_4+ cell count), and most concomitantly administered drugs have little or no effect on ABT-378 clearance, as outlined below.

2.6.7.1 ABT-378 and Ritonavir Dose Size after Single Dosing

The single dose pharmacokinetics of ABT-378 co-administered with ritonavir at varying dose combinations were investigated in healthy subjects in Study M96-552. Doses of ABT-378 ranging from 100 to 800 mg were co-administered with ritonavir doses ranging from 50 to 300 mg. Additionally, single 200 and 800 mg doses of ABT-378 were administered in the absence of ritonavir. All doses were administered with a meal, with the exception of the food effect segment of the study. The plasma concentrations of ABT-378 were profoundly increased when co-administered with ritonavir. CL/F for a single dose of ABT-378 200 mg in the absence of ritonavir was > 2000 L/h, and the CL/F for a single 800 mg dose was approximately 360 L/h. In general, after single dosing, ABT-378 concentrations increased relatively proportionally with dose for a fixed ritonavir dose. For a fixed ABT-378 dose, increasing the ritonavir dose did not consistently result in proportional increase in ABT-378 AUC or C_{max}. The increase in ABT-378 concentrations 24 hours after dosing (C_{24}) tended to be proportional or more than proportional with respect to ritonavir dose. Ritonavir also significantly reduced the intersubject variability in ABT-378 pharmacokinetics. In general, the pharmacokinetics of ritonavir did not appear to be greatly influenced by ABT-378.

2.6.7.2 Time and Dose Effects After Multiple Dosing

The multiple dose pharmacokinetics of ABT-378 co-administered with ritonavir were investigated in two studies conducted in healthy subjects, M97-650 and M97-806, and in HIV-infected subjects in M97-720 and M97-765. Both QD and BID regimens were administered to healthy subjects. For BID regimens, doses of ABT-378 ranged from 200 to 400 mg and doses of ritonavir ranged from 50 to 200 mg. For a fixed ritonavir dose, increases in the ABT-378 dose provide less than proportional increases in ABT-378 concentrations. Also, for a fixed ABT-378 dose, increasing the ritonavir dose increases ABT-378 concentrations; but the increase in ABT-378 concentration appears to be less than proportional to the increase in ritonavir dose. However, when both the ABT-378 and ritonavir doses are increased proportionally, concentrations of ABT-378 increase relatively proportionally. In healthy subjects, for a fixed total daily dose of ABT-378/ritonavir 600/200 mg, the 300/100 mg q12h regimen has a more attractive pharmacokinetic profile than the 600/200 mg QD regimen, with the q12h regimen yielding an estimated 2.6-fold higher mean C_{min}.

Similar to ritonavir when it is given alone, the pharmacokinetics of ABT-378/ritonavir are time-dependent, probably due to induction of CYP3A isoenzymes and possibly other mechanisms such as induction of P-glycoprotein. The decreases in mean ABT-378 C_{min}, AUC, and C_{max} values from Day 5 to Day 16 in Study M97-650 were approximately 50% for C_{min}, 30% for AUC, and 21% for C_{max}. Across studies, steady state was reached in both healthy and HIV-infected subjects after dosing for approximately 10 days to 2 weeks, and remained stable in HIV-infected subjects through measurements at 24 weeks. Plasma protein binding of ABT-378 remained unchanged after multiple dosing, being approximately 98.8 to 99.2% bound at concentrations ranging from 2.1 to 12.6 µg/mL.

2.6.7.3 Body Weight, Gender, Race and Age

No formal studies were conducted to assess the effect of body weight, gender, age and race on the pharmacokinetics of ABT-378 co-administered with ritonavir. An across-study examination of seven bioavailability studies was performed in which single doses of co-formulated capsule formulations of ABT-378/ritonavir 400/100 mg or 400/200 mg

were administered under nonfasting conditions. Analysis of covariance including study, weight, gender, race and age as explanatory variables was performed on log-transformed AUC, C_{max} and C_{24}/C_{max}. In the analysis of covariance, body weight was a significant factor for both ABT-378 C_{max} and AUC. This is not surprising since the adult clinical dose of ABT-378/ritonavir is not adjusted based on body weight. Subjects with a higher body weight tend to have lower AUC and C_{max}; however, the effect of weight appears to be small. With weight as an explanatory variable in the model, gender was not a significant factor for ABT-378 pharmacokinetic parameters. Within the range of 18 to 55 years, no significant age effect on ABT-378 pharmacokinetic parameters was detected. Due to the lack of trend in this age range, and low prevalence of AIDS above age 65, a formal study in elderly has not been conducted. The predominant race represented in Phase I studies and clinical trials was Caucasian (157), with a limited number of Blacks (20) and Hispanics (17). Based on limited data, Blacks appeared to have marginally significantly lower AUCs (14%) and lower C_{max} values (14%) than Caucasians. The differences were small and unlikely to be clinically important. No statistically significant efficacy differences between Blacks and Caucasians were noted in the pivotal trial, M98-863.

2.6.7.4 HIV Infection

In spite of no restrictions with regard to dosing with food at the time of pharmacokinetic sample collection in the early Phase II studies and the less controlled study conditions for a multi-center Phase II study compared to a Phase I healthy volunteer study, there appear to be no important differences between the pharmacokinetics in healthy subjects and in HIV-infected subjects. In the Phase II studies, M97-720 and M97-765, an analysis of covariance (ANCOVA) was performed on the natural log-transformed pharmacokinetic parameters, including C_{max}, C_{min} and AUC. The initial model included effects of baseline HIV RNA level and baseline CD_4 cell count. Neither of these covariates was found to be statistically significant factors in defining pharmacokinetic differences between subjects.

2.6.7.5 Renal and Hepatic Impairment

Since approximately 10% of a [^{14}C]dose of 400 mg ABT-378 in combination with ritonavir 100 mg was eliminated in the urine (2.2% as parent), renal impairment should not have a significant effect on ABT-378 clearance or the elimination of its metabolites. With both ABT-378 and ritonavir, unchanged parent is the overwhelmingly predominant drug-associated entity in circulation. Accordingly, no formal study of the pharmacokinetics has been performed in renally impaired HIV-infected subjects.

No formal study of the effect of hepatic impairment on the pharmacokinetics of ABT-378 administered in combination with ritonavir has been conducted. Based on historical observations for other CYP3A substrates in hepatic disease, a reduction in clearance would be expected.

2.6.7.6 Interactions Produced by Other Drugs

Since ABT-378 is co-administered with ritonavir, a potent inhibitor of the CYP3A-mediated metabolism of ABT-378, additional effects on ABT-378 by administration of other potent inhibitors of CYP3A-mediated metabolism would be expected to be minimal. In confirmation of this expectation, ketoconazole, a potent CYP3A inhibitor, did not increase the AUC of ABT-378.

Since ABT-378 is extensively metabolized, enzyme induction by other agents may theoretically increase ABT-378 clearance. Confirmation of the susceptibility of ABT-378 to induction effects was observed in the interaction study with the potent CYP3A inducer, rifampin. The AUC and C_{min} of ABT-378 were decreased by 75% and 99% during rifampin co-administration. As a result, rifampin and ABT-378/ritonavir should not be co-administered. Additionally, the nonnucleoside reverse transcriptase inhibitor (NNRTI) efavirenz, an inducer of CYP3A, decreased ABT-378 AUC and C_{min} by 19% and 39%, respectively, during co-administration. Nevirapine, another inducing NNRTI, also appears to decrease ABT-378 C_{min}. Dosage increase of ABT-378/ritonaivr by 1/3 should be considered in highly antiretroviral experienced patients receiving either efavirenz or nevirapine. Interestingly, rifabutin, also an inducer of CYP3A, did not decrease the concentrations of ABT-378.

2.6.7.7 Inter- and Intrasubject Variability

Generally, the intersubject coefficient of variation (CV) in AUC was 35 to 40% across bioavailability studies when ABT-378/ritonavir was administered under nonfasting conditions (regular meal, 25-30% Kcal from fat). The intersubject CV for AUC was higher (45 to > 50%) when ABT-378/ritonavir formulations were administered under fasting conditions. Note that intersubject CV here really corresponds to total variance, which is the sum of intrasubject and intersubject variablility. An estimate of intrasubject CV was obtained from the analysis of variance performed on log-transformed AUC and C_{max} of a bioequivalence study (M99-072). The intrasubject CV was estimated by the square root of the residual mean square from the analysis of the logarithms (*i.e.*, the estimate of error term standard deviation). In Study M99-072, the intrasubject CV was estimated to be 35% for AUC and 32% for C_{max}. This is likely an overestimation of the true intrasubject variability because both nonfasting and fasting administration were included in the model.

In the Phase II Study M97-720, both the intersubject and intrasubject CVs were estimated for the pharmacokinetic data obtained at Weeks 3, 6 and 24 during multiple dosing. In this study in HIV-infected subjects, the estimated intrasubject CV for C_{max} and AUC was 26% and 31%, respectively, considerably lower than the estimated intersubject CV, 51% and 59% for C_{max} and AUC, respectively. Intrasubject variability in Study M97-720 could be overestimated because doses were taken without regard to meals, which may increase both intrasubject and intersubject variability. The ABT-378 intersubject variability in pharmacokinetic parameters in HIV-infected subjects in Study M97-720 calculated in this multiple dose Phase II study tended to be higher than that noted in the single-dose bioavailability studies and in previous multiple dose studies conducted in healthy subjects. Higher intersubject variability in Phase II compared to Phase I studies could be attributed to multiple factors including variation in study environment, dietary variation , timing of dosing in relationship to meals (*i.e.*, fasting *vs.* nonfasting), adherence to the regimen (missed doses) and pharmacokinetic sampling in relation to dosing.

2.6.8 Pharmacokinetic Interactions Produced by ABT-378/Ritonavir

Since ABT-378 is co-administered with the potent CYP inhibitor, ritonavir in the clinic, the effect of the ABT-378 and ritonavir combination on isoform-specific cytochrome P450-dependent monooxygenase activities in human liver microsomes was examined using concentration ratios (3:1 and 29:1 ABT-378:ritonavir) that would encompass the range of ratios likely to occur in a clinical setting. These data were then compared with data obtained for ritonavir alone.

Effect of ABT-378/Ritonavir Combination on Isoform-Specific Cytochrome P450 Activities

Isoform	Assay	IC_{50} (μM)		
		ABT-378:ritonavir ratio		Ritonavir (historical data)
		3:1	29:1	
CYP1A2	Phenacetin *O*-deethylation	No effect	No effect	>50
CYP2A6	Coumarin 7-hydroxylation	No effect	No effect	No effect
CYP2B6	(S)-Mephenytoin *N*-demethylation	> 30	> 30	8.1
CYP2C9	Tolbutamide hydroxylation	13.7	23.0	8.0
CYP2C19	(S)-Mephenytoin 4'-hydroxylation	28.7	38.0	13.0
CYP2D6	Dextromethorphan *O*-demethylation	13.5	29.0	2.5
CYP2E1	Chlorzoxazone 6-hydroxylation	No effect	No effect	No effect
CYP3A	Terfenadine hydroxylation	1.1	4.6	0.14

CYP assays were conducted at approximate K_m concentrations.

The combination of the two drugs resulted in a less potent inhibition of CYP3A, CYP2D6, CYP2C9 and CYP2C19 than ritonavir alone. CYP1A2, CYP2A6 and CYP2E1 isoforms were not inhibited by the ABT-378:ritonavir combination, with only a marginal effect on CYP2B6 activity. Some predictions of likely *in vivo* interactions that might be expected have been made using an accepted published approach. Based on *in vitro* IC_{50} values for inhibition of the various CYP isoforms, it is expected that only metabolism mediated by CYP3A will be inhibited at clinically relevant concentrations of ABT-378 and ritonavir. Note that the clinically observed ratio of ABT-378:ritonavir concentrations is 15 to 20:1. In humans, trough plasma concentrations and the AUC values for ritonavir were found to decrease on multiple dosing, suggesting that ritonavir

is a metabolic inducer, inducing its own metabolism *in vivo*. A similar observation has been made with ABT-378 co-dosed with ritonavir.

Drug-drug interaction studies in humans were selected primarily based on those drugs with a high likelihood of co-administration with ABT-378/ritonavir which posses a high potential for interaction with ABT-378/ritonavir. Those with high potential of interaction are primarily CYP3A substrates with a high intrinsic clearance and drugs susceptible to induction by ABT-378/ritonavir. Interaction studies have demonstrated large inhibitory effects of ABT-378/ritonavir on the clearance of the following CYP3A substrates: other HIV-protease inhibitors (saquinavir, indinavir, nelfinavir and amprenavir), rifabutin and its 25-O-desacetyl metabolite (combined AUC increases of 5.7-fold), atorvastatin (parent AUC increased 5.9-fold) and ketoconazole (AUC increased 3-fold). Dosage modification when these drugs are co-administered with ABT-378/ritonavir is recommended. Additionally, decreases in AUC of other drugs were noted during co-administration of ABT-378/ritonavir with ethinyl estradiol (42% decrease) and methadone (53% decrease), suggesting induction of glucuronidation and CYP-mediated metabolism, respectively.

Overall, ABT-378/ritonavir inhibits CYP3A-mediated metabolism at clinically relevant concentrations and is also an inducer of CYP-mediated metabolism and glucuronidation.

2.6.9 Antiviral Effects of ABT-378

ABT-378 is virologically ten-fold more active than ritonavir, with an EC_{50} of 0.07 µg/mL against HIV-1_{IIIB} activity in MT_4 cells in a medium containing 50% human serum and 10% calf serum. The protein binding corrected EC_{50} against wild-type HIV for ritonavir under the same conditions is 0.9 µg/mL. Against ritonavir-resistant HIV, ABT-378 displays potency similar to that observed by ritonavir against wild-type HIV. In the Phase II and Phase III trials, ABT-378 has been tested in HIV protease inhibitor (PI)-naïve subjects, as well as HIV-infected subjects with PI experience who have developed various degrees of genotypic and phenotypic resistance to PIs and to nucleoside reverse transcriptase inhibitors (NRTI).

Pharmacokinetic/pharmacodynamic modeling of the antiviral effect of ABT-378 has shown little relationship between exposure and virologic outcome. From the Phase II trial M97-720, 92% and 98% of antiretroviral-naïve subjects on treatment demonstrated HIV RNA levels < 400 copies/mL after 48 weeks and 72 weeks of treatment, respectively. For the HIV-infected subjects with more extensive pharmacokinetic sampling, there was no apparent relationship between concentration and the initial rate of viral load decline or viral load at various times. Similarly for the full cohort, there appeared to be no relationship between administered dose and therapeutic success. In part this is due to the high exposures of ABT-378 in this study, with mean minimum concentrations exceeding the EC_{50} of wild-type virus by a factor of greater than 50. In the Phase II trial in single PI-experienced HIV-infected subjects (M97-765), the average ABT-378 C_{min} values determined from the 12 subjects participating in the pharmacokinetic subgroup exceeded the estimated protein binding-corrected EC_{50} for HIV by > 4-fold in 91% of the 57 baseline viral isolates.

Although previous studies with the first generation PIs have described a relationship between magnitude and duration of antiviral effect and C_{min}, the issues become more complicated as more potent therapy becomes available. With registered PIs given singly with NRTI support, average C_{min} were near the viral EC_{50}, and maladherence and/or abnormally high clearance resulted in a significant fraction of the treated HIV-infected subjects experiencing virologic rebound. With ABT-378, the C_{min}/EC_{50} ratios are greatly improved. From the data of Phase II studies in naïve and single PI-experienced subjects, there appeared to be no predictive relationship between the degree and duration of viral suppression and the pharmacokinetics of ABT-378 or the phenotype of virus, although it is clear that the failure rates were higher in the subjects with greater antiviral experience. In Study M98-957, a substantially more difficult to treat population of multiply experienced HIV-infected subjects was evaluated. In that study, stepwise logistic regressions were performed with a variety of possible explanatory variables, including genotype, phenotype, pharmacokinetic parameters, and "inhibitory quotients" (IQ), which are ratios of a pharmacokinetic variable (*e.g.*, C_{min}, AUC, etc.) and the EC_{50} of the pretreatment viral isolate. Both phenotype and genotype were found to be predictive of Week 24 success. In a stepwise logistic regression using IQ ratios that

included genotype as an explanatory variable, but excluded phenotype (since it is contained in the IQ ratio), the trough to EC_{50} ratio was an important explanatory variable for durable suppression. Several other variables were found to also be important, such as viral load, subject weight, and nucleoside coverage. From the results of all of these studies, it has become evident that ABT-378 provides superb coverage of wild-type and minimally mutated virus, and that there is little correlation between pharmacokinetics and therapeutic success. However, with extensively mutated virus, a concentration-effect relationship is observed, at least as is reflected by the IQ:C_{trough} ratio. Undoubtedly, as more data become available, a stronger relationship will emerge, particularly if HIV-infected subject adherence, pharmacokinetics, and genotype are simultaneously considered.

2.6.10 Secondary Pharmacologic Concentration/Effect or Dose/Effect Relationships

Dose/concentration relationships were explored with the most commonly observed gastrointestinal adverse events and changes in cholesterol and triglyceride levels, which may potentially affect adherence, and long-term cardiovascular risk, respectively.

The incidence of diarrhea showed increased rates with increased dose within individual studies; however, no statistically significant dose group differences were observed. Also, no apparent difference was observed in the incidence of diarrhea between the antiretroviral-naïve and -experienced groups. The incidence of nausea was higher for treatment naive subjects who received ABT-378/ritonavir 400/200 mg than subjects who received 400/100 mg dose. In addition, across-study comparisons suggested that naïve subjects receiving ABT-378/ritonavir 400/200 mg dose tended to have higher incidence rates of nausea compared to experienced subjects receiving the same dose.

For the ABT-378 effects on cholesterol and triglyceride levels, it should first be recognized that serum samples were collected without regard to fasting in the Phase II ABT-378 trials. Nonetheless, elevations of cholesterol and triglyceride levels were observed in all studies. The dose-response as a function of time is found to be both complex and ambiguous, with a relatively rapid early increase followed by stabilization. An exploration of the concentration effect relationship based on M97-720 and M98-957

data found no apparent correlation between ABT-378 AUC and the maximum changes in cholesterol or triglyceride levels over an approximately 10-fold range of AUC values. These results indicate that the increase of lipid levels is mechanistically complex and intersubject variablity in concentrations explains only a very small fraction of total variance. Pharmacologic interventions with HMG-CoA reductase inhibitors, principally atorvastatin and cerivistatin, appeared to be effective in lowering cholesterol in subjects with very high cholesterol or triglyceride levels.

10. Virology Overview (1-38)

ABT-378/ritonavir

Documents for Application Summary

Section 2.7

Virology Overview

R&D/00/280

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2.7 Virology Overview

This overview summarizes the results contained in seven virology reports[1-7] covering both *in vitro* and *in vivo* virologic studies with ABT-378/ritonavir. The information contained in these reports provides the basis for understanding the virologic activity of ABT-378/ritonavir *in vivo* as well as the development of resistance to ABT-378/ritonavir and its implications.

2.7.1 Inhibition of HIV Protease by ABT-378

ABT-378 has been shown to be a potent inhibitor of HIV protease, with ≥200,000-fold selectivity over related human enzymes.

2.7.1.1 Enzymatic Inhibitory Potency and Selectivity of ABT-378.

The Ki values of ABT-378 and ritonavir for inhibition of recombinant wild type and mutant HIV proteases at pH 4.7 were determined in a fluorogenic assay (Table 1). At this pH, the potency of ABT-378 (1.0 pM) against the wild type enzyme was approximately 12-fold higher than that of ritonavir. The activities against two site-directed mutant proteases representing the initial steps of the ritonavir resistance pathway *in vivo* were also examined. Whereas the potency of ritonavir declined by 12- and 60-fold against the V82A and V82F mutant proteases, respectively, the activity of ABT-378 diminished only slightly (2- and 3-fold, respectively) and remained higher than that observed for ritonavir against the wild-type protease.

As a measure of selectivity, the inhibitory potency of ABT-378 against several human aspartic proteinases was examined. IC_{50} values for plasma renin, cathepsin D and cathepsin E were measured at >10,000 nM, 2000 nM and 200 nM, respectively, representing an ~200,000-fold selectivity for HIV-1 protease. Based on this high selectivity, it is unlikely that inhibition of human aspartic proteinases by ABT-378 *in vivo* is of any physiological consequence.

Table 1. Inhibition of Aspartic Proteinases by ABT-378

	Ki (nM)	
Enzyme	**ABT-378**	**Ritonavir**
Wild type HIV protease	0.0010	0.012
V82A mutant HIV protease	0.0022	0.14
V82F mutant HIV protease	0.0030	0.72
Human plasma renin	>10,000	>10,000
Human cathepsin D	2,000	20
Human cathepsin E	200	8

2.7.2 Antiviral Activity of ABT-378 *in Vitro*

The antiviral activity of ABT-378 *in vitro* has been evaluated against both laboratory strains of HIV and primary subject isolates. The effect of human serum on the *in vitro* activity of ABT-378 has also been investigated. Finally, the antiviral activity against mutant clones and isolates has been assessed.

2.7.2.1 Antiviral Activity of ABT-378 Against Wild Type HIV Laboratory Strains

The anti-HIV activity of ABT-378 against HIV-1_{IIIB} was evaluated in MT4 cells using cytopathic effect as an endpoint (Table 2). EC_{50} values (expressed below as mean ± standard deviation) were determined in the absence of human serum and in the presence of 50% human serum in order to gauge the effect of serum protein binding on the antiviral activity (each assay also contained 10% fetal calf serum). In the presence of 50% human serum, the EC_{50} of ABT-378 (0.102 µM) was 6-fold higher than in the absence of human serum. By comparison, the EC_{50} of ritonavir increased by 18-fold in the presence of 50% human serum. Thus, in the presence of human serum, the antiviral potency of ABT-378 was 10-fold higher than that of ritonavir.

Table 2. Antiviral Activity of ABT-378 Against HIV-1_{IIIB} *in Vitro*

		Mean EC_{50} (μM)	
Inhibitor	**n**	**0% Human Serum**	**50% Human Serum**
ABT-378	10	0.017 ± 0.004	0.102 ± 0.044
Ritonavir	10	0.058 ± 0.014	1.044 ± 0.306

In separate experiments, the antiviral activity of ABT-378 against five laboratory HIV-1 strains (pNL4-3, HXB2, HIV-1_{IIIB}, HIV-1 RF and HIV-1 MN) was determined in MT4 cells. The mean EC_{50} in the absence of human serum ranged from 10 to 27 nM with an average EC_{50} against the five strains of 19 nM. In the presence of human serum, the average EC_{50} of ABT-378 increased by 9-fold to 163 nM (range 65-289 nM). ABT-378 displayed equal activity against both HIV-1 and HIV-2, with EC_{50} values of 25 and 104 nM against one laboratory HIV-2 strain (HIV-2 MS) in the absence or presence of 50% human serum, respectively.

The cytotoxic effect of ABT-378 in MT4 cells uninfected with HIV was also assessed. The $CCIC_{50}$ value for ABT-378 in the absence of human serum was 22 ± 7 μM (n = 10). The *in vitro* selectivity index ($CCIC_{50}/EC_{50}$) of ABT-378, calculated using the EC_{50} value from Table 2, was 1294.

2.7.2.2 Antiviral Activity of ABT-378 Against Wild Type Clinical Isolates

The antiviral activity of ABT-378 against HIV isolated from subjects prior to ritonavir therapy was determined by PBMC coculture using p24 antigen production as an endpoint (no human serum was added to this assay). The average EC_{50} for ABT-378 for 6 isolates was 6.5 nM. The average EC_{50} for ritonavir for the same six isolates was 20 nM.

2.7.2.3 Activity Against HIV Isolated from Subjects on Ritonavir Therapy

The activity of ABT-378 was examined against viral isolates from subjects who experienced a rebound in plasma HIV RNA during ritonavir monotherapy. EC_{50} values

against selected strains are provided in Table 3. In general, multiply mutant strains with *in vitro* resistance to ritonavir (RTV) also displayed reduced susceptibility to ABT-378, but to a lesser degree.

Table 3. Activity of ABT-378 Against Mutant HIV from Subjects on Therapy with Ritonavir

			EC_{50} (nM)	
Pt. No.	**Day**	**Resistance Mutations in Sequence**	**ABT-378**	**RTV**
129	-1	E35D, N37S, L63P	5	24
129	140	K20K/R, E35D, M36M/I, I54V, A71A/V, V82T, V77V/G	29	677
131	-28	L63P, I93T	4	18
131	200	K20K/R, E35D, M36I, I54I/V, L63P, V82A	52	731
224	-6	E21Q, R41G, F53F/L	6	29
224	190	I13G/T/V, K20K/N/R, E21Q, L24L/S, L33L/F, M36M/I, L38W, R41S, I47I/R, I54V/M, V82S	52	496
313	1	L63P	4	12
313	85	I13V, G16E, M36M/I, I54I/V, Q61Q/H, L63P, V82S/F/A/T	25	274
410	1	R41K, L63P	6	29
410	85	L33I, E35D, M36I, N37I, I54V, L63P, V82A, N88I	97	545

2.7.3 *In Vitro* Selection of HIV Resistant to ABT-378

2.7.3.1 Selection for ABT-378 Resistant HIV-1 by *in Vitro* Passage of ABT-378 Alone and ABT-378/Ritonavir Mixtures

HIV variants resistant to ABT-378 *in vitro* were selected by serial passage of the pNL4-3 strain in MT 4 cells in the presence of increasing concentrations of ABT-378 alone (experiments C and D) and two fixed concentration ratios (5/1 and 15/1) of ABT-378/ritonavir (experiments A and B, respectively). Virus was initially grown in the presence of 1 nM (selection A-C) or 20 nM (selection D) of ABT-378 (passage P1), and during the course of the 5-month selection procedure, the concentration of ABT-378 was gradually increased to 3000- 4000 nM (passage P17-26). Similarly, 0.2 and 0.067 nM concentrations of RTV were used in passage 1 of selection experiments A (A1) and B (B1) respectively, and the concentrations were then gradually increased to keep constant ratios of ABT-378/ritonavir (5/1=A and 15/1=B) at each successive passage. The phenotypic susceptibilities of some of the passaged variants to ABT-378 and RTV are provided in Table 4. The susceptibility to both inhibitors decreased with successive passages in each experiment, irrespective of whether RTV was present in the passage experiment. In the two selection experiments that included RTV (A, 5/1 and B, 15/1), the EC_{50} for RTV against each passaged virus was > 8-fold higher than the concentration of RTV used at each corresponding passage (Tables 3 & 4), indicating little or no selective pressure by RTV in the selection experiments.

Table 4. Susceptibility of the Passaged Variants to ABT-378 and Ritonavir

	Passage Concentration (μM)		Mean EC50 μM (Fold Change)	
Virus	ABT-378	Ritonavir	ABT-378	Ritonavir
pNL4-3	n/a	n/a	0.06 (1)	0.16 (1)
A11	0.72	0.144	0.68 (11)	1.51 (9)
A17	1.5	0.3	≥ 3.31(≥ 55)	≥ 8.1 (≥ 51)
A25	4.0	0.8	4.02 (67)	6.05 (38)
B11	0.72	0.048	0.49 (8)	2.21 (14)
B17	1.5	0.1	≥ 3.06 (≥ 51)	> 8.33 (> 52)
B26	4.0	0.267	> 4.2 (> 70)	> 8.33 (> 52)
C11	0.72	0	0.55 (9)	1.18 (7)
C24	4.0	0	3.85 (64)	3.8 (24)

2.7.3.2 Sequence Analysis of Variants Selected *in Vitro* by ABT-378

A compilation of the common amino acid substitutions observed following selected passages is shown in Table 5. Both selection A (passage with 5/1 of ABT-378/ritonavir) and D (passage with ABT-378 alone) produced a similar pattern of mutations. At passage 6, the I84V mutation emerged and remained present in all clones sequenced after passage 7 (A7, D7). Following this change, a sequential accumulation of mutations to produce the I84V-L10F-M46I-V32I-I47V-Q58E and I84V- L10F-M46I-T91S/T-V32I-I47A-G16E/G-H69Y/H genotypes was observed in selection experiments A (A9 to A25) and D (D6 to D25), respectively. Thus, although the variants from selection A and D were generated with ABT-378 either in the presence of ritonavir (A, 1:5 ritonavir:ABT-378) or alone (D), they shared the same initial mutation (I84V) and 4 other mutations (L10F, M46I, V32I, and I47V). Likewise, selection with 15/1 of ABT-378/ritonavir (selection B) and a second passage with ABT-378 alone (selection C) also generated the same initial mutations: I50V/M46I. These two mutations were retained in the subsequent passages of

both selection B (B9-B26) and C (C9-C24). However, they were followed in subsequent passages by a different set of mutations (Table 5). The differences between the observed patterns of mutations in the four passage experiments is likely to reflect the stochastic nature of *in vitro* selection rather than specific causative effects of low concentrations of ritonavir present in two of the experiments.

Table 5. Common Mutations Observed During *in Vitro* Selection with ABT-378 and ABT-378/ritonavir

Virus	Mutations in HIV Protease
A11	I84V, L10L/F, M46M/I, V32V/I
A17	I84V, L10F, M46I, V32I, I47V, Q58E
A25	I84V, L10F, M46I, V32I, I47V, Q58E
B11	I50I/V, M46M/I, V82V/L, K45K/I
B17	I50V, M46I, A71V, V82F
B26	I50V, M46I, A71V, V82F, L33F, I54V, K45I
C11	I50V, M46I
C17	I50V, M46I, L10F, I47V
C24	I50V, M46I, L10F, I47V, I32V, E34Q, Q61H, E65Q
D11	I84V, L10F, M46I, T91S/T
D17	I84V, L10F, M46I, T91S/T, I47A, G16E
D25	I84V, L10F, M46I, T91S/T, V32V/I, I47A, G16G/E, H69Y/H

2.7.3.3 Susceptibility of Mutant Clones to Protease Inhibitors

Viral clones with specific mutations that were observed during selection experiments A, B, and C were constructed, and their susceptibility to ABT-378 and other protease inhibitors was evaluated. All constructed mutants (A25-clone 2, B26-clone 12 and C24-clone 1) that contained more than 5 mutations with either I84V or I50V as a primary mutation were highly resistant to both ABT-378 and RTV, with EC_{50} values ranging from

27- to 49-fold and 9- to 72-fold, respectively, above those for wild type virus. These viruses were also highly cross-resistant to amprenavir (APV) (42- to 65-fold), and of intermediate resistance to indinavir (IDV) and nelfinavir (NFV) (ranging from 2- to 18-fold). However, all mutant clones remained susceptible to saquinavir (SQV).

2.7.4 Characterization of Phenotypic Cross-Resistance between ABT-378 and Other Protease Inhibitors *in Vitro*

The relative potencies of ABT-378 and other protease inhibitors have been compared against two different sets of subject isolates. The first set consisted of the combined baseline isolates from Study M97-765 (single PI-experienced subjects) and a panel of viral isolates selected by VIRCO. The R^2 values for comparisons of the log fold EC_{50} of RTV or IDV *vs.* that of ABT-378 were high (0.70 and 0.64, respectively), indicating a high correlation of phenotypes. In contrast, the correlation of fold EC_{50} between ABT-378 and either SQV or NFV was low (0.29 and 0.26, respectively). These results suggest that the patterns of phenotypic susceptibility to ABT-378 resemble those of RTV and IDV more closely than those of NFV or SQV. This observation was confirmed in a second analysis of a panel of 56 viral isolates from multiple PI-experienced subjects (Study M98-957 baseline isolates). As shown in Figure 1, the correlation of fold EC_{50} of ABT-378 against the viruses in this panel with the fold EC_{50} of RTV and IDV was very high ($R^2 = 0.90$ and 0.82, respectively). As with the previous panel, the correlation between susceptibility to ABT-378 and to either NFV or SQV ($R^2 = 0.68$ and 0.55, respectively) was significantly lower than to RTV or IDV. Finally, in this panel the phenotypic pattern of susceptibility to ABT-378 also varied substantially from that of the pattern of susceptibility to APV ($R^2 = 0.58$).

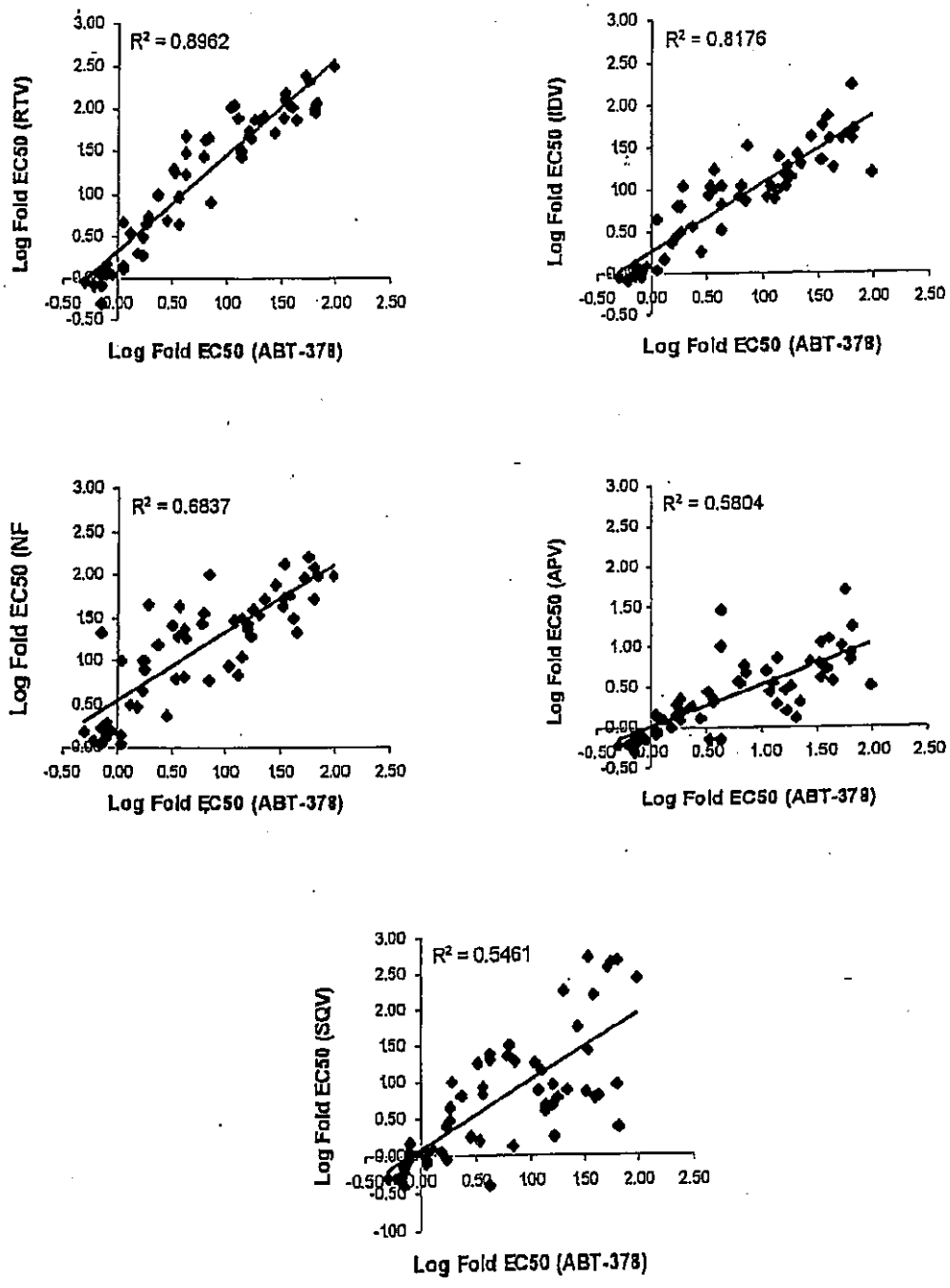

Figure 1. Relative Phenotype of M98-957 Baseline Viruses to ABT-378 and Other Protease Inhibitors

2.7.5 Correlation of Genotype and Reduced Phenotypic Susceptibility to ABT-378

The association of genotypic changes in HIV protease with reduced *in vitro* susceptibility to ABT-378 was explored using a panel of 112 viral isolates from subjects failing PI therapy. This panel consisted of all of the baseline isolates from Study M97-765 (single PI-experienced subjects) and Study M98-957 (multiple PI-experienced subjects) for which HIV protease sequence and phenotypic susceptibility to ABT-378 were available. The EC_{50} of ABT-378 against this panel ranged from 0.6- to 95.8-fold compared to that against the wild-type standard virus. Characterization of the isolates from the two studies is presented in Table 6.

Table 6. Characterization of the Baseline Viral Isolates Used in the Correlation of Genotype and Phenotypic Susceptibility to ABT-378

	Study M97-765	Study M98-957
Number of baseline isolates included	56	56
Median number of previous PIs	1	3
Mean fold EC_{50} (ABT-378)	2.8	16.1
Median fold EC_{50} (ABT-378)	1.1	5.2
Range of fold EC_{50} (ABT-378)	0.7 – 25.9	0.6 – 95.8

2.7.5.1 Identification of Mutations in HIV Protease Associated with Reduced *in Vitro* Susceptibility to ABT-378

Two statistical tests (Wilcoxon Rank Sum Test and ANOVA), both of which consider the EC_{50} as a continuous response variable, were used to compare the phenotype of viruses containing a mutation at a particular amino acid position with the phenotype of viruses with the wild-type sequence at that position. Using both of these methods, the same eleven amino acid positions in HIV protease (10, 20, 24, 46, 53, 54, 63, 71, 82, 84 and 90) were found to be statistically correlated with reduced *in vitro* susceptibility to ABT-378. Since the sequence at some positions can mutate to more than one different amino

acid, the specific amino acid mutations found within the panel were assigned based on literature reviews and searches of three online databases as either being 'likely to contribute' to reduced susceptibility to ABT-378 or of 'unknown contribution'. Following the identification of the subset of amino acid mutations assigned as 'likely to contribute', these data were reanalyzed using the Wilcoxon Rank Sum Test and ANOVA considering only this subset of specific amino acid mutations. The mutations associated with reduced *in vitro* susceptibility to ABT-378 by this method, along with p-values for each association, are provided in Table 7.

Table 7. Median Fold EC_{50} Against Viral Isolates Containing Mutations Associated with Reduced *in Vitro* Susceptibility to ABT-378

	Isolates Containing Mutation			Isolates Lacking Mutation			p-value	
		Fold EC_{50}			Fold EC_{50}			
Mutations	n	Median	Mean	n	Median	Mean	Wilcoxon	ANOVA
L10F, I, R or V	69	4.6	14.5	43	0.9	1.4	<0.0001	<0.0001
K20M or R	14	30.5	29.3	98	1.6	6.6	0.0001	<0.0001
L24I	10	14.0	26.7	102	1.7	7.8	0.0011	0.0008
M46I or L	41	4.2	15.8	71	1.1	5.8	0.0003	0.0002
F53L	13	4.6	24.6	99	1.6	7.5	0.0019	0.0014
I54L, T or V	38	16.0	23.7	74	1.1	2.1	<0.0001	<0.0001
L63P	88	2.5	11.5	24	1.1	2.1	0.0026	0.0023
A71I, L, V or T	65	3.6	11.2	47	1.1	7.1	0.0002	0.0001
V82A, F or T	40	14.0	21.5	72	1.1	2.8	<0.0001	<0.0001
I84V	19	6.1	14.8	93	1.4	8.4	0.0007	0.0004
L90M	48	3.4	12.4	64	1.1	7.3	0.0004	0.0003

2.7.5.2 Analysis of *in Vitro* Susceptibility to ABT-378 with Respect to the Number of Mutations

The susceptibility of the combined panel of isolates, expressed as the log of the fold EC_{50} of ABT-378, as a function of the number of mutations at the eleven amino acid positions in HIV protease identified above as 'likely to contribute to reduced susceptibility' to ABT-378 ('ABT-378 mutation score') is shown in Figure 2. One or more primary mutations at positions 82, 84 or 90 occurred in 71/73 (97%) of the isolates containing 3 or more mutations. Linear regression analysis for the subset of isolates containing 2 or more mutations provided a model for the estimation of phenotype based on genotype. The estimated slope for the regression model was 0. 241 log fold EC_{50}/mutation (95% CI 0.201, 0.280), providing the algorithm $1.74^{(\text{ABT-378 mutation score}-2)}$ for the estimation of phenotype.

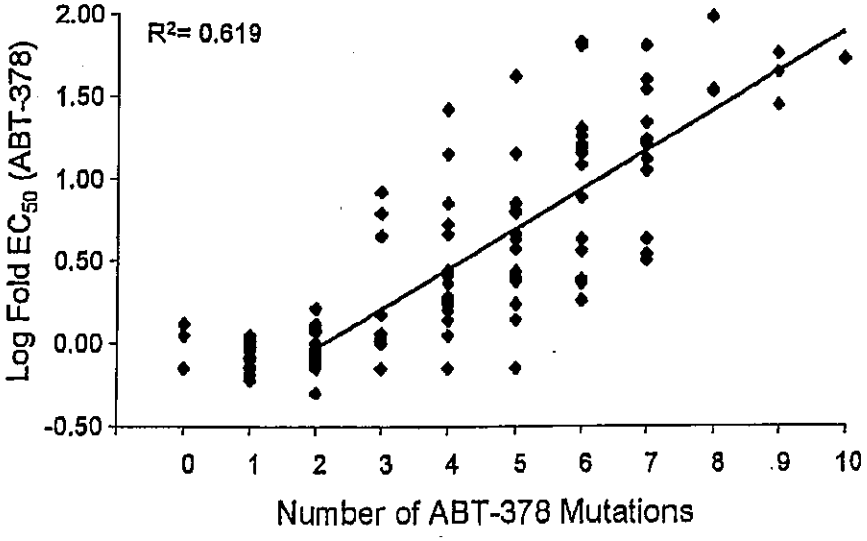

Figure 2. Log Fold EC_{50} of ABT-378 with Respect to the Number of Mutations Associated with Reduced *in Vitro* Susceptibility to ABT-378

The median fold EC_{50} values of ABT-378 *in vitro* against the combined panel with respect to the number of mutations associated with reduced susceptibility to ABT-378 are

shown in Figure 3. Against isolates with 0-3, 4-5, 6-7 and 8-10 mutations, the median EC_{50} was 0.8-, 2.7- 13.5- and 44.0-fold higher than the EC_{50} against wild type HIV, respectively. The 16 viruses that displayed >20-fold change in susceptibility all contained mutations at positions 10, 54, 63 plus 82 and/or 84. In addition, they contained a median of 3 mutations at amino acid positions 20, 24, 46, 53, 71 and 90.

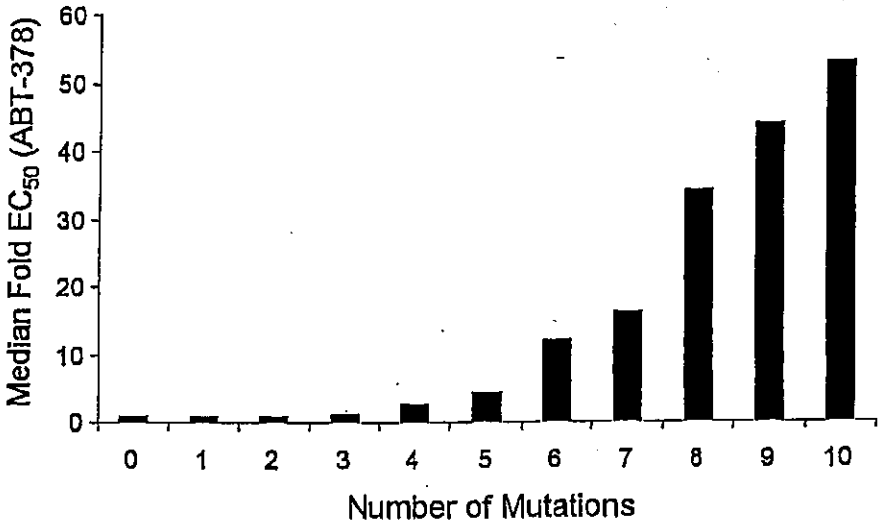

Figure 3. Median Fold EC_{50} of ABT-378 with Respect to the Number of Mutations Associated with Reduced *in Vitro* Susceptibility to ABT-378

2.7.6 Virologic Response of PI-experienced Subjects to Therapy with ABT-378/ritonavir with Respect to Baseline Phenotype and Genotype

Retrospective studies with protease inhibitor regimens have determined that a 2- to 4-fold change in baseline phenotype, compared to wild-type HIV, and/or the presence of 2-3 mutations in HIV protease is associated with a diminished response to salvage therapy with PI-containing regimens. [8-12] These relatively low susceptibility breakpoints for PI regimens that have been studied are likely to be a consequence of relatively low

inhibitory quotients (C_{trough} /EC_{50} ratios) for many PIs. Given the high, sustained ABT-378 plasma concentrations in subjects, the determination of phenotypic and genotypic susceptibility breakpoints for ABT-378/ritonavir is important in order to provide for the accurate interpretation of HIV resistance tests. Accordingly, the virologic response to ABT-378/ritonavir therapy with respect to baseline phenotype and genotype has been examined both in single (Study M97-765) and multiple (Study M98-957) PI-experienced subjects. The results of these studies have not only established the basis for interpretation of HIV resistance testing, but also provide valuable insight into the genetic barrier to *in vivo* resistance to ABT-378/ritonavir.

2.7.6.1 Virologic Response in Single PI-experienced Subjects (Study M97-765)
Study M97-765 enrolled 70 subjects with plasma HIV RNA between 1,000 and 100,000 copies/mL on PI-based triple therapy at the time of screening for this study. At baseline, samples were collected for analysis of genotype and phenotype. During the first two weeks of the study, only the PI in the previous regimen was switched to ABT-378/ritonavir. After the Week 2 visit, nevirapine was added to the regimen, and the NRTI component of the regimen was switched to contain at least one new NRTI. Baseline phenotype and protease genotype data were available for 57/70 and 60/70 subjects, respectively. Among the 57 subjects for whom baseline phenotype was available, the identity of the PI at study entry was as follows: IDV—24 (42%), NFV—21 (37%), SQV—9 (16%), RTV—3 (5%).

All but two baseline viruses displayed a quantifiable (≥4-fold) change in susceptibility to at least one of the drugs in the previous regimen. The median EC_{50} of the previous PI against the baseline isolates was 7.6-fold compared to wild type HIV, and 36/57 (63%) of baseline viruses displayed a ≥4-fold change in susceptibility to the previous PI. Changes in phenotype to the RTIs of the previous regimen were even more evident: 50/56 (89%) of baseline viruses displayed a ≥4-fold change in susceptibility to at least one previous NRTI, and 12/56 (21%) displayed a ≥4-fold change in susceptibility to two previous

NRTIs. The mean fold change in EC_{50} of ABT-378 was 2.8-fold, and 11/57 (19%) of baseline viruses displayed a ≥4-fold change in susceptibility compared to wt virus. The baseline protease genotypes were characterized according to the specifications of the Data Analysis Plan (DAP) of the HIV Resistance Collaborative Working Group. Using this definition, the number of PI mutations in the baseline isolates ranged from 0 to 6. Approximately one third of baseline viruses showed little or no evidence of genotypic resistance to PIs. The remainder contained a median of 4 mutations associated with resistance to the PI class. A median of 2.5 NRTI-associated mutations was found in this set of baseline viral isolates.

2.7.6.1.1 Analysis of Week Two HIV RNA Response in the Context of Baseline Phenotype

The HIV RNA decline between baseline and Week 2 for 54 subjects who completed two weeks of therapy in Study M97-765 is plotted in Figure 4 as a function of the fold change in baseline EC_{50} of ABT-378 (Week 2 RNA values of <400 copies/mL were scored as 400 copies/mL). There was no observed relationship between initial decline in viral load and baseline phenotype (Spearman correlation coefficient = 0.099, p = 0.475). The mean and median viral load decline in the 43 subjects with susceptible (<4-fold change in EC_{50}) baseline viruses (–1.21 and –1.25 log copies/mL, respectively) was not different from that observed in the 11 subjects whose baseline viruses showed a ≥4-fold loss of susceptibility to ABT-378 (–1.11 and –1.19 log copies/mL, respectively).

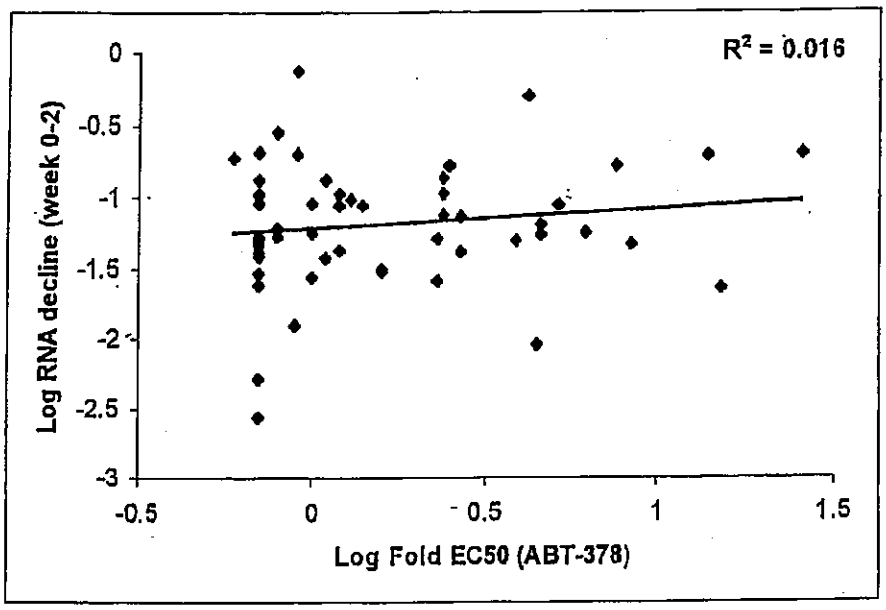

Figure 4. Week 2 HIV RNA Decline as a Function of Fold Change in EC_{50} of ABT-378

2.7.6.1.2 Analysis of Week 24 and 48 HIV RNA Response in the Context of Baseline Phenotype and Genotype

The Week 24 and Week 48 virologic response in Study M97-765, with respect to the baseline phenotype and genotype, was also examined. This analysis considered not only baseline parameters relating to ABT-378/ritonavir but also to the other drugs in the treatment regimen at these time points. In general, the retrospective multivariate analyses followed the guidelines of the DAP, and response was analyzed using both 'dropouts as censored' (DAC) and 'dropouts as failure' (DAF) definitions. Baseline HIV RNA was found to be most commonly associated with virologic response to <400 copies/mL. This association was stronger at Week 24 than at Week 48, and was similar using both DAC and DAF definitions of response. In contrast, the Week 24 response to <50 copies/mL was not predicted by baseline viral load. Instead, response at this level was found to be associated with the number of new drugs in the regimen and the number of baseline

NRTI mutations, using both univariate and multivariate models. Importantly, the response at both time points was not diminished in those subjects whose baseline viruses displayed greater than 4-fold reduced susceptibility to ABT-378, using an HIV RNA cut-off of either 400 or 50 copies/mL (Table 8). The results of the Week 24 and 48 analyses, as well as those of the Week 2 analysis above, strongly suggest that the clinically relevant susceptibility breakpoint(s) for ABT-378 are much higher than 4-fold, compared to wild-type HIV.

Table 8. Virologic Response in Study M97-765 with Respect to Baseline Phenotype to ABT-378

Week	HIV RNA Cut-off (copies/mL)	Virologic Response (percent) <4-Fold Change in Baseline EC_{50}	Virologic Response (percent) ≥4-Fold Change in Baseline EC_{50}	p-value
24	400	37/43 (86%)	11/11 (100%)	0.96
	50	31/43 (72%)	8/10 (80%)	0.61
48	400	34/41 (83%)	8/10 (80%)	0.83
	50	28/41 (68%)	6/10 (60%)	0.62

Similarly, there was no relationship between the number of baseline mutations associated with PI resistance and the virologic response at Week 24 and Week 48 in Study M97-765, in spite of the fact that 40% of the baseline viruses contained 4-6 of those mutations (Table 9). This lack of correlation suggests that the genotypic breakpoint for clinical response to ABT-378/ritonavir is greater than 4 or 5 mutations. Further, these results suggest that viral isolates from subjects with even more antiretroviral treatment experience than those subjects enrolled in M97-765 will be required to determine both the genotypic and phenotypic susceptibility breakpoints.

Table 9. Virologic Response in Study M97-765 with Respect to the Number of Mutations Associated with Protease Inhibitor Resistance

	Virologic Response			
HIV RNA Cut-off	400 copies/mL		50 copies/mL	
No. of Mutations	Week 24	Week 48	Week 24	Week 48
0	14/18	13/18	13/18	11/18
2	9/9	8/8	7/9	7/8
3	7/7	5/7	5/7	5/7
4	8/8	7/8	7/8	6/8
5	10/12	9/11	8/12	6/11
6	3/3	2/2	2/2	½
Total	51/57	44/54	42/56	36/54
p-value	0.25	0.38	0.79	0.82

2.7.6.2 Virologic Response in Multiple PI-experienced Subjects (Study M98-957)

The virologic response in Study M98-957 was also examined with respect to baseline genotype and phenotype. Study M98-957 is an ongoing open-label Phase I/II study of the antiviral activity of ABT-378/ritonavir plus efavirenz (EFV) and NRTIs in multiple PI-experienced, NNRTI-naïve subjects with plasma HIV RNA > 1,000 copies/mL. The median number of prior PIs, prior NRTIs and prior antiretroviral agents was 3, 4 and 7.5, respectively, and 16/57 subjects (29%) had previous treatment with 4 PIs. After 24 weeks of therapy, plasma HIV RNA <400 copies/mL was observed in 86 % of subjects (on treatment analysis). Phenotype and genotype were obtained on the baseline isolates from 56/57 subjects. The previous PI experience among those 56 patients was the following: IDV—86%, RTV—77%, SQV—71%, NFV—57%.

The 56 baseline viruses displayed quantifiably (>2.5-fold) reduced susceptibility to a median of 5 PIs, and 46 (82%) isolates showed reduced susceptibility to at least one PI. The susceptibilities of the baseline isolates to ABT-378 ranged from 0.6- to 96-fold

compared to the standard wild-type virus. The median and mean EC_{50} values of ABT-378 were 5.2-fold and 16.2-fold, respectively. The frequency of >10-fold, >20-fold and >40-fold reduced susceptibility to ABT-378 was 24/56 (43%), 15/56 (27%) and 8/56 (14%), respectively. In general, broad *in vitro* resistance to the PI class correlated with substantially reduced susceptibility to ABT-378. Thus, all but two of the 24 isolates that displayed >10-fold loss of susceptibility to ABT-378 also displayed >10-fold reduced susceptibility to at least 3/5 of the remaining PIs tested. In addition to displaying substantial *in vitro* phenotypic resistance, the majority of the baseline isolates contained multiple mutations associated with PI resistance (Figure 5).

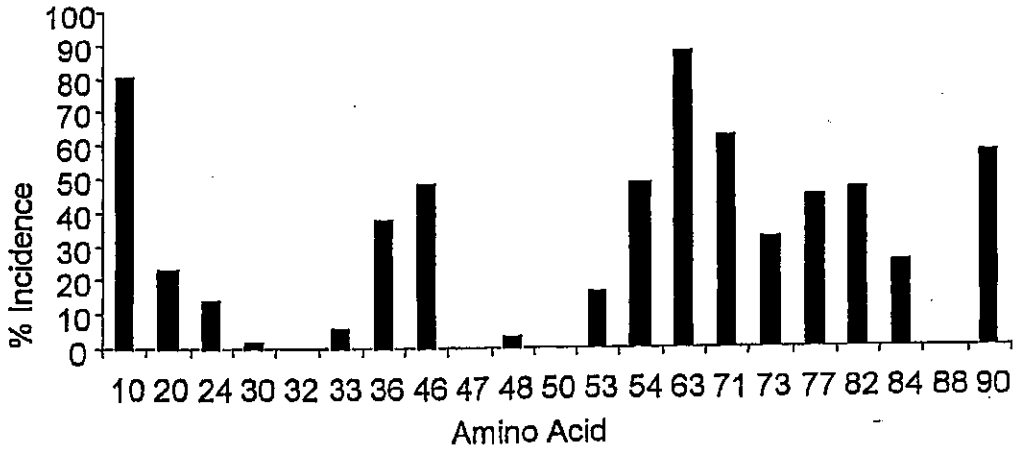

Figure 5. Incidence of Mutations Associated with Reduced Susceptibility to Protease Inhibitors among the M98-957 Baseline Viral Isolates

2.7.6.2.1 Analysis of Virologic Response at Week 24 in the Context of Baseline Phenotype and Genotype

Two statistical approaches to analyzing the virologic response at Week 24 in Study M98-957 were employed: univariate analysis with respect to baseline phenotype and genotype, and stepwise logistic regression of the above parameters plus 17 additional covariates. A

total of 52 of the 56 subjects for whom baseline data were available qualified for the Week 24 analysis by virtue of completing at least 8 weeks of ABT-378/ritonavir therapy. Of these 52 subjects, 42 (81%) had HIV RNA <400 copies/mL at Week 24 and 10 (19%) had experienced virologic failure.

Virologic response in Study M98-957 was clearly related to both baseline phenotype and genotype, as summarized in Table 10. Uniformly high response (27/29, 93%) was observed among subjects whose baseline isolate displayed <10-fold reduced *in vitro* susceptibility to ABT-378, compared to wild type HIV. The response rate diminished somewhat in those whose baseline viral susceptibility to ABT-378 was either 10- to 20-fold or 20- to 40-fold (78% and 67%, respectively). A further decrement in the response rate (50%) was observed among those subjects with baseline isolates with >40-fold reduced *in vitro* susceptibility to ABT-378 (p=0.019, Fisher's exact test for the 2X4 table of response versus baseline fold EC_{50}). Virologic response was also related to baseline genotype. In particular, a high response rate (24/25, 96%) was observed among subjects whose baseline isolate contained five or fewer of the eleven mutations associated with reduced *in vitro* susceptibility to ABT-378 (amino acid positions 10, 20, 24, 46, 53, 54, 63, 71, 82, 84 and 90 in HIV protease). The response rate diminished in those subjects with six or seven baseline mutations (76%), and a substantially lower rate (33%) was observed in the six subjects whose baseline isolates contained 8 or more mutations associated with reduced susceptibility to ABT-378 (p=0.0024, Fisher's exact test for the 2X3 table of response versus number of baseline mutations)

Table 10. Virologic Response at Week 24 in Study M98-957 with Respect to Baseline Phenotype and Genotype

Baseline Fold EC_{50} (ABT-378)	Response Rate
<4-fold	21/23 (91%)
4- to 10-fold	6/6 (100%)
10- to 20-fold	7/9 (78%)
20- to 40-fold	4/6 (67%)
>40-fold	4/8 (50%)
Number of Baseline Mutations	**Response Rate**
0 to 5	24/25 (96%)
6 or 7	16/21 (76%)
8 to 10	2/6 (33%)

Univariate logistic regression models of the virologic response at Week 24 as a function of baseline phenotype and genotype were constructed. The estimated response produced by these analyses are shown in the solid lines in Figures 6 and 7, respectively, with corresponding 95% confidence intervals (dashed lines). Point estimates for virologic response for 4-fold, 10-fold, 20-fold and 40-fold reduced susceptibility to ABT-378 at baseline were 90%, 81%, 72% and 59%, respectively. Similarly, point estimates for virologic response for 3, 5, 7 and 9 baseline mutations were 96%, 88%, 70% and 43%, respectively. Using a conservative measure of response (i.e., the lower 95% confidence limit), the probability of response was at least 50% with baseline susceptibilities up to 23-fold above that of wild type HIV. Similarly, the probability of response was at least 50% or greater in subjects with up to 7 baseline mutations (out of the 11 that are associated with reduced susceptibility to ABT-378).

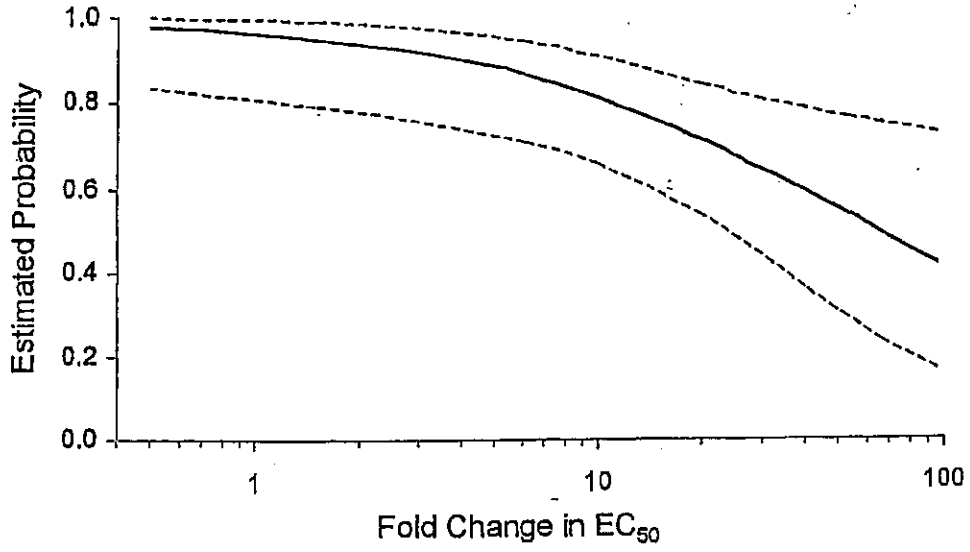

Figure 6. **Plot of Predicted Probability of Virologic Response at Week 24 with Respect to Log Baseline Phenotypic Susceptibility to ABT-378**

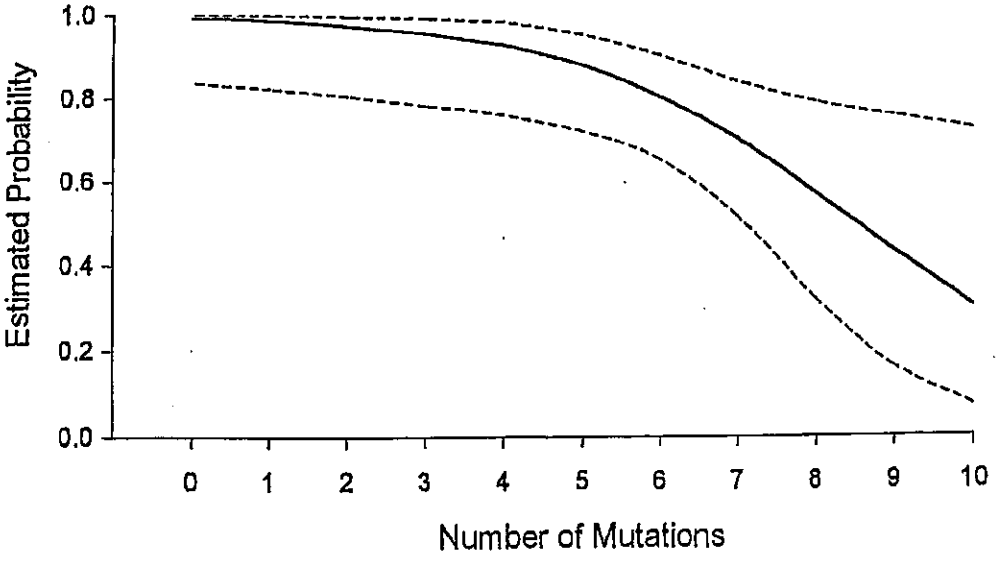

Figure 7. **Plot of Predicted Probability of Virologic Response at Week 24 with Respect to the Number of Baseline Mutations Associated with Reduced Susceptibility to ABT-378**

In order to assess the effect of baseline phenotype and genotype in the context of other baseline parameters that might be expected to impact virologic response, we also performed a stepwise logistic regression using a total of 19 parameters. These included demographic factors (age, gender, weight), medical and treatment history (time since HIV diagnosis, time since the initiation of PI therapy, number of previous antiretroviral agents, number of previous NRTIs, number of previous PIs), immunological factors (CD4 level, CD8 level), baseline virological parameters [HIV RNA, log fold ABT-378 EC_{50}, log fold EFV EC_{50}, NRTI phenotypic susceptibility (2.5-fold cut-off), number of ABT-378 mutations, NRTI genotypic susceptibility score and NNRTI genotypic susceptibility score], the number of new NRTIs in the treatment regimen, and ABT-378/ritonavir dose. The baseline phenotypic susceptibility to ABT-378 entered the regression model first and remained in the model at all subsequent evaluations. This variable was most closely associated with the Week 24 virologic response ($p = 0.0161$) in the "final" model. Other variables constituting the final model were the NRTI phenotypic susceptibility score (2.5-fold cut-off), years since HIV+ diagnosis, baseline weight and the number of new NRTIs. Since the phenotypic susceptibility to ABT-378 and the number of ABT-378 mutations are highly interrelated, the two parameters could not co-exist within the stepwise logistic regression model. Therefore, to assess the effect of baseline protease genotype, a second stepwise logistic regression, excluding the baseline ABT-378 phenotype, was performed. In this case, the number of ABT-378 mutations entered the model first and remained in the model as a significant predictor of Week 24 response. As with the previous model, the years since HIV+ diagnosis, NRTI phenotypic susceptibility score, baseline weight and the number of new NRTIs were also associated with response.

2.7.7 Analysis of the Genotype and Phenotype of Isolates from Subjects Experiencing Rebound in HIV RNA on Therapy with ABT-378/ritonavir

To investigate the selection of resistance *in vivo* by ABT-378/ritonavir, the genotype and phenotype of 23 post-rebound viral isolates were examined for those subjects in three

Phase I/II clinical trials (Studies M97-720, M97-765 and M98-957) who experienced a sustained rebound in plasma HIV RNA to >400 copies/mL while on therapy with ABT-378/ritonavir. Study M97-720 examined the activity of ABT-378/ritonavir plus stavudine (d4T) and lamivudine (3TC) in antiretroviral-naïve subjects. Study M97-765 examined the activity of ABT-378/ritonavir plus nevirapine (NVP) plus two nucleoside reverse transcriptase inhibitors (NRTIs) in single PI-experienced, non-nucleoside RT inhibitor (NNRTI)-naïve subjects. Study M98-957 examined the activity of ABT-378/ritonavir plus efavirenz (EFV) plus NRTIs in multiple PI-experienced, NNRTI-naïve subjects.

2.7.7.1 Analysis of Rebound Samples from Study M97-720

Rebound isolates were examined from 4 subjects in Study M97-720. No detectable change in susceptibility to ABT-378 and no new mutations in HIV protease, compared to the baseline isolates, were observed. In contrast, evidence of the evolution of resistance to 3TC (M184V mutation in reverse transcriptase) was found in the rebound isolates from two subjects from Study M97-720. Concurrently, a change in phenotypic susceptibility to 3TC (3.3- and 39.1-fold, respectively) was also observed.

2.7.7.2 Analysis of Rebound Samples from Study M97-765

The rebound isolates from 9/10 subjects examined from Study M97-765 showed evidence of resistance development to at least one drug in the study regimen. Within the rebound viruses of these 9 subjects two genotypic and phenotypic patterns were observed. In 5 subjects, there was no detectable development of resistance to ABT-378 during plasma HIV RNA rebound. Baseline genotype and phenotype were available for 4/5 of these subjects; rebound viruses from all five were characterized. The 4 baseline viruses were fully susceptible to ABT-378 (EC_{50} ranged from 0.7- to 1.3-fold, compared to wt HIV). Following rebound, there was no detectable change in susceptibility to ABT-378 from that of the baseline isolates. This lack of change in phenotype was corroborated by the protease genotype. The four available baseline isolates contained 3 or fewer mutations associated with PI-resistance (2 or fewer of the 11 mutations associated with

reduced susceptibility to ABT-378). No new mutations associated with resistance to PIs appeared following rebound. However, the rebound isolates from all 5 subjects displayed high-level phenotypic resistance to NNRTIs and contained 1-3 mutations in reverse transcriptase associated with NNRTI resistance.

In contrast, the protease sequence in each of the baseline isolates from the remaining 4 subjects contained 4 of the 11 mutations associated with reduced *in vitro* susceptibility to ABT-378. Moreover, the baseline viruses from 3 of the 4 subjects also displayed >8-fold reduced susceptibility to NVP, and one contained the K103N mutation in reverse transcriptase. The mean baseline EC_{50} values for ABT-378 and NVP against the 4 baseline isolates were 8.6 and 11.6-fold, respectively, compared to wt. Following rebound, the mean EC_{50} of ABT-378 against the isolates that could be cultured (from 3/4 subjects) was 27.8-fold compared to wt HIV. Concurrent with the increase in the EC_{50} of ABT-378, new mutations associated with reduced *in vitro* susceptibility to ABT-378 and/or resistance to other PIs (positions 10, 24, 33, 36, 46, 54, 63 and 82 in HIV protease) appeared in the sequences of the rebound isolates. High-level phenotypic resistance (mean >50.9-fold) to NVP was also observed in the rebound isolates along with mutations associated with NNRTI resistance.

2.7.7.3 Analysis of Rebound Samples from Study M98-957

The baseline isolates from 8/9 subjects examined from Study M98-957 displayed >10-fold (range 12- to 96-fold) reduced susceptibility to ABT-378 and contained a median of 7.5 (range 6-9) of the 11 mutations associated with reduced susceptibility to ABT-378. Following rebound, either no or marginal changes in ABT-378 susceptibility, compared to the matched baseline isolates, were observed in those 8 subjects. Concurrently, there was little evidence of evolution in the protease gene as a consequence of rebound during ABT-378/ritonavir therapy. In contrast, the baseline isolate from one subject displayed only marginally reduced (2.8-fold above wt EC_{50}) susceptibility to ABT-378 and the protease sequence contained only 4 mutations associated with reduced susceptibility to

ABT-378. Following rebound, the EC_{50} of ABT-378 increased to 99-fold above the wt EC_{50} and four new mutations in protease (I54A, A71V, I72R, and V82A) were detected. In addition, the rebound isolates that emerged in all 9 subjects were resistant to EFV.

2.7.7.4 Cross-resistance of Isolates Selected by ABT-378/ritonavir *in Vivo* to Other Protease Inhibitors

The cross-resistance to other PIs of seven rebound isolates from the four subjects in whom increased resistance to ABT-378 during ABT-378/ritonavir therapy was noted (3/4 M97-765 subjects for whom rebound isolates could be cultured and one M98-957 subject) was examined (Table 11). Notably, each of these 4 subjects were PI-experienced prior to ABT-378/ritonavir therapy, and baseline isolates from each subject contained multiple mutations in HIV protease associated with resistance (and cross-resistance) to the PI class. Thus, the evaluation of cross-resistance of viruses selected *in vivo* only by ABT-378/ritonavir (or with ABT-378/ritonavir as the only PI) is not feasible at this time. The rebound viruses from each of the 4 subjects either remained (if cross-resistant at baseline) or developed cross-resistance to ritonavir, indinavir, and nelfinavir. In the 2 subjects that were naïve to saquinavir therapy, there was no change in susceptibility to saquinavir between the baseline and rebound isolates (all displayed wt susceptibility to SQV concurrent with up to 78.7-fold resistance to ABT-378). Each of the 4 subjects was naïve to amprenavir therapy. Following rebound a modest level of decreased susceptibility to amprenavir was observed (the mean EC_{50} against the 7 isolates was 5.1-fold above wt EC_{50}). Notably, the rebound virus from one subject, which displayed 52.5- to 205-fold reduced susceptibility to the other five PIs tested, including 99-fold resistance to ABT-378, demonstrated only an 8.5-fold reduced susceptibility to APV. Finally, the investigational protease inhibitor tipranavir[13] was found to maintain full activity against the 3 rebound isolates tested.

Table 11: Cross-resistance of the Viruses Selected by ABT-378 to Other Protease Inhibitors

		Fold EC_{50} change						
Pt. #	Day	ABT-378	RTV	SQV	IDV	NFV	APV	TPV
311	-1	5.2	12.1	1.5	10.2*	7.4	1.4	-
	84	20.3	101.9	1.9	15.6*	17.8	4.6	-
	140	33.6	37.9	2.4	9.3*	27.4	2.5	-
401	-1	2.5	22.6	32.4*	12.6	33.5	3.4	-
	253	3.7	15.4	16.3*	8.2	20.5	5.5	1.2
	341	9.4	19.5	>29.8*	10.6	30.1	5.4	1.1
415	-1	0.7	4.5	3.4	15.7	>54.2*	0.6	-
	337	46.3	16.2	0.6	8.7	1.6*	2.9	0.8
	501	78.7	26.6	0.4	6.8	8.5*	6.6	-
548	-1	2.8	5.0*	1.8*	1.8*	2.3	1.3	-
	153	99.0	260.9*	52.0*	156.0*	205.0	8.5	-

*Denotes PI with which the subject had been treated prior to ABT-378/ritonavir therapy

2.7.8 Integrated Interpretation of Virologic Studies with ABT-378/ritonavir

The preceding sections of this overview summarize observations concerning the virologic activity of, and development of resistance to, ABT-378/ritonavir both *in vitro* and *in vivo*. Taken together, these observations provide support for several conclusions that may have clinical significance. The support for these conclusions, from the perspective of the integrated virologic studies performed to date, is discussed below.

2.7.8.1 ABT-378 Provides the Antiviral Activity of ABT-378/ritonavir

The ABT-378/ritonavir combination contains two active agents: ABT-378 to inhibit HIV protease (thus to block the formation of infectious virus) and ritonavir to inhibit human cytochrome P450 (CYP) 3A4 (thus to enhance and prolong the plasma levels of ABT-

378). Since ritonavir also inhibits HIV protease as well as CYP 3A4, the possible role of ritonavir in contributing to the antiviral activity of the ABT-378/ritonavir combination has been investigated. Across studies, plasma ABT-378 concentrations are 15- to 20-fold higher than those obtained for ritonavir following administration of ABT-378/ritonavir at 400 mg/100 mg BID.[14] In addition, the EC_{50} of ABT-378 in the presence of 50% human serum is approximately one tenth of that for ritonavir. Since the antiviral activity of each agent *in vivo* should be related to the ratio of the plasma levels for each agent to its respective EC_{50}, one can assume qualitatively that the activity of the ABT-378/ritonavir combination is derived almost exclusively from ABT-378. Further, one can conclude that the contribution of ritonavir to the overall activity is clinically insignificant. Quantitative phamacodynamic modeling provides support for this conclusion.[14] Thus, the difference between the calculated antiviral activity for the ABT-378/ritonavir combination (assuming an additive virologic interaction) and the calculated activity of only ABT-378 at the same plasma levels was only 0.0037%.[14]

In vitro passage experiments also suggest that ritonavir does not contribute significantly to the antiviral activity of the ABT-378/ritonavir combination. In the two selection experiments using constant ratios of ABT-378 and ritonavir (5/1 and 15/1), the discordance between the concentration of ritonavir at various passages and the EC_{50} of ritonavir against the passaged virus was > 8-fold, indicating little or no selective pressure by ritonavir in the selection experiments. Moreover, the presence of ritonavir in two of the four passage experiments did not appear to affect the pattern of mutations that emerged. On a theoretical basis, the presence of ritonavir alone at persistently sub-inhibitory concentrations would be expected to select for protease mutations. However, the obligatory, simultaneous presence of the mechanistically identical inhibitor ABT-378 in far greater concentrations eliminates this selective pressure. Finally, any selective pressure exerted by ritonavir in the ABT-378/ritonavir combination, however minor, is unlikely to be of clinical consequence, since there is a high level of *in vitro* cross-resistance between ABT-378 and ritonavir in the two panels of isolates examined in these

studies. Thus, the combined data from both virologic and pharmacokinetic studies suggest that the antiviral activity of the ABT-378/ritonavir combination arises nearly exclusively, if not solely, from ABT-378.

2.7.8.2 Activity of ABT-378/ritonavir in Protease Inhibitor-Experienced Subjects is a Consequence of the High Plasma Levels of ABT-378 Rather Than a Lack of Cross-Resistance Between ABT-378/ritonavir and Other Protease Inhibitors

The results of Studies M97-765 and M98-957 demonstrate that ABT-378/ritonavir displays substantial antiviral activity in subjects who have failed at least one PI regimen. Better adherence to the ABT-378/ritonavir regimens in these studies, compared to the previous regimen(s), could provide one rationale for a portion of the observed activity in these patient populations. However, it is clear that ABT-378/ritonavir supplies a level of antiviral activity, above that of the previous regimens, that leads to the suppression of viral load in the majority of subjects. The potent antiviral activity of ABT-378/ritonavir, rather than suppression due solely to the other components of the regimens (in particular NVP or EFV in Studies M97-765 or M98-957, respectively), is demonstrated by the decline in plasma HIV RNA that occurred during the first two weeks of Study M97-765, in which only the previous PI was changed to ABT-378/ritonavir. Further, the statistical correlation of baseline phenotypic susceptibility (to ABT-378) and genotype (number of protease mutations) to virologic response in Study M98-957 is inconsistent with the premise that virologic response in that study was due only to EFV and the NRTI portion of the study regimen.

Since it can be concluded that ABT-378/ritonavir exerts substantial antiviral activity in PI-experienced subjects, it is important to examine the mechanistic basis for that activity. Two distinct mechanisms should be considered: (1) lack of virologic cross-resistance between ABT-378 and other PIs, and (2) activity due to plasma drug levels that are sufficient to inhibit viral strains with substantially reduced phenotypic susceptibility to ABT-378. Although some evidence for the first mechanism exists [a higher percentage of

baseline viruses in Study M97-765 were phenotypically susceptible (<4-fold change in EC_{50}) to ABT-378 than to the previous PI], there is overwhelming evidence for the second mechanism. The phenotypic patterns of reduced susceptibility to ABT-378 correlated with those of both RTV and IDV. Moreover, ten of the eleven mutations in HIV protease identified as being associated with reduced susceptibility to ABT-378 have previously been shown to be selected during IDV and/or RTV therapy or to be associated with resistance to one of those PIs. The observation that ABT-378/ritonavir exerts substantial antiviral activity in subjects that have previously failed IDV- and/or RTV-based therapy strongly argues that, because phenotypic resistance is always relative to the drug concentrations present (both *in vitro* and *in vivo*), high sustained ABT-378 plasma levels are primarily responsible for overcoming the resistance of the baseline viruses in the majority of Study M97-765 and M98-957 subjects. In contrast to results obtained with other PI-based regimens,[8-11] this conclusion is further supported by the observation that a 4-fold change in baseline susceptibility to ABT-378 was not associated with diminished virologic response in either Study M97-765 or M98-957. Instead, diminished response only became evident in subjects whose baseline isolates displayed greater than 10- to 40-fold reduced *in vitro* susceptibility to ABT-378. Since the ratio of the ABT-378 trough level to its serum-adjusted EC_{50} is much higher (>75 for wild-type HIV when dosed as ABT-378/ritonavir at 400/100 mg BID) than those produced by the other PI-based regimens examined in other retrospective studies,[8-11] this provides additional support for the conclusion that ABT-378/ritonavir displays activity in PI-experienced subjects primarily because of high ABT-378 plasma levels rather than because of lack of virologic cross-resistance.

2.7.8.3 Results of HIV Resistance Testing for ABT-378/ritonavir Should Be Interpreted in the Context of Clinical Results Rather than Solely Based on *in Vitro* Data

Results from the combined virologic studies with ABT-378 provide valuable information for the interpretation of phenotypic and genotypic HIV resistance testing for antiretroviral-experienced patients considering treatment with ABT-378/ritonavir.

Current phenotype tests report the *in vitro* susceptibility of clinical isolates to available antiretroviral drugs, compared with the susceptibility of a standard wild type isolate. These data are provided in the absence of clinical interpretation concerning the degree of reduced phenotypic susceptibility, or (perhaps worse) accompanied by an interpretation based only on consideration of assay variability (*e.g.* sensitive if <4-fold, intermediate if 4- to 10-fold, and resistant if >10-fold change in EC_{50}, compared to the wild type standard). Unfortunately, this interpretation, which uses the same criteria to define phenotypic "resistance" to all antiretroviral drugs, ignores substantial differences in the pharmacology of individual agents as well as the potential drug-drug interaction between these agents. In retrospective studies, a change in baseline phenotype of 4-fold or less (defined by the sensitivity limits of the phenotypic assays) has been associated with diminished virologic response to several PI-based regimens,[8-10] illustrating the potential utility of assigning some degree of phenotypic "resistance" using a 4-fold cutoff. However, that association is likely to be coincidental, a consequence of the fact that the inhibitory quotient (i.e., the plasma trough level to EC_{50} ratio) of those PIs generally range from 1- to 5-fold above the respective serum-adjusted EC_{50} values against wild type HIV. Thus, a 4-fold change in baseline susceptibility would reduce those inhibitory quotients to 1 or less, and would be expected to affect virologic response. In contrast, the inhibitory quotient for ABT-378/ritonavir, dosed at 400/100 mg BID, exceeds 75 for wild type HIV. Therefore, viruses that display 4-fold reduced susceptibility to ABT-378 would be expected to be strongly inhibited *in vivo* by ABT-378/ritonavir (*i.e.*, the inhibitory quotient for a 4-fold mutant could approach 20). Thus, the lack of an association of 4-fold reduced baseline susceptibility with the virologic response observed in Studies M97-765 and M98-957 is not unexpected. In contrast, the mean inhibitory quotient for ABT-378 against viruses with 40-fold reduced susceptibility would be expected to be low (*ca.* 2). Thus, the observed correlation between diminished response and clinical isolates with greater than 10- to 40-fold reduced baseline viral susceptibility in Study M98-957 can be readily explained by the inhibitory quotient of ABT-378. The results of phenotypic

resistance testing with ABT-378/ritonavir are therefore best interpreted using the probabilistic model developed in Study M98-957, since detection of reduced *in vitro* susceptibility *per se* does not necessarily constitute clinical resistance.

Similarly, results from current genotypic resistance tests may be accompanied by "interpretation," suggesting evidence of resistance if a key primary mutation associated with a particular PI is present. This interpretation is supported by retrospective studies demonstrating diminished response in subjects whose baseline isolates contained one or two mutations associated with resistance to the PIs in their salvage regimen.[11,12] In contrast, no diminution of response was observed in subjects from Study M98-957 whose baseline isolated contained at most 5 of the 11 mutations associated with reduced *in vitro* susceptibility to ABT-378. Furthermore, the presence of a primary mutation(s) at position 82 and/or 84 plus three secondary mutations at positions 10, 54 and 63 was insufficient to predict virologic failure. Instead, response was partially diminished in subjects with 6-7 baseline mutations, and further diminished in those with 8 or more baseline mutations. These results suggest that subjects with up to 7 of the 11 mutations associated with reduced susceptibility to ABT-378 might receive at least some level of antiviral activity from therapy with ABT-378/ritonavir. Consequently, the results from these integrated virologic analyses provide specific guidance for the use of ABT-378/ritonavir in antiretroviral-experienced patient populations.

2.7.8.4 The Genetic Barrier to the Emergence of Resistance *in Vivo* to ABT-378/ritonavir is High but Finite

Although most of the resistance data collected thus far in this development program concerns the *in vitro and in vivo* activity of ABT-378 against viruses selected by other protease inhibitors, the information gathered in this context also provides insight into the genetic barrier to the *in vivo* evolution of resistance to ABT-378/ritonavir. In this context, the genetic barrier to resistance can generally be defined as the number of mutations needed to overcome the suppression of the drug *in vivo*. This is obviously an oversimplification because individual mutations can contribute differently to phenotypic

changes. Nonetheless, provided there is at least one primary (active site) mutation present, the overall susceptibility to PIs tends to decrease with increasing numbers of mutations in the protease gene sequence. It is evident that the genetic barrier is dependent on the incremental change in drug susceptibility produced by a given mutation, as well as the local concentration of drug at the site of replication (with PIs, approximated by plasma levels). Thus, the genetic barrier might be more appropriately referred to as the pharmacological barrier to resistance.

As with many protease inhibitors, the incremental change in EC_{50} of ABT-378 per additional mutation is relatively modest. Thus, the median EC_{50} of ABT-378 against isolates containing 4, 5, 6 or 7 of the 11 mutations associated with reduced *in vitro* susceptibility was only 2.4-, 4.2-, 12- and 16-fold, respectively, above the EC_{50} against wild type HIV. In contrast to other PIs, however, plasma ABT-378 levels maintained *in vivo* represent many multiples of the EC_{50} against wild type HIV. Therefore, in viruses selected during therapy with other PIs, phenotypic changes that begin to approach plasma ABT-378 trough levels are generally only observed when more than 7 mutations are present. The clinical significance of this conclusion is manifested in Study M98-957, wherein the virologic response in subjects initiating ABT-378/ritonavir therapy with 6-7 baseline mutations was greater than in subjects initiating therapy with 8 or more mutations, albeit less than those initiating therapy with 5 or fewer mutations. These analyses help delineate the range and limits of response to ABT-378/ritonavir therapy. In subjects with viruses demonstrating high level resistance to ABT-378, providing higher drug concentrations should theoretically compensate for diminished activity to some extent, but it is likely that this strategy will have finite potential.

Provided that an alternate, as of yet undiscovered, mutation pathway to ABT-378/ritonavir resistance is not favored, the above results with isolates selected by other PIs suggest that the genetic barrier to *in vivo* resistance in subjects receiving ABT-378/ritonavir as their first PI is high. This conclusion is supported by the genotypic and

phenotypic patterns of the rebound isolates from subjects experiencing viral rebound in Studies M97-720 and M97-765. Subjects who initiated ABT-378/ritonavir therapy with baseline isolates containing 2 or fewer of the 11 mutations associated with reduced susceptibility to ABT-378 displayed no genotypic or phenotypic evidence of resistance to ABT-378 following increases in HIV RNA to >400 copies/mL. Concurrently, genotypic and phenotypic resistance to either 3TC or nevirapine, two agents with known low genetic barriers, was observed in the majority of those subjects. In contrast, viruses containing 4-5 mutations, although retaining susceptibility to ABT-378 plasma levels achieved *in vivo*, would be expected to have a genetic platform from which more complete resistance can evolve with relative ease. This was confirmed in Studies M97-765 and M98-957, wherein subjects initiating therapy with ABT-378/ritonavir with 4-5 baseline mutations evolved higher level resistance to ABT-378 (along with additional mutations in protease) in the same time frame during which resistance to nevirapine or efavirenz, respectively, emerged. Taken together, these results suggest that in individuals instituting initial antiretroviral therapy with ABT-378/ritonavir, the genetic barrier to resistance will be high, provided they have not received a PI-resistant virus through transmission. A high genetic barrier might be expected to translate clinically into a highly durable response to therapy, provided that adherence is not seriously compromised. However, this perceived relationship will require clinical validation.

2.7.8.5 Because of Limited Data, the Cross Resistance of HIV Selected by ABT-378/ritonavir to Other Protease Inhibitors is not Well-defined

There is a relative paucity of data on the development of resistance to ABT-378/ritonavir *in vivo* and the corresponding cross-resistance, or lack thereof, of the viruses selected by ABT-378/ritonavir to other PIs. The data accumulated to date on the activity of ABT-378/ritonavir against HIV selected by other PIs provide considerable information on *in vivo* resistance to ABT-378/ritonavir. However, that information does not entirely replace the characterization of viral isolates from previously untreated subjects who have experienced rebound on therapy with documented emergence of drug-resistance. These

data are limited partly as a consequence of the high virologic response rate in the Phase I/II studies used to study rebound viruses. However, the fact that actual genetic selection by ABT-378/ritonavir could be documented in only a small subset of subjects who experienced rebound also hampered the identification of the mutation pattern(s) for ABT-378/ritonavir. In short, rebound isolates from subjects who began ABT-378/ritonavir therapy with at most 2 of the 11 mutations associated with reduced susceptibility to ABT-378 showed no evidence of increased genotypic or phenotypic resistance to ABT-378, compared to the baseline isolates. Moreover, those subjects whose baseline isolates contained 6 or more mutations also showed little or no evidence of evolution in the protease gene during rebound, presumably because the selective pressure by ABT-378 was relatively low. Thus, evolution during rebound was observed only in a few subjects whose baseline isolates were relatively susceptible to ABT-378 (<10-fold reduced baseline susceptibility in all but one of these subjects) yet contained 4 or more baseline mutations associated with reduced susceptibility to ABT-378.

Obviously, since *in vivo* selection of resistance to ABT-378/ritonavir in antiretroviral-naïve subjects has yet to be documented, the cross-resistance of such isolates to other PIs cannot be evaluated at this time. The lack of development of high-level resistance to saquinavir or amprenavir during rebound in previously PI-treated subjects suggests the potential use of these agents, in conjunction with ritonavir to achieve adequate plasma concentrations, after failure of ABT-378/ritonavir therapy. Moreover, it is consistent with the relatively low correlation between the patterns of *in vitro* susceptibility to ABT-378 and either saquinavir or amprenavir. However, at present there is no information on the success of regimens containing either saquinavir or amprenavir in subjects failing therapy with ABT-378/ritonavir. Thus, additional vigilance will be required to identify resistant viruses selected by ABT-378/ritonavir *in vivo* and to evaluate the potential for salvage therapy of such individuals.

2.7.9 References

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