CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-226

21-251

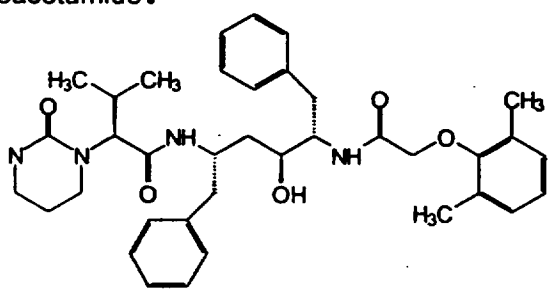
MICROBIOLOGY REVIEW

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA # N021226 **Serial #** 000 **REVIEWER:** Julian J. O'Rear, Ph.D.
Date Submitted: 12/28/99 **Date Assigned:** 1/10/00
Date Received: 12/29/99 **Date Reviewed:** 5/1/00

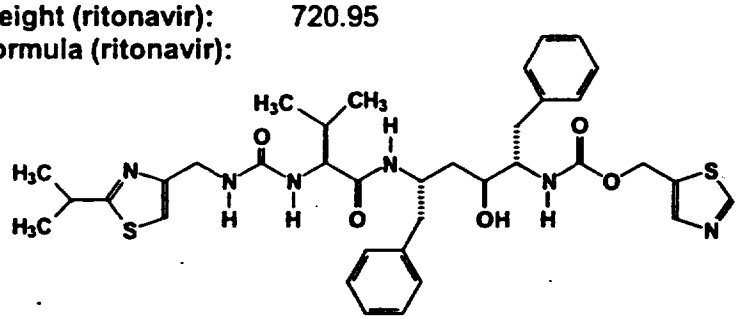
Sponsor: Abbott Laboratories
100 Abbott Park Road
D-491, AP6B-1SW
Abbott Park, IL 60064-3500

Product Names: lopinavir, ABT-378, Abbott 157378
Chemical Name: [1S-[1R*,(R*),3R*,4R*]]-N-[4-[[(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenyl-1- (phenylmethyl)pentyl]tetrahydro-α-(1-methylethyl)-2-oxo-1(2H)-pyrimidineacetamide.
Structural Formula:



ABT-378

Empirical Formula: $C_{37}H_{48}N_4O_5$
Molecular Weight: 628.82
Drug Category: Antiviral
Chemical Name (ritonavir): 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8.11-*bis*(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester, [5S-(5R*,8R*,10R*,11R*)].
Empirical Formula (ritonavir): $C_{37}H_{48}N_6O_5S_2$
Molecular Weight (ritonavir): 720.95
Structural Formula (ritonavir):



RITONAVIR

Indication: Treatment of HIV infection
Dosage Form/Route of administration: Tablets and soft gel capsule/Oral

Supporting Documents: IND#'s [redacted] and supplements and amendments.

Abbreviations: AAG, α-1 acidic glycoprotein; AIDS, acquired immunodeficiency syndrome; APV, amprenavir; AZT, zidovudine; CCIC, cytotoxic concentration; ddI, didanosine; DLV, delavirdine; d4T, stavudine; EC, effective concentration; EFV, efavirenz; ELISA, enzyme linked immunosorbent assay; HAART, highly active anti-retroviral therapy, HIV-1, human immunodeficiency virus-1; HS, human serum; IC, inhibitory concentration; IDV, indinavir; IN, integrase; NFV, nelfinavir; NVP, nevirapine; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; PMEA, adefovir dipivoxil; RT, reverse transcriptase; RTI, reverse transcriptase inhibitor; RTV, ritonavir; SAR, structure activity relationships; SQV, saquinavir; 3TC, lamivudine; WT, wild type;

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BACKGROUND

Human immunodeficiency virus-1 (HIV-1) was discovered by Luc Montagne at the Institute Pasteur in Paris because of its association with an acquired immunodeficiency syndrome (AIDS). The virus is transmitted by exchange of bodily fluids, primarily by sexual contact or use of a contaminated needle. Prior to the development of a sensitive test for the virus, it was transmitted through blood transfusion and the use of clotting factor by hemophiliacs.

HIV-1 is a human retrovirus. Retroviruses have a lipid bilayer membrane with a ribonucleoprotein core. The virus is diploid, having two copies of its single-stranded RNA genome. Genome sizes range from 8-10 kb for replication competent viruses.

The retrovirus life cycle begins with its attachment to the outer cell membrane by a specific interaction between the viral encoded envelope glycoprotein and specific proteins on the cell surface, *e.g.* CD4 and accessory proteins in the case of HIV-1. The viral and outer cell membranes fuse, in a reaction catalyzed by the envelope protein, releasing the viral core into the cytoplasm of the cell. Single-stranded genomic RNA in the core is copied into unintegrated linear viral DNA by the viral encoded enzyme reverse transcriptase (RT). The resulting linear viral DNA migrates into the nucleus and becomes covalently attached (integrates into) to the host cell genomic DNA forming the provirus. The integration reaction is catalyzed by the viral encoded integrase (IN). Transcription from the single viral promoter forms a full-length RNA molecule which can serve as genomic RNA, mRNA, or as a precursor to spliced subgenomic mRNAs. Translation of the full-length and subgenomic RNAs produces polyproteins. The polyprotein from the envelope-glycoprotein subgenomic RNA is directed to the secretory pathway where it is cleaved and posttranslationally modified by cellular enzyme functions. The Gag and Gag-Pol polyproteins, encoded by the full-length RNA, associate with full-length genomic viral RNA to form the virus core which buds from the outer cell membrane creating progeny virus particles. Cleavage of the Gag and Gag-Pol polyproteins by the viral encoded protease (PR), found in Gag and/or Gag-Pol in different retroviruses, matures the released particles into virus (Debouck et al., 1987; Darke et al., 1988; Graves et al., 1988).

The discovery and molecular characterization of HIV-1 led to the development of a sensitive enzyme linked immunosorbent assay (ELISA) to identify infected individuals based upon the detection of antibodies to the p24 capsid protein. This assay proved useful in identifying individuals prior to the onset of AIDS and helped in making blood supplies and clotting factor preparations safer. More recently, levels of virus are quantified by determining the amount of viral RNA in plasma. These assays are based upon the polymerase chain reaction (PCR) and are capable of detecting as little as 50 RNA molecules/ml. Viral loads correlate well with the progression of the disease and are frequently used to assess the effectiveness of therapeutics.

Studies of clinical isolates and laboratory experiments to determine why infected individuals failed to mount an effective immune response led to the discovery that HIV-1 mutates at a very high rate. The viral RT is error prone and the envelope protein shows great versatility in its ability to change while retaining function. Changes in the envelope protein enable the virus to evade the host immune system. In addition, the high rates of mutation and replication of the virus have complicated the development of

effective vaccines and therapeutics. Many of the viral proteins can undergo multiple amino acid changes enabling the virus to evade antiviral agents, and yet these proteins retain sufficient activity for virus replication.

Traditionally, drugs directed against infectious agents are designed to target activities not found within or not essential to the host. The earliest therapeutics directed against HIV-1 target the RT. These inhibitors are nucleoside analogs that are phosphorylated *in vivo* and competitively inhibit RT and/or result in chain-termination. The impact upon host cells is not as great because cellular DNA polymerases are more capable of discriminating between the correct nucleotides and the inhibitors. This class of drugs as a whole is known as nucleoside reverse transcriptase inhibitors (NRTIs).

These nucleoside-based drugs significantly inhibit replication of HIV-1 *in vitro* but are of limited effectiveness *in vivo* when administered individually. This limit is due in part to the high mutation and replication rates, mentioned above, that result in great genetic diversity within the virus population. Administration of a single drug can rapidly select resistant viruses from within the virus population. Furthermore, these resistant viruses can rapidly evolve into viruses that replicate more efficiently in the presence of the anti-retroviral agent(s) and/or are resistant to higher concentrations. (The term resistance is relative in its use herein.) An example of the selection of a resistant virus from the population is the appearance of lamivudine resistance. High levels of lamivudine resistance can be achieved by mutation of a single nucleotide base leading to the M184V substitution. On the other hand, high level resistance to some agents is more complex requiring the accumulation of multiple mutations which individually confer stepwise increases in resistance. Identification of common mutations associated with resistance to particular anti-retroviral agents combined with the characterization of HIV genotypes from baseline and study isolates may help to predict the clinical outcomes of patients.

More recently, non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been developed for the treatment of HIV-1 infection. FDA approved therapeutics to date include efavirenz (EFV), delavirdine (DLV), and nevirapine (NVP). NNRTIs bind to a site different from the nucleotide-binding site and may complement nucleoside analogs. NRTIs and NNRTIs together are referred to as RTIs.

The effectiveness of some NNRTIs is compromised because of their sequestration by serum proteins (Corbett et al., 2000). EFV is >99% bound by a serum protein resulting in a 16-fold reduction in its *in vitro* antiviral activity. Likewise, DLV is also bound by serum proteins and is reduced 38-fold in these assays. Only a modest effect (2-fold) is observed on NVP.

In the mid-1990s, a new class of drugs targeting the viral PR was demonstrated to be effective in the treatment of HIV infection. The development of these drugs was based upon the observations that the viral PR was distantly related to the renin family of aspartate proteases which have the active site motif Asp-Thr-Gly (Pearl and Taylor, 1987a; 1987b), and that site-directed mutagenesis of the active site aspartate residue to an asparagine residue resulted in the production of noninfectious viral particles having immature morphology when viewed in the electron microscope (Kohl et al., 1988; LeGrice et al., 1988). These noninfectious particles contain full-length viral Gag and Gag-Pol polyproteins.

The first approved protease inhibitor (PI), saquinavir (SQV), was developed starting with a noncleavable peptide backbone mimetric of the substrate cleavage site and optimizing its inhibitory activity using the classical method of structure activity relationships (SAR; Roberts et al, 1990; Kempf et al., 1993). Later PI development employed the use of X-ray crystallographic structure (Navia et al., 1989; Wlodawer et al., 1989), which showed that the active site was made by the dimerization of the protein monomer. These dimers have C_2 point group symmetry. Inhibitors based upon this approach are mimetrics of the amino terminal portion of the cleavage site joined in a symmetrical manner. There are four approved PIs based upon the dimer structure of the viral PR: indinavir (IDV), ritonavir (RTV), nelfinavir (NFV), and amprenavir (APV).

There has been recent success in treating HIV-1 infected individuals using highly active anti-retroviral therapy (HAART) which uses combinations of FDA approved drugs to inhibit replication of the virus. All other things being equal, the probability of viruses having resistance mutations to two or more drugs is predicted to be the product of their individual frequencies, about 10^{-9} for two drugs, assuming resistance acquired from single base mutations (Mansky and Temin, 1995). In theory, the frequency of the development of resistance to combinations of drugs acting independently could exceed the total viral load in an individual and prevent virus rebound. However, it has been found that resistance mutations to one member of a class may confer some degree of resistance to other members of the same class. In an effort to develop new drug classes, inhibitors of two other viral enzymatic activities are being studied. One target is the envelope glycoprotein-catalyzed fusion reaction of the viral and cell membranes at virus entry. Another interesting target is integrase which catalyzes the formation of the provirus. The development of pharmaceuticals targeting other virus functions may increase the effectiveness of HAART. However, these inhibitors all target replicating virus and would not eliminate from the infected individual cells harboring an HIV provirus.

During the development of PIs, it was found that some very potent inhibitors *in vitro* had little or no activity *in vivo*. Investigation of this phenomenon let to the discovery that serum proteins bind many PIs (Molla et al., 1998). Most PIs are primarily bound by α-1 acidic glycoprotein (AAG). Shown below in Table 1 is the percentage of the indicated approved PI bound by serum proteins and the serum protein to which it is bound (Lazdins et al., 1997). SC-52151 has high affinity for, and is a strong inhibitor of, HIV-1 protease *in vitro*, but has much less activity *in vivo* (Fischl et al., 1997).

Table 1 Binding of HIV-1 Protease Inhibitors by Serum Proteins

	% bound	protein bound to
Saquinavir	98-99 %	AAG
Indinavir	60	AAG
Ritonavir	98-99	AAG
Amprenavir	93	AAG
Nelfinavir	98-99	AAG
SC-52151	98-99	AAG

Viruses resistant to the approved PIs have been isolated and characterized. Genotypic analysis has identified amino acid changes associated with resistance to

specific PIs, and three dimensional structure determination of protease-PI complexes has provided insight in some cases into how these changes result in resistance (Ho et al., 1994). The development of ABT-378 began after such an analysis of RTV (Sham et al., 1998).

SUMMARY

Abbott Laboratories is submitting this NDA (#21-226) for the approval of ABT-378/co-dosed with RTV, denoted by ABT-378/r, for the treatment of HIV-1 infection in adults. According to the sponsor, this drug was developed to circumvent the major resistance mutation at amino acid 82 associated with the use of RTV (Schmit et al., 1996) and to improve upon some of the limitations of the currently utilized PIs. One of the improvements of ABT-378 over RTV and some of the other PIs is a reduction in its sequestration by serum proteins.

ABT-378 is co-dosed with sub-therapeutic levels of RTV. RTV previously was discovered to be an effective inhibitor of CYP3A4 with a K_i in the nM range. CYP3A4 is a liver P450 enzyme that rapidly clears ABT-378 and many other drugs from humans. As a result of inhibiting CYP3A4, the AUC and the C_{min} for ABT-378 are significantly increased with trough values that are much greater than its EC_{50}. Trough values far in excess of the EC_{50} and the reduced sequestration by serum proteins may play an important role in reducing the appearance of resistant virus.

This application contains supporting information and data about the mechanism of action of ABT-378, its *in vitro* anti-viral activity against laboratory adapted and clinical virus isolates, its *in vitro* anti-viral activity against viruses resistant to ABT-378 and viruses resistant to other anti-retroviral agents, its *in vitro* cytotoxicity, the genetic characterization of resistant virus selected *in vitro*, the cross-resistance to other PIs by viruses resistant to ABT-378, and the cross-resistance to ABT-378 by viruses resistant to other PIs.

Clinical Studies

The reader is referred to the clinical and statistical reviews for information on the safety and efficacy of ABT-378/r.

Mechanism of Action

The mechanism of action of ABT-378 is the same as the other approved PIs. As mentioned above, PIs competitively inhibit the cleavage of Gag and Gag-Pol polyproteins by the viral encoded protease. Processing of these polyproteins is necessary for the efficient cellular release and maturation of viral particles into virus. The K_i's of ABT-378 for wild type protease, PR_{WT}, and RTV resistant mutants, PR_{V82A} and PR_{V82F}, are in the 1-3 pM range (Enclosure #1). These were determined using an assay for viral PR activity based upon a fluorogenic substrate (Matayoshi et al.,1990). In contrast to the potency of ABT-378 against the viral PR, inhibition of the distantly related human aspartyl proteases, plasma renin and cathepsins D & E, occurs at

200,000-fold or higher concentrations (Enclosure #2) indicating a high selectivity for its target protein.

An additional feature of ABT-378 is reduced sequestration by serum proteins providing greater availability for its target protein. The selection process for ABT-378 incorporated a screen for the retention of anti-retroviral activity *in vitro* in the presence of the human serum proteins, which, as mentioned above, sequester many PIs. The activity of ABT-378 *in vitro* is only modestly affected in the presence of 50% human serum (Table 2) compared to other PIs.

Table 2. Median effect of serum on the effective concentration of PIs

	Median fold reduction (EC_{50} 50% HS[a]/ EC_{50} 0% HS)
Ritonavir	24
Saquinavir	33
Indinavir	1.8
Nelfinavir	38
Amprenavir	8.7
ABT-378	5.3

[a]HS, human serum

RTV was developed as a PI, but its mechanism of action here is different. ABT-378 administered alone is rapidly cleared from the body. The sponsor plans to co-dose ABT-378 with RTV, which results in an increase in ABT-378's AUC (77-fold) and trough values (25-100X EC_{50}). RTV acts by inhibiting CYP3A4 P450, the liver enzyme that rapidly clears ABT-378 and other PIs from the body (Kempf et al., 1997; Koudriakova et al., 1998; Kumar et al., 1999a; 1999b). These increases in AUC and C_{min} likely play a significant role in ABT-378 effectiveness as a therapeutic agent. An inverse correlation has been observed between the appearance of resistant virus and AUC, and between the appearance of resistant virus and C_{min} (Molla et al., 1996; Burger et al., 1998; Acosta et al., 1999; Descamps et al., 2000; Fletcher et al., 2000). Trough values much greater than the EC_{50} value may minimize the window of opportunity in which virus may replicate and thereby generate virus having decreased susceptibility.

Conclusion: The data and background information support the proposed mechanism of action of ABT-378 as a potent and specific inhibitor of HIV protease. RTV is acting as an inhibitor of CYP3A4.

Antiviral Activity of ABT-378 *In Vitro*
a) Against wild type virus

A comparison of the activities of ABT-378 and other PIs against patient isolates (referred to as WT by the sponsor) was performed using PBMC co-culture and detection of inhibition of virus production by measuring p24 antigen levels (Enclosure #3). The EC_{50} values in this assay were 6.5 ± 2.5, 22 ± 10, 14 ± 14, and 3.5 ± 2.2 nM for ABT-378, RTV, IDV, and SQV, respectively.

ABT-378 showed anti-viral activity against WT HIV-1 (laboratory strains) when measured in MT4 host cells using a standard cytopathicity assay which is based upon

the uptake of MTT tetrazolium dye (Pauwels et al., 1988). The mean EC_{50} value for $HIV\text{-}1_{IIIB}$ grown on MT4 cells was 0.017 μM in 0 % serum (Enclosures #4 and #5) and 0.102 μM in 50 % human serum (Enclosures #4 and #6). ABT-378's activity against WT HIV-1 is affected by serum proteins slightly more than IDV and slightly less than APV. In contrast, the activities of RTV, SQV, and NFV are greatly reduced in the presence of serum proteins. The mean EC_{50} value for ABT-378 grown on MT4 cells in 0 % serum for five laboratory strains of HIV-1 was 0.019 μM, and was 0.025 μM for one strain of HIV-2 (Enclosures #7 and #8). These same viruses had a mean EC_{50} value in the presence of 50 % serum of 0.163 μM and 0.104 μM, respectively.

Conclusion: The data presented show that ABT-378 has anti-viral activity against laboratory (n=5) strains and clinical (n=6) isolates of HIV-1 in the absence or presence of serum proteins, and support the sponsor's proposition that ABT-378 may have improved efficacy *in vivo* due to reduced sequestration by serum proteins. The mean EC_{50} values for clinical and laboratory strains were similar, 10-20 nM in the absence of serum and 100-200 nM in the presence of 50 % serum, even though they were measured using different culture and assay systems. The inhibition of a single laboratory strain of HIV-2 is the only data supporting ABT-378 activity against HIV-2.

b) Against virus resistant to antiretroviral drugs from other classes
Antiviral activity of ABT-378 against clinical isolates resistant to AZT

As might be expected based upon the different target proteins, ABT-378 was active against clinical isolates of virus resistant to the NRTI AZT. The susceptibility to ABT-378 of AZT-resistant viruses (n=7) grown on MT4 cells and measured with the MTT assay was similar to that observed against wild type virus isolates. The mean EC_{50} values were 20 nM and 186-188 nM in the absence and presence (50 %) of human serum, respectively (Enclosures #9 and #10).

The activity of ABT-378 against viruses resistant to other PIs is reviewed below in the section on cross-resistance.

Conclusion: Virus resistant to the RT inhibitor AZT shows similar susceptibility *in vitro* to ABT-378 as WT virus.

***In Vitro* Cytotoxicity of ABT-378**

ABT-378 shows a greater than 5 $\log_{10}$ difference in inhibitory activity between human aspartyl proteases and its target protein, HIV-1 protease, suggesting good selectivity for its target. *In vitro* cytotoxicity assays of ABT-378 are consistent with this expectation. The $CCIC_{50}$ in MT4 cells measured with the MTT assay is 22 μM in 0% human serum and >100 μM in the presence of 50% serum. The resulting selectivity index ($CCIC_{50}$/ EC_{50}) for ABT-378 is 1290 in 0% serum and >1000 in 50% serum (Enclosure #11).

Conclusion: The selectivity (therapeutic) index indicates that the inhibition by ABT-378 of virus replication in cell culture, described above in the "*in vitro* antiviral activity" section, is not likely due to cytopathic effects of the drug.

Combination Antiviral Activity *In Vitro*

Combining drugs as done in HAART therapy can result in a synergistic, additive, or antagonistic effect upon the drugs. The sponsor has not presented *in vitro* combination antiviral activity data addressing possible antagonistic or synergistic acitivy with other approved anti-retroviral agents.

Summary of ABT-378 Resistance Studies

The sponsor has conducted multiple studies to examine the development of resistance to ABT-378. Four separate experiments selecting ABT-378 resistant virus in cell culture failed to identify a common pathway to ABT-378 resistance. These studies indicate that multiple mutations are required to reduce susceptibility to ABT-378. In a study of >100 treatment naïve patients, there were few failures and no common PR mutations identified (M97-720). The sponsor therefore decided to look for cross-resistance mutations by phenotypic and genotypic analyses of 1) a bank of previous PI resistant isolates and 2) baseline isolates in PI experienced patients, first in a study of subjects who had failed a single PI (M97-765) and then in subjects who had failed multiple PIs (M98-957). The data from the latter two clinical studies were combined by the sponsor to identify 12 mutations associated with cross-resistance to ABT-378. Four subjects in these two studies were virologic failures and showed a shift in susceptibility to ABT-378. A comparison of baseline isolates and isolates at failure identified 8 additional mutations possibly associated with ABT-378 resistance.

ABT-378 Resistance Development *In Vitro*, Phenotypic and Genotype Analysis

The sponsor has selected viruses resistant to ABT-378 and ABT-378/r by serial passage in cell culture of the parental WT virus pNL4-3 on MT4 cells in the presence of increasing concentrations of the drug(s) (Enclosure #12). In the two selections in which RTV was included with ABT-378, the ratio of ABT-378 concentration to RTV concentration was chosen to reflect the high and low extremes of the *in vivo* range. Growth of virus was detected by measuring p24 antigen concentrations and monitoring cytopathic effects. Medium was harvested, filtered to remove cells and cell debris, and frozen at –80° C as a virus stock. Infected cells were washed, lysed, and frozen for proviral DNA analysis at a later time. Viruses showing a >100-fold decrease in susceptibility, up to 338-fold in one case, were obtained in this manner (Enclosure #13 and Carrillo et al., 1998).

ABT-378 and ABT-378/r resistant virus from different stages of the selection process in four different selections has been subjected to genotypic analysis to study the evolution of resistance. The PR genes in proviral DNA from infected cells were amplified by PCR, subcloned, and subjected to DNA sequence analysis to deduce the amino acid sequences of the variant PRs. In one analysis, five to ten subclones were analyzed for each step in the selection process, and in the others, two to five isolates from passages 6, 7 or 8, 11, 17, and either 24, 25 or 26. The first mutations to appear in virus from one selection using ABT-378 alone were I84V and L10F (passage 6; Enclosures #14 and #15), followed by M46I and T91S (passage 11), then I47V and

G16E (passage 14), and finally I47A and H69Y (passage 17). A summary of the frequency of these mutations is shown in the enclosure (#16). The V32I mutation was not consistently associated with ABT-378 selection in this study, appearing, disappearing, and then re-emerging. In another selection, the pattern of appearance of mutations was I50V and M46I (passage 7), L10F and I47V (passage 17), followed by V32I, E34Q, Q61H, and E65Q (passage 24). These results suggest that there may be multiple genetic pathways to resistance to ABT-378. The sponsor suggests that characterization of clinical isolates from patients failing therapy may be a better way to study resistance profiles than characterization of resistant virus selected *in vitro*.

In a selection containing RTV at 1/5 the concentration of ABT-378, many of the mutations found in the first ABT-378 isolates were found in the ABT-378/r isolates. First to appear was I84V (passage 6; Enclosures #14 and #15), then L10F and M46I (passage 9), then V32I (passage 11), and finally I47V and Q58E (passage 17). When RTV was present at 1/15 ratio, I50V and M46I first appeared (passage 8), then V82L (passage 11), followed by A71V and V82F (passage 17), and finally L33F and I54V (passage 26). Like V32I, the K45I mutation was not consistently observed. The only amino acid residue observed to mutate in all four studies was 46M. Residues 10L and 47I were found in three of the studies.

Conclusion: WT virus serially passaged in cell culture in the presence of increasing concentrations of ABT-378 can select variants with increasing phenotypic resistance. Variants selected to date have shown >330-fold decrease in susceptibility to ABT-378. The genetic analysis indicates that high level resistance (≥10 fold change in susceptibility) requires multiple mutations and may require mutations at the protease cleavage sites in the Gag and Gag-Pol polyproteins (Carrillo et al., 1998). The methionine residue at position 46 may play an important role in ABT-378's interaction with the protease, and its change may be important to the development of high level resistance. It is not, however, essential as shown by the analysis of clinical isolates (below). The leucine and isoleucine residues at positions 10 and 47, respectively, may also be important. There does not appear to be a residue 46 rule similar to the strong association of residue 82 and RTV resistance.

ABT-378 Resistance Development *In Vivo*: Virologic Failures from Studies M97-720, M97-765, and M98-957

Clinical isolates at baseline and at virologic failure from 4 PI naïve, 10 single and 9 multiple PI-experienced subjects who had failed ABT-378/r treatment, as evidenced by a rise in plasma HIV-1 RNA levels, were examined for genotypic and phenotypic changes (Studies M97-720, M97-765, M98-957) which may be associated with ABT-378 resistance. Three groups of resistance isolates were identified. Group 1 (n=7) isolates contained ≤2 of the 12 mutations found in PI-experienced patients and that were statistically associated with reduced *in vitro* susceptibility (The 12 mutations are identified below in the section entitled "Phenotypic and Genotypic Analysis of Clinical Study Virus Isolates."). These isolates retained susceptibility *in vitro* to ABT-378 but had acquired reduced susceptibility to lamivudine or NVP at failure. The reduced susceptibility to ABT-378 *in vivo* is not understood. Group 2 (n=5) baseline isolates, all from PI experienced patients, contained 4-5 of the mutations associated with reduced susceptibility to ABT-378, had a shift in susceptibility *in vitro* to ABT-378, and had

acquired 1-4 additional mutations above baseline at amino acid residues 4, 10, 16, 24, 33, 36, 46. 54, 62, 63, 70, 71, 72, 82, and 88. Eight of these mutations (residues 4, 16, 33, 36, 62, 70, 72, and 88) were not among the 12 mutations associated with cross-resistance and may be associated with ABT-378 resistance. It is conceivable that any reduced susceptibility to ABT-378 conferred by these mutations functions in the context of cross-resistance mutations and not in the wild type protease. Group 3 (n=8) baseline virus contained 6-9 mutations associated with reduced susceptibility to ABT-378, showed reduced susceptibility *in vitro* to ABT-378 at baseline and displayed a less than twofold change in susceptibility at failure, and, had developed resistance to EFV. According to the sponsor, the development of resistance *in vivo* occurs in individuals having 4-5 baseline mutations associated with reduced susceptibility *in vitro* as a result of the acquisition of additional mutations.

Cross-resistance

a) Antiviral activity *in vitro* of ABT-378 against viruses with resistance to other proteases inhibitors

Archived clinical isolates from 7 patients experiencing viral rebound after being treated for varying lengths of time with RTV monotherapy were tested *in vitro* for susceptibility to ABT-378 (Enclosure #17) using the MTT cytopathicity assay and MT4 infected cells. Isolates from two patients showed little or no change in the EC_{50} values for ABT-378 while experiencing a 3- to 7-fold decrease in susceptibility to RTV. The other five patient isolates had decreases in ABT-378 susceptibility ranging from 6- to 16-fold. This is in contrast to the change for RTV that ranged from 17- to 41-fold.

The activity of ABT-378 against a panel of genotypically and phenotypically characterized clinical isolates (n=38; [redacted]) resistant (>10-fold increase in EC_{50}) to individual and multiple approved PIs was studied. The mean fold change in EC_{50} for each group of viruses relative to the standard WT virus $HIV\text{-}1_{IIIB}$ is shown (Enclosures #18, #19, and #20). There was little if any difference in ABT-378 activity against isolates that exhibited resistance to a single PI (group C and group F, NFV and SQV resistant viruses, respectively) compared to the WT virus standard. ABT-378 was less effective against isolates resistant to a broad range of PIs (group A, mean increase in EC_{50} of 7.23), isolates resistant to the combination of IDV, RTV, and NFV (group B, mean increase in EC_{50} of 5.00), and isolates resistant to the combination of RTV, NFV, and SQV (group E, mean increase in EC_{50} of 6.46).

In clinical study M97-765, cross-resistance of baseline viruses to ABT-378 and approved PIs was assessed using an increase in the EC_{50} value of ≥ 4 fold as an arbitrary cutoff defining cross-resistance (Enclosure #21). These viruses were isolated from patients in treatment failure, the majority of whom had been treated with IDV or NFV. The data are presented as the mean fold change in the EC_{50} value and as the number of viruses with a quantifiable change in the EC_{50} value. Examined individually (data not shown), eighteen (32%) of the 57 viruses were uniformly sensitive to the 4 approved PIs tested (IDV, NFV, SQV, and RTV), 13 (23 %) were resistant to one PI, and another 13 to 3 of 4 PIs. Nine of the 13 isolates resistant to one PI were resistant to NFV, and 9 of the 13 resistant to 3 PIs were from treatment with IDV. Three of the 5 viruses showing resistance to all the approved PIs were from patients who had previously received SQV. These data were combined with a similar analysis done with

the 38 virus isolates from ——— (above) to determine which PI had the greatest shift in susceptibility to ABT-378 for a given shift in susceptibility to an approved PI in virus resistant to one or more PIs, or, in other words, the development of resistance to which protease inhibitor produced the greatest resistance to ABT-378. For each isolate, the change in the EC_{50} values for ABT-378 was plotted on the abscissa and the change for the approved PI, on the ordinate. Subjecting the data to linear regression analysis (Enclosure #22) indicates that ABT-378 is more like RTV and IDV than NFV and SQV in its development of phenotypic resistance since the slopes for these are closer to 1.

Conclusion: ABT-378 shows *in vitro* anti-viral activity against NFV and SQV resistant viruses. RTV and IDV resistant viruses showed low level resistance to ABT-378. The sponsor has not determined the *in vitro* antiviral activity of ABT-378 against APV resistant virus.

b) Effect of serum on ritonavir and ABT-378 activity against ritonavir resistant isolates

The phenotypic susceptibility to ABT-378 of RTV resistant virus selected by serial passage of pNL4-3 virus in cell culture in the presence of increasing concentrations of RTV (Markowitz et al., 1995) was also examined in the presence and absence of human serum. The EC_{50} value for RTV in P22 virus (M46I, L63P, A71V, V82F, I84V) increased, compared to parental pNL4-3 virus, 44-fold in the absence of serum and >72-fold in the presence of 50 % serum (Enclosure #23). The increase in the EC_{50} value for ABT-378 in the absence and presence of serum was 11- and 14-fold, respectively.

The effects of serum on the phenotypic susceptibility to ABT-378 and RTV of the parental viruses pNL4-3 and HXB2 carrying mutations found in RTV resistant viruses is shown in the enclosure (#24). RTV resistance mutations were introduced into a pNL4-3 genetic background by ligating PCR amplified DNA, generated with primers and containing mutant sequences, into a cassette shuttle vector derived from the pNL4-3 provirus, transforming competent bacteria, and transfecting DNA isolated from the resultant subclones into MT4 cells. HXB2 recombinant virus was constructed by subcloning the RT/PCR product made with plasma RNA, and then co-transfecting subcloned DNA and an HXB2 protease deletion clone into MT4 cells which selects recombinants between the PCR amplified DNA and cloned proviral DNA. The EC_{50} values were determined by growing recombinant viruses on MT4 cells and measuring cytopathic effects with the MTT assay. Individual mutations had little effect upon pNL4-3's sensitivity to ABT-378 including 4 different substitutions of the valine at residue 82. However, the combination of I54V and V82T mutations resulted in an approximately 9- to 12-fold decrease in susceptibility to RTV compared to the parental virus and a 9- to 10-fold decrease for ABT-378. Multiple RTV mutations in an HXB2 background showed a 15- to 22-fold decrease in RTV sensitivity compared with a 7- to 13-fold decrease in ABT-378 sensitivity. These studies using virus stocks generated from molecular clones indicate that some RTV resistant mutations, or combinations of mutations, may confer a similar level of resistance to ABT-378.

Conclusion: These studies indicate that virus with a high level of resistance to RTV possesses cross-resistance to ABT-378. The presence of serum reduces RTV's activity more than that of ABT-378, consistent with its greater sequestration by serum proteins.

c) Antiviral activity *in vitro* of other protease inhibitors against virus resistant to ABT-378

i) Activity of approved protease inhibitors against ABT-378 resistant virus selected in cell culture

ABT-378 resistant virus derived from pNL4-3 by serial passage in the presence of increasing concentrations of the drug was tested for sensitivity to other approved PIs (Enclosure #25). The EC_{50} value of ABT-378 for the P17 (passage 17) stock was 338-fold higher than for the parental pNL4-3 virus. An increase of approximately 20-fold was observed for IDV and RTV. Susceptibility to SQV was only slightly decreased with an observed four-fold increase in the EC_{50} value.

ii) Activity of approved protease inhibitors against pNL4-3 parental virus carrying mutations associated with resistance to ABT-378

The effectiveness of other PIs against parental pNL4-3 virus carrying mutations associated with ABT-378 resistance was evaluated (Enclosures #26 and #27). Parental virus carrying 10F/32I/46I/47V/58E/84V mutations had a 27-fold increase in resistance to ABT-378 and 19-, 42-, 13-, and 4-fold increase with respect to RTV, APV, IDV, and NFV, and no change with respect to SQV. Viruses carrying 10I/33F/45I/46I/50V/54V/71V/82F and 10F/23I/32I/34Q/26I/47V/50V mutations had increases of 49-, 72-, 65-, 18-, and 11-fold, and 43-, 9-, 42-, 2-, and 2-fold with respect to ABT-378, RTV, APV, IDV, and NFV, and no change with respect to SQV. A high level of cross-resistance to RTV and APV, and an intermediate to low level to IDV and NFV, may be associated with ABT-378 resistance. ABT-378 resistant virus was susceptible to SQV.

In another experiment, *in vitro* selection of virus showing high level resistance to ABT-378 (64-fold increase in the EC_{50} value), resulted in a 24-fold decrease in susceptibility to RTV (Enclosures #28 and #29). Likewise, in *in vitro* selections for ABT-378 resistant virus, which also contained low RTV concentrations (far below its EC_{50} value), 67- and 70-fold reductions in susceptibility to ABT-378 resulted in 38- and >52-fold reductions in susceptibility to RTV, respectively). It is unlikely that the presence of RTV applied selective pressure since the EC_{50} value for RTV was 8-fold higher than the concentration employed in the selection.

Conclusion: These results show that cross-resistance to RTV, APV, and IDV occurs with ABT-378 resistant virus; whereas ABT-378 resistant virus may remain susceptible to NFV and SQV.

Phenotypic and Genotypic Analysis of Clinical Study Virus Isolates
Overview

The sponsor has conducted many experiments in an attempt to develop relationships between *in vitro* susceptibility to ABT-378, virus genotype, and the virologic response to ABT-378/r therapy. The multiple isolations of ABT-378 resistant virus in cell culture failed to identify a common genetic pathway to resistance. In addition, no ABT-378 resistant viruses were identified in PI naïve individuals failing ABT-378/r therapy (Study M97-720). Failing to identify primary mutations associated with resistance to ABT-378, the sponsor characterized baseline virus isolates from subjects who had

previously failed treatment with a single PR inhibitor (Study M97-765). Baseline virus isolates from each patient were characterized by DNA sequence analysis of the PR gene and by susceptibility in cell culture to ABT-378 and other approved PIs. Again, there was an insufficient number of individuals failing treatment in Study M97-765 to obtain statistically significant data. In a further effort to identify mutations associated with reduced susceptibility to ABT-378, the sponsor characterized baseline virus isolates from Study M98-957 in which individuals had on average failed treatment with 3 PIs. A statistical analysis of the combined data from both clinical studies indicated that the 16 of 112 virus isolates with a >20-fold decrease in susceptibility to ABT-378 contained the L10F/I/R/V, I54L/T/V, L63P, and either V82A/F/T or I84V mutations, and a median of 3 of the following mutations: K20M/R, L24I, M46I/L, F53L, A71I/L/T/V, and L90M.

a) Study M97-765

i) Protocol Synopsis

This protocol was entitled "Assessment of the Pharmacokinetics from a Phase I/II Study of ABT-378/Ritonavir in Combination with Nevirapine and Nucleoside Reverse Transcriptase Inhibitors in Protease Inhibitor Experienced HIV-Infected Subjects (Protocol M97-765)." Seventy subjects with plasma HIV RNA levels between 1,000 and 100,000 copies/mL were enrolled in this study. NNRTI naïve patients, who were failing therapy in their previous PI regimen, were subsequently treated with ABT-378/r plus 2 NRTIs for 2 weeks and then switched to ABT-378/r plus one NRTI and the NNRTI NVP. Antiviral drugs administered to study M97-765 participants at entry are shown in (Enclosure #30).

ii) Virology Substudy

Viral Phenotyping and Genotyping: Plasma samples for viral genotyping and phenotyping were collected at screening or at Day –1. Baseline viral phenotyping was performed by [redacted] using the experimental Antivirogram® assay (Version 3.0). Genotypic changes were reported with respect to the baseline nucleotide sequence of the PR gene from pHXB2 WT laboratory strain.

Samples for HIV RNA levels and flow cytometry [including but not limited to CD4 and CD8 cell counts (absolute and percent)] were obtained at the initial screen, and additionally at < 13 and > 1 days before day –1 if screened > 15 days before day 1, day –1, weeks 1, 2, 4, 6, 8, 10, 12,16, 20, 24, 36, 48, and quarterly visits thereafter until discontinuation from the study.

Plasma HIV RNA levels were measured by the Roche Amplicor HIV-1 Monitor™ assay performed by a central laboratory. Samples measuring ~400 copies/mL were re-assayed using the Roche Amplicor HIV-1 Monitor™ Ultrasensitive assay. It should be noted that the Roche Amplicor HIV-1 Monitor™ Test standard sample preparation has not been shown to be comparable to that same test using the ultrasensitive method of sample preparation.

Baseline for antiviral and immunologic activity parameters were defined as the mean of two measurements taken within 15 days of the subject starting ABT-378/r. If the subject's screening HIV RNA and flow cytometry measurement is within 15 days of

Day 1, the subject's baseline was the mean of screening and Day - 1. If the subject's screening HIV RNA and flow cytometry measurement was more than 15 days prior to Day 1, the subject had to return to the clinic for a second HIV RNA and flow cytometry measurement. The second measurement was taken within 15 days of Day 1 and at least 24 hours before the subject's Day -1 visit.

For all subjects who experienced an increase in viral load to levels > 400 copies/mL after the Week 24 visit, a plasma viral genotyping/phenotyping sample and a repeat viral load sample was obtained within two weeks of the reported elevation. These samples were drawn prior to any change in antiretroviral therapy.

Plasma viral genotyping/phenotyping samples were also to be obtained at all subsequent visits following the initial elevation of viral load to levels >400 copies/mL until the subject's viral load had returned to less than 400 copies/mL.

Specific instructions for preparation and storage of flow cytometry, HIV RNA, archive and other virology samples were provided by the central laboratory, Abbott Laboratories, or their designee.

iii) Results

Baseline phenotypic susceptibility data to all of the drugs in the previous treatment regimen were performed on virus isolates from 56 of 70 individuals in the study. All but two of the virus isolates showed a ≥ 4-fold change in susceptibility for at least one drug, with 36 %, 45 %, and 16 % having a change in one, two, or three drugs in their previous regimen (Enclosure #31).

Cross-resistance of baseline viruses to ABT-378 and approved PIs was described in the "Cross-resistance" section above.

DNA sequence analysis of the PR and RT genes in the virus population was done for viruses having a ≥ 4-fold change in the EC_{50} value for the PI in the treatment regimen at study entry. The most commonly changed amino acids, those found in the PR in > 1 out of 3 viruses, are presented (Enclosure #32). Missense mutations were frequently (>50%) found at amino acid residues 10L, 46M, 63L, 71A, 82V, and 93I in isolates from patients treated with IDV, at amino acid residues 10L, 63L, 71A, 84I, and 90L in SQV treatment isolates, and at amino acid residues 10L, 54I, 62I, 63L, 82V and 93I in RTV isolates. The 13 NFV isolates could be subdivided into those sensitive to the other PIs (n=9) and those showing a significant loss in activity of 3 or 4 PIs (n=3) (data not shown). Those isolates in the first group all contained a change at residue 30 and a >50% frequency at positions 63L, 64I, 71A and 88N. The latter group did not contain a change at position 30, but all of these did contain changes at positions 10L, 63L, 71A, 90L, and 93I. The one patient treated with APV had changes at 46M, 50I, 63L and 71A (data not shown). Fourteen of the 18 viruses sensitive to all the approved PIs had two or more mutations at positions commonly associated with resistance: 10L, 20K, 32V, 36M, 46M, 48G, 54I, 63L, 71A, 77V, 88N and 90L (data not shown).

DNA sequence analysis was also done on the PR gene from the 25 baseline viruses resistant to two or more of the approved PIs (≥4-fold change in EC_{50}; data not shown). Mutations occurring with > 33 % frequency were found at amino acid residues

10L (19/25, 76%), 35E (10/25, 40%), 46M (11/25, 44%), 54I (10/25, 40%), 62I (10/25, 40%), 63L (24/25, 96%), 71A (16/25, 64%), 82V (13/25, 52%), 90L (10/25, 40%) and 93I (16/25, 64%). Four of the 5 viruses showing *in vitro* a quantifiable loss of susceptibility to all approved PIs had changes at positions 10L, 63L, 71A, 84I, and 90L.

Mutations associated with the loss of susceptibility *in vitro* (≥4-fold increase in EC_{50}) to ABT-378 (11/56) were identified in ABT-378 naïve patient isolates (data not shown). Changes occurring at > 33 % frequency were found at amino acid residues 10L (9/11, 82%), 35E (5/11, 45%), 36M (4/11, 36%, 46M (6/11, 55%), 54I (6/11, 55%), 62I (6/11, 55%), 63L (10/11, 91%), 71A (7/11, 64%), 82V (9/11, 82%), and 93I (5/11, 45%). By combining these data with the data from the 38 viruses selected by [redacted] for the *in vitro* study described above, 4 amino acid residues were identified which undergo changes that are statistically associated with ABT-378 loss of susceptibility *in vitro* ($p<0.05$): #'s 10L, 54I, 71A and 82V. The five viruses having a ≥ 10 fold increase in their EC_{50} value had a > 80% incidence of these mutations and a mutation at position 63L. Three of these five viruses (#'s 305, 371, 471, 102366, and 102695) were resistant to the four approved PIs when examined *in vitro*, and the other two were resistant to three of the four and to two of the four.

Characterization of the baseline genotype and phenotype was done in an effort to predict clinical outcomes in treatment with ABT-378/r. Two weeks after the initiation of the clinical study, HIV RNA levels were quantified to see if there was a correlation between baseline resistance phenotype and decline in viral load below the limit of detection (400 copies/ml). This analysis was done for all baseline viruses (Enclosure #33) and for the baseline viruses for those patients who failed to go below 400 copies of viral RNA/ml (Enclosure #34). There was no observed correlation between baseline genotype and clinical outcome measured in either manner. The sponsor suggests that the lack of a correlation may be due to the high *in vivo* concentrations of ABT-378 relative to the EC_{50} values for clinical isolates (Enclosure #35) and the effectiveness of high concentrations of ABT-378 against viruses resistant to low concentrations of ABT-378. This hypothesis remains to be proven.

b) Study M98-957

i) Protocol Synopsis

This protocol was entitled "A Phase II Study of ABT-378/Ritonavir and Efavirenz in HIV Infected Subjects Experienced with Multiple Protease Inhibitors (Protocol M98-957)." Fifty-seven subjects who had failed therapy with two or more PIs, had plasma HIV RNA levels > 1,000 copies/mL, and were NNRTI naïve, were enrolled in this study. Patients were treated with ABT-378/r, EFV and NRTIs.

ii) Virology Substudy

Viral Phenotyping and Genotyping: Plasma samples for viral genotyping and phenotyping were collected at screening and at Day –1. Baseline viral phenotyping was performed by [redacted] using the PhenoSense™ HIV assay. Genotypic changes were reported with respect to the baseline nucleotide sequence of the protease gene from pNL4-3 WT laboratory strain.

Samples for HIV RNA levels and flow cytometry [including but not limited to CD4 and CD8 cell counts (absolute and percent)] were obtained at the initial screen, day –1, weeks 2, 5, 8, 12, 16, 20, 24, 32, 40, 48, and quarterly visits thereafter until discontinuation from the study..

Plasma HIV RNA levels were measured by the Roche Amplicor HIV-1 Monitor™ assay performed by a central laboratory. Samples measuring ~400 copies/mL were re-assayed using the Roche Amplicor HIV-1 Monitor™ Ultrasensitive assay.

For all subjects who achieved a viral load < 400 copies/mL while on study and subsequently experienced an increase in viral load to levels > 400 copies/mL, a repeat viral load determination was made within two weeks of the reported elevation.

Plasma viral genotyping/phenotyping samples were also to be obtained at all subsequent visits following the initial elevation of viral load to levels >400 copies/mL or >0.5 $\log_{10}$ copies/mL above nadir until the subject's viral load had returned to less than 400 copies/mL or <0.5 $\log_{10}$ copies/mL above nadir.

Specific instructions for preparation and storage of flow cytometry, HIV RNA, archive and other virology samples were provided by the central laboratory, Abbott Laboratories, or their designee.

iii) Results

Study M98-957 used NNRTI naïve subjects who had failed on average 3 PIs. Subjects had previously been treated with IDV (86%), RTV (77%), SQV (71%) and NFV (57%; Enclosure #36). Baseline virus isolates were obtained and individuals to be treated with ABT-378/r, EFV, and NRTIs were determined by the investigator.

Baseline virus isolates were characterized with respect to phenotypic susceptibility in cell culture to ABT-378 (Enclosure #37) and other approved PIs, and to viral PR gene nucleotide sequence (Enclosure #38). A statistical analysis of these data combined with data from Study M97-765, and using the Wilcoxon Rank Sum Test, identified amino acid positions associated with reduced susceptibility to PIs *in vitro* (Enclosure #39). Specific amino acid changes in the PR gene that are likely to contribute to reduced susceptibility and of unknown contribution were identified based upon comparisons with previously characterized resistance mutations (Enclosure #40) and the median and mean fold change in EC_{50} associated with each statistically significant mutation determined (Enclosure #41).

Three models were used to search for a possible relationship between fold change in EC_{50} and the number of mutations associated with reduced *in vitro* susceptibility to ABT-378. Model 1 included the subset of baseline isolates containing two or more mutations, Model 2 included all baseline isolates, and Model 3 included only those isolates containing mutations at positions 82, 84 and/or 90 (Enclosures #42 and #43). The results with Models 1 and 3 were very similar. Model 1 intercepts the *x*-axis at approximately 2 mutations (y = 0.0) while model 2 poorly describes the susceptibility of isolates containing 0-2 mutations. Model 1 more successfully calculates the predicted susceptibility to ABT-378 for viruses having 3 or more mutations (Enclosure #44). A model incorporating all sequence variations in the protease gene (Enclosure #45) had a

lower correlation coefficient and slope. This analysis indicated that increased resistance to ABT-378 correlated with an increased number of mutations statistically associated with reduced *in vitro* susceptibility to ABT-378 (Enclosure #46).

A categorical analysis using only data for virus isolated from Study M98-957 participants was also performed to look for statistically significant mutations associated with reduced susceptibility. The EC_{50} cut-off values chosen were 4-, 10-, 20-, and 40-fold (Enclosure #47). Mutations at amino acids 10, 54, and 82 were statistically associated with 4- and 10-fold increases in resistance, mutations at 20, 54, and 82 were associated with a 20-fold increase, and mutations at 54 and 82 were associated with a 40-fold increase. These results indicated that a continuous variable combining data from Studies M97-765 and M98-957 might better identify mutations associated with reduced susceptibility to ABT-378.

A total of 16 virus isolates from both studies (M97-765 and M98-957) displaying a >20-fold reduction in *in vitro* susceptibility (Enclosure #48) were checked for the mutations identified in the continuous variable analysis. Each of these viruses contained mutations at the codons for amino acid residues 10, 54, 63, and 82 or 84, and a median of 3 mutations at the codons for residues 20, 24, 46, 53, 71 and 90. Their median fold change in EC_{50} for ABT-378 was 40, for IDV 40, for NFV 56, RTV 92, SQV 18, and APV 6.5 (15 isolates). 14/16, 16/16, 16/16, 9/16, and 1/15 of these viruses had a >20-fold reduced susceptibility to IDV, NFV, RTV, SQV, and APV, respectively.

Conclusion: A continuous variable statistical analysis of the combined phenotypic and genotypic data for baseline isolates from PI experienced patients (Studies M97-765 and M98-957) identified 12 mutations associated with reduced *in vitro* susceptibility to ABT-378. The sponsor concludes that the genetic threshold for ABT-378 resistance *in vitro* appears to be high, usually requiring the acquisition of a core group of 4 mutations at amino acids 10, 54, 63, and 82/84, and a median of 3-4 additional mutations at residues 20, 24, 46, 53, 71, 73 and 90.

The legitimacy of combining the data from the phenotypic and genotypic analysis of the baseline viruses obtained in Studies M97-765 and M98-957 has yet to be established. The analysis of baseline viruses from Study M97-765 was done with the Virco Antivirogram™ method (version 3.0) using pHXB2 laboratory strain as the wild-type virus standard for comparison. Study M98-957 utilized the [redacted] HIV assay and pNL4-3 laboratory strain as the standard. Both the [redacted] assays are not FDA approved. Without appropriate comparability data submitted to the FDA for review, it is not logical to evaluate these data as a single set. The relative resistance of pHXB2 and pNL4-3 WT standard viruses to ABT-378 is not provided and possible differences in linearity of the assays is not addressed. Therefore, the sponsors conclusion here has to be regarded with legitimate discretion.

An internal categorical analysis of these data was conducted by FDA statisticians. Baseline virus isolates from protease inhibitor experienced patients in Studies M97-765 and M98-957 (n=111) were segregated into those having <10-fold and ≥10-fold reduced *in vitro* susceptibility to ABT-378, and mutations statistically associated with ≥10-fold reduced susceptibility due to cross-resistance ($p \leq 0.001$) identified. Mutations at three amino acid residues were found: 20K, 54I, and 82V.

5 page(s) of revised draft labeling has been redacted from this portion of the review.

CONCLUSIONS

Abbott Laboratories has submitted an original NDA #21-226 for a new protease inhibitor, ABT-378, to treat HIV infection in adults. Data supporting its mechanism of action, and defining its *in vitro* anti-retroviral activity and cytotoxicity are presented. ABT-378 has high specific activity and a high selectivity index. In addition, the levels of cross-resistance of ABT-378 resistant virus to other approved PIs, and of cross-resistance of viruses resistant to approved PIs to ABT-378 have been quantified. Mutations frequently associated with ABT-378 resistance have been characterized. It is likely that ABT-378 will be used in combination with other approved anti-retroviral agents. The sponsor has not looked for possible antagonistic effects among these and ABT-378. With respect to microbiology, approval of this NDA submission is supported pending acceptance of the final draft of the label.

ABT-378 is a peptidometric inhibitor of HIV protease, which is necessary for cleavage of the Gag and Gag-Pol polyproteins and maturation of virus particles. It was derived from RTV by incorporating changes to circumvent the major mutation at 82V leading to RTV resistance, to avoid binding by serum proteins, and to increase its solubility.

ABT-378 has potent *in vitro* anti-viral activity against WT virus (EC_{50} = 17 ± 4 nM in 0% serum and 102 ± 44 nM in 50 % serum), and similar activity against NFV and SQV resistant virus with high selectivity indices. Viruses resistant to IDV and RTV show low level resistance *in vitro* to ABT-378, but may still be susceptible to ABT-378 *in vivo* due to the high (relative to the EC_{50} value) effective concentrations maintained, *i.e.*, the trough values are much greater than the EC_{50} value. Antiviral activity against APV resistant virus has yet to be determined.

Amino acid residues that are frequently mutated in ABT-378 resistant virus isolates obtained by *in vitro* passage are #'s 10L, 46M and 47I. Isolation of virus resistant to high concentrations of ABT-378 in four independent experiments identified the methionine residue at position 46 as being associated with ABT-378 resistance. However, the difference amino acid residues mutated in the evolution of highly resistant virus in multiple experiments in cell culture indicated multiple pathways and suggested that analysis of clinical isolates might be more informative. Characterization of clinical isolates resistant to one or more PIs indicates that there is not an absolute rule for mutation at residue 46 for resistance. These results are consistent with a high genetic threshold for the development of reduced susceptibility to ABT-378.

ABT-378 is not as tightly bound by serum proteins as are many of the other approved PIs. The decreased serum binding and the increase in AUC and trough levels due to co-dosing of RTV, which results in trough values several fold above the EC_{50} for

ABT-378, may be important in avoiding the appearance of resistant virus and virus rebound.

The development of resistance to ABT-378 appears to have a high genetic threshold. Genotypic and phenotypic analyses of baseline isolates in individuals who have failed treatment with other PIs indicate that 4-5 mutations associated with resistance to other PIs and the acquisition of 1-4 additional mutations, some of which have previously been associated with resistance to other PIs, can lead to reduced susceptibility *in vitro* to ABT-378.

PHASE IV COMMITMENTS

1. Analyze isolates from patients with virologic failure on KALETRA to determine associations between protease mutations and *in vitro* shifts in susceptibility to define the resistance profile of lopinavir.
2. Continue genotypic and phenotypic analysis of isolates from patients in study 765 and 957 who experience loss of virologic response.
3. Assessment of the genotypic basis of drug susceptibility attributable to extragenic sites, such as the protease cleavage sites.
4. Conduct *in vitro* combination activity studies with other protease inhibitors.
5. Evaluate the cross-resistance potential between KALETRA and amprenavir.

/S/

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CONCURRENCES

/S/

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cc:
HFD-530/Original NDA #21-226
HFD-530/Division File
HFD-530/Review Micro
HFD-530/S. Lynche, RPM
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