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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-814

Microbiology Review(s)

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21814 **SN:** 000 **DATE REVIEWED:** 6/15/05
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 21814 (capsules) **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address: Boehringer Ingelheim Pharmaceuticals, Inc.
900 Ridgebury Rd
Ridgefield, CT 06877

Initial Submission Dates:

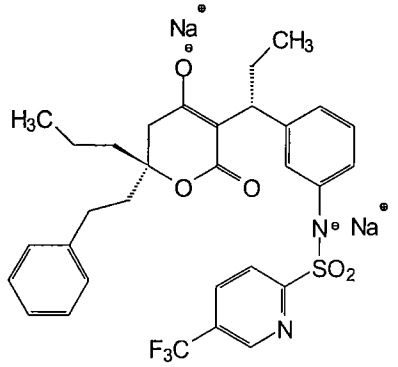
Correspondence Date:	12/21/2004
CDER Receipt Date:	12/22/2004
Assigned Date:	10/19/2004
Review Complete Date:	6/15/2005
PDUFA Date:	6/22/2005

Amendments:
Related/Supporting Documents: IND51979

Product Name(s)
Proprietary: Aptivus
Non-Proprietary/USAN: tipranavir (TPV)
Code Name/Number: PNU-140690

Empirical formula: $C_{31}H_{31}F_3N_2O_5SNa_2$
Chemical Name: [R-R(*,R*)]-N-[3-[1-[5,6-Dihydro-4-hydroxy-2-oxo-6-(2-phenylethyl)-6-propyl-2H-pyran-3-y1]propyl]phenyl]-5-(trifluoromethyl)-2-pyridinesulfonamide disodium salt
Molecular mass: 646.63

Structural Formula:



TIPRANAVIR

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21814 **SN:** 000 **DATE REVIEWED:** 6/15/05
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

Drug category: antiviral
Dosage Form(s): 250-mg soft elastic capsules/Oral; co-administration of ritonavir as 100-mg soft gelatin capsules; 500 TPV/200 RTV mg BID
Route(s) of Administration: Oral
Indication(s): Combination antiretroviral treatment of HIV-1 infected adult patients with evidence of viral replication, who are heavily treatment-experienced or have HIV-1 strains resistant to multiple protease inhibitors.

Dispensed: Rx _X_ **OTC**___

Abbreviations: ABC, abacavir; APV, amprenavir; ATV, atazanavir; AZT, zidovudine; CPI, comparator protease inhibitor; ddI, didanosine; d4T, stavudine; DLV, delavirdine; EFV, efavirenz; FTC, emtricitabine; HAART, highly active antiretroviral therapy; HIV-1, human immunodeficiency virus-1; IC, inhibitory concentration; IDV, indinavir; LOCF, last observation carried forward; LPV, lopinavir; NFV, nelfinavir; NVP, nevirapine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OBT, optimized background therapy; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; /r, ritonavir boosted; RT, reverse transcriptase; SQV, saquinavir; T20, enfuvirtide; TNF, tenofovir; TPV, tipranavir; 3TC, lamivudine;

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Executive Summary

Tipranavir (TPV), an HIV-1 protease inhibitor, has 50% inhibitory concentrations (IC_{50} value) ranging from 40 to 390 nM against laboratory HIV-1 strains grown in vitro in PBMCs and cell lines. The average IC_{50} value for multi PI-resistant clinical HIV-1 isolates was 240 nM (range 50 to 380 nM). Human plasma binding resulted in a 4-fold decrease in the antiviral activity. Ninety percent (94/105) of HIV-1 isolates resistant to APV, ATV, IDV, LPV, NFV, RTV, or SQV had $\leq$3-fold decreased susceptibility to TPV.

Because TPV will be administered to HIV-positive patients as part of a HAART regimen comprising several antiretroviral agents, the activity of TPV in combination with other antiviral drugs was determined in cell culture to assess the impact of potential in vitro drug interactions on overall antiviral activity. Additive to antagonistic relationships were seen with combinations of TPV with other PIs. Combinations of TPV with the NRTIs were generally additive, but additive to antagonistic for TPV in combination with ddI and 3TC. Combinations of TPV with the NNRTIs DLV and NVP were additive and with EFV were additive to antagonistic. Activity of TPV with the fusion inhibitor enfuvirtide (T20) was synergistic.

In Vitro Selection of TPV-Resistant Viruses
TPV-resistant viruses were selected in vitro when wild-type HIV-1_{NL4-3} was serially passaged in the presence of increasing concentrations of TPV in tissue culture. Amino acid substitutions L33F and I84V emerged initially at passage 16 (0.8 μM), producing a 1.7-fold decrease in TPV susceptibility. Viruses with >10-fold decreased TPV susceptibility were selected at drug concentrations of 5 μM with the accumulation of six protease mutations (I13V, V32I, L33F, K45I, V82L, I84V). After 70 serial passages (9 months), HIV-1 variants with 70-fold decreased susceptibility to TPV were selected and had 10 mutations arising in this order: L33F, I84V, K45I, I13V, V32I, V82L, M36I, A71V, L10F, and I54V. Mutations in the CA/P2 protease cleavage site and transframe region were also detected by passage 39. TPV-resistant viruses showed decreased susceptibility to all currently available protease inhibitors except SQV. SQV had a 2.5-fold reduced susceptibility to the TPV-resistant virus with 10 protease mutations.

Clinical TPV Resistance
The efficacy of ritonavir boosted tipranavir (TPV/r) was examined in treatment-experienced HIV-infected subjects in two pivotal phase III trials, study 012 (RESIST 1) and study 048 (RESIST 2). Genotypes from 1482 isolates and 454 phenotypes from both studies were submitted for review. In the comparator PI arm (CPI/r), most patients received LPV/r (n=358) followed by APV/r (n=194), SQV/r (n=162) and IDV/r (n=23). The patient populations in RESIST 1 and 2 were highly treatment-experienced with a median number of 4 (range 1-7) PIs received prior to study. In the combined RESIST trials at baseline, 97% of the isolates were resistant to at least one PI, 95% of the isolates were resistant to at least one NRTI, and >75% of the isolates were resistant to at least one NNRTI. The treatment arms from both studies were balanced with respect to baseline

genotypic and phenotypic resistance. Baseline phenotypic resistance was equivalent between the TPV/r arm (n=745) and the CPI/r arm (n=737) with 30% of the isolates resistant to TPV at baseline and 80-90% of the isolates resistant to the other PIs - APV, ATV, IDV, LPV, NFV, RTV or SQV. The number of PI-resistance mutations was equivalent between the TPV/r and CPI/r arms in RESIST 1 and 2 and the median number of baseline PI, NRTI and NNRTI mutations was equivalent between arms in both studies.

Mutations Developing on TPV Treatment
TPV/r-resistant isolates were analyzed from treatment-experienced patients in the phase II study 052 (n=32) and the phase III studies RESIST 1 and 2 (n =59) who experienced virologic failure. The most common mutations that developed in greater than 20% of these TPV/r virologic failure isolates were L33V/I/F, V82T and I84V. Other mutations that developed in 10 to 20% of the TPV/r virologic failure isolates included L10V/I/S, I13V, E35D/G/N, I47V, K55R, V82L and L89V/M/W. In RESIST 1 and 2, TPV/r resistance developed in the virologic failures (n=59) at an average of 38 weeks with a median decrease of >14-fold in TPV susceptibility from baseline. The resistance profile in treatment-naive subjects has not been characterized.

Baseline Genotype/Phenotype and Virologic Outcome Analyses
The FDA analyses of virologic outcome by baseline resistance are based on the As-Treated population from studies RESIST 1 and 2. To assess outcome, several endpoints including the primary endpoint (proportion of responders with confirmed 1 $\log_{10}$ decrease at Week 24), DAVG24, and median change from baseline at weeks 2, 4, 8, 16, and 24 were evaluated. In addition, because subjects were stratified based on enfuvirtide (T20) use, we examined virologic outcomes in three separate groups - overall (All), subjects not receiving T20 (No T20), and subjects receiving T20 (+T20) as part of the optimized background regimen. We focused on the No T20 group in order to assess baseline resistance predictors of virologic success and failure for TPV/r without the additive effect of T20 use on the overall response.

Both the number and type of baseline PI mutations affected response rates in RESIST 1 and 2. Virologic responses were analyzed by the presence at baseline of substitutions at each of 25 different protease amino acid positions using both the primary endpoint (>1$\log_{10}$ decrease from baseline) and DAVG24. Reduced virologic responses were seen in TPV/r-treated subjects when isolates had a baseline amino acid substitution at position I13, V32, M36, I47, Q58, D60 or I84. The reduction in virologic responses for these baseline substitutions was most prominent in the No T20 subgroup. Virologic responses were similar or greater than the overall responses for each subgroup (All, No T20, +T20) when these amino acid positions were wild-type. In addition, virologic responses to substitutions at position V82 varied depending on the amino acid substitution. Interestingly, substitutions V82S or F or I or L, but not V82A or T or C, had reduced virologic responses compared to the overall response.

Analyses were also conducted to assess virologic outcome by the number of PI mutations present at baseline. In these analyses, any changes at protease amino acid positions -

D30, V32, M36, M46, I47, G48, I50, I54, F53, V82, I84, N88 and L90 were counted if present at baseline. These PI mutations were used based on their association with reduced susceptibility to currently approved PIs, as reported in various publications.

Regardless of the endpoint used for these analyses, the response rates were greater for the TPV/r treatment arm compared to the CPI/r arm. Within each treatment arm, response rates were similar to or greater than the overall response rates for subjects with one to four PI mutations at baseline. Response rates were reduced if five or more PI-associated mutations were present at baseline. For subjects who did not use T20, 28% in the TPV/r arm and 11% in the CPI/r arm had a confirmed 1 $\log_{10}$ decrease at Week 24 if they had five or more PI mutations in their HIV at baseline. The subjects with five or more PI mutations in their HIV at baseline and not receiving T20 in their OBT achieved a 0.86 $\log_{10}$ median DAVG24 decrease in viral load on TPV/r treatment compared to a 0.23 $\log_{10}$ median DAVG24 decrease in viral load on CPI/r treatment. In general, regardless of the number of baseline PI mutations or T20 use, the TPV/r arm had approximately 20% more responders by the primary endpoint (confirmed 1 $\log_{10}$ decrease at Week 24) and greater declines in viral load by median DAVG24 than the CPI/r arm.

An examination of the median change from baseline of HIV RNA at weeks 2, 4, 8, 16 and 24 by number of baseline PI mutations (1-4 and 5+) showed the largest decline in viral load by Week 2 for all groups with the greatest decline observed in the TPV/r arms . A 1.5 $\log_{10}$ decrease in viral load at Week 2 was observed for subjects receiving TPV/r regardless of the number of baseline PI mutations (1-4 or 5+). Sustained viral load decreases (1.5 – 2 $\log_{10}$) through Week 24 were observed in subjects receiving TPV/r and T20. However, subjects who received TPV/r without T20 and who had five or more baseline PI mutations group began to lose antiviral response between Weeks 4 and 8.

Proportion of Responders by Baseline TPV Phenotype
TPV/r response rates were also assessed by baseline TPV phenotype. Again, we focused on the No T20 group in order to more accurately assess the effect of baseline phenotype on virologic success for TPV/r. With no T20 use, the proportion of responders was 45% if the shift in IC_{50} value from reference of TPV susceptibility was 3-fold or less at baseline. The proportion of responders decreased to 21% when the TPV baseline phenotype values were >3- to 10-fold and 0% when TPV baseline phenotype values were >10-fold.

Conclusions

TPV is a novel protease inhibitor with antiviral activity against multi PI-resistant clinical HIV-1 isolates. The most common protease amino acid substitutions that developed in >20% of isolates from treatment-experienced subjects who failed on TPV/r treatment were L10I/V/S, I13V, L33V/I/F, M36V/I/L V82T, V82L, and I84V. The resistance profile in treatment-naive subjects has not been characterized. Both the number and type of baseline PI mutations affected response rates to TPV/r in RESIST 1 and 2. Virologic response rates in TPV/r-treated subjects were reduced when isolates with substitutions at

amino acid positions I13, V32, M36, I47, Q58, D60 or I84 and substitutions V82S/F/I/L were present at baseline. Virologic responses to TPV/r at week 24 decreased when the number of baseline PI mutations was 5 or more. Subjects taking enfuvirtide with TPV/r were able to achieve >1.5 $\log_{10}$ reductions in viral load from baseline out to 24 weeks even if they had 5 or more baseline PI mutations. Virologic responses to TPV/r in RESIST 1 and 2 decreased when the baseline phenotype for TPV was a >3 shift in susceptibility with respect to wild-type reference virus.

1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for is approvable with respect to microbiology for combination antiretroviral treatment of HIV-1 infected adult patients with evidence of viral replication, who are heavily treatment-experienced or have HIV-1 strains resistant to multiple protease inhibitors

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

1. Evaluate drug resistance in viruses from patients with virologic rebound on initial ART (in the 1182.33 naïve study), please submit data in resistance template.

   Protocol Submission: Completed
   Final report Submission: September 30, 2006

2. Evaluate cleavage site mutations in rebound samples on tipranavir.

2. Summary of OND Microbiology Assessments

2.1. Brief Overview of the Microbiological Program

2.1.1. Non-clinical

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combination with other antiviral drugs was determined in cell culture to assess the impact of potential in vitro drug interactions on overall antiviral activity. Additive to antagonistic relationships were seen with combinations of TPV with other PIs. Combinations of TPV with the NRTIs were generally additive, but additive to antagonistic for TPV in combination with ddI and 3TC. Combinations of TPV with the NNRTIs DLV and NVP were additive and with EFV were additive to antagonistic. Activity of TPV with the fusion inhibitor enfuvirtide (T20) was synergistic.

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2.1.2. Clinical Microbiology

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3. Administrative

3.1. Reviewer's Signature(s)

_____
Lisa K. Naeger, Ph.D.
Sr. Microbiologist, HFD-530

3.2. Concurrence

HFD-530/Signatory Authority _____ Signature _____ Date
HFD-530/Micro TL _____ Signature _____ Date

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY DRAFT REVIEW
NDA: 21814 **SN:** 000 **DATE REVIEWED:** 6/15/05
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

OND Microbiology Review

1. Introduction and Background

1.1. Important Milestones in Product Development

1.1.1. Methodology

Genotypic Methods
Genotypes from 1482 isolates were submitted for review. Genotypes were determined by two different methods in RESIST 1 and 2. In RESIST 1, the TruGene assay version 1.0 was used. If a sample could not be amplified and genotyped, version 1.5 was used. In RESIST 2, the Virco Virtual Phenotype assay was used for samples from Europe and the TruGene assay was used for samples from Latin America and Australia. Genotypic resistance testing was used to stratify patients according to pre-selected protease inhibitors (APV/r, IDV/r, LPV/r, SQV/r). For the purpose of stratification, protease inhibitor sensitivity was interpreted from genotypic reports as not resistant, possibly resistant or resistant. Differences in interpretation between the two studies could be attributed to the different algorithms used in the TruGene and Virtual Phenotype assays. In addition, phenotypic cut-offs used to determine the resistance strata are largely based on unboosted PI data, whereas ritonavir-boosted PIs were used in the RESIST trials.

Phenotypic Methods
Phenotypes (n= 454) were submitted for review with 361 from the TPV/r arm and 93 from the CPI/r arm. Both the Virco Antivirogram® and the Virologic Phenosense™ assays were used to determine phenotypes. The Antivirogram assay was used for the randomly selected baseline samples from the phase III trials. Baseline TPV phenotypes were measured with both assays in the Phase II studies. In study 051, the Antivirogram was used for baseline samples and the Phenosense assay was used for 80 randomly selected baseline samples. In study 052, both assays were used for all the baseline samples and the Phenosense assay was used for selected on-treatment samples. The fold change in IC_{50} values for TPV and the other PIs was similar whether assayed by the VIRCO Antivirogram or Virologic Phenosense assay ($r = 0.83$ and $r = 0.92$, respectively).

1.1.2. Major microbiological issues that arose during product development.
In vitro combination studies were requested because of the drug interactions between TPV and other PIs seen in study 051. Data examining the activity of TPV against different clades of HIV-1 and HIV-2 was also requested.

We requested analyses of on-treatment samples from virologic failures on TPV treatment because the applicant did not intend to submit this data until traditional approval.

1.2. State of antimicrobials used for the indication (s) sought:

An estimated 40 million people worldwide were infected with HIV in 2001 and 3 million died from AIDS. Since HAART regimens have been introduced, the number of AIDS cases has decreased dramatically. HAART does not eradicate HIV from patients completely and even though the number of HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that most HIV-infected patients will require antiretroviral therapy throughout their lives.

There are currently twenty FDA-approved anti-HIV drugs including seven PIs (amprenavir/fosamprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir), eight NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), three NNRTIs (delavirdine, efavirenz, nevirapine) and the fusion inhibitor T-20 (enfuvirtide). PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV protease enzyme, which is necessary for the production of mature virions, resulting in defective particles that are unable to infect new cells. NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and by incorporating into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. Enfuvirtide (T-20) is a gp41 fusion inhibitor preventing the joining of the viral and cellular membranes necessary for virus entry.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis), and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important determinant of successful virologic suppression for patients on HAART. Regimens that are well-tolerated and easy to administer with a few pills once daily are likely to aid in patient compliance and improve clinical outcomes. There is a need for new anti-HIV drugs that are well-tolerated and easy to use with new modes of action and low likelihood of viral resistance development. Additionally, drugs that are effective against viruses resistant to all currently approved drugs are needed for the heavily treatment-experienced population.

2. Non-clinical Microbiology

Mechanism of Action

Tipranavir is a non-peptidic protease inhibitor of HIV belonging to the class of 4-hydroxy-5,6-dihydro-2-pyrone sulfonamides. In enzymatic assays, TPV demonstrates inhibition of the cleavage of a peptidic substrate by the HIV-1 protease with an inhibition constant (K_i) of 8.9 + 6.8 pM. Using the same assay, TPV also inhibits the activities of HIV-2 protease (K_i <1 μM) and of mutant HIV-1 proteases carrying the mutations V82A (K_i = 0.003 μM) or V82F/184V (K_i = 0.25 μM). Selectivity for the HIV protease was demonstrated by high K_i values against the human aspartyl proteases pepsin (K_i = 2 μM), cathepsin D (K_i = 15 μM), and cathepsin F (K_i = 9 μM).

Antiviral Activity In Vitro

The in vitro antiviral activity of TPV against laboratory HIV strains and clinical HIV isolates was evaluated in acutely and chronically infected lymphoblastic and monocytic cell lines and peripheral blood lymphocytes (PBMC). Cell culture toxicity was determined using a MTT assay. The activity of TPV against laboratory strains of wild type HIV is shown in Table 1 (Report U04-3215, page 52).

Table 1. Antiviral activity of TPV against wild type laboratory HIV strains in acute and chronic models of infection using PBMC and different cell lines

Assay[1]	IC_{50}[2]	IC_{90}[2]	$CCTD_{50}$[3]	$CCTD_{90}$[3]	Selectivity Index[4]
Acute H9/HIV-1 $_{IIIB}$	0.04 ± 0.01	0.16 ± 0.07	21.1	38.7	528
Acute PBMC/HIV-1 $_{JR-CSF}$	0.05	0.18	17.5 ± 0.05	34.8 ± 7.4	350
Acute U397/HIV-1 $_{IIIB}$	0.11	0.55	7.4	16.8	67
Chronic H9/HIV-1 $_{IIIB}$	0.39	1.9	Not done	Not done	Not done

1 Results represent means and standard errors of at least two repeated experiments. Concentrations expressed in μM.

2 Compound concentration required to inhibit 50% or 90% of HIV-1 p24 antigen production compared with drug free controls.

3 Cell culture toxicity dose (CCTD) required to inhibit 50 or 90% of metabolism as determined by an MTT assay.

4 Calculated by dividing $CCTD_{50}$ by IC_{50}.

Since one of the target populations for TPV is treatment-experienced HIV positive patients, the antiviral activity of TPV has been tested against HIV-1 isolates resistant to currently available protease inhibitors. The average IC_{90} value for multidrug resistant clinical HIV-1 isolates was 619 nM (range 31 to 860 nM) and the average IC_{50} value for multi PI-resistant clinical HIV-1 isolates was 240 nM (range 50 to 380 nM). The isolates shown in Table 2 (Report U04-3215, page 53) had an IC_{50} value of >0.1 μM for indinavir or nelfinavir and had been obtained from patients who had increasing viral loads while taking these drugs.

Table 2. Phenotypic susceptibility to tipranavir of HIV-l isolates resistant to IDV and/or NFV

Clinical isolate[1]	IC_{50}	IC_{90}
006	0.191 ± 0.01	0.860 ± 0.04
003	0.280 ± 0.02	0.560 ± 0.05
007	0.046 ± 0.01	0.310 ± 0.02
008	0.097 ± 0.04	0.650 ± 0.01
004	0.315 ± 0.01	0.740 ± 0.07
010	0.103 ± 0.07	0.340 ± 0.02
001	0.355 ± 0.05	0.670 ± 0.01
002	0.363 ± 0.03	0.660 ± 0.08
009	0.301 ± 0.09	0.668 ± 0.04
005	0.383 ± 0.05	0.730 ± 0.07

1 Results (mean ± standard error) of three experiments are presented.

In addition, the TPV susceptibility of 134 isolates obtained from multiple PI-experienced HIV-positive patients (127 different subjects) were tested using the VIRCO Antivirogram method. Identified were 105 variants resistant (10-fold or greater increase in IC_{50} value) to at least three protease inhibitors and 29 variants resistant (10-fold or greater increase in IC_{50} value) to a single protease inhibitor. Of the 105 highly cross-resistant variants, 98% had less than a 10-fold decrease in susceptibility to TPV and 90% had less than a 4-fold decrease in susceptibility to TPV.

TPV demonstrates antiviral activity in vitro against a broad panel of HIV-1 group M non-clade B isolates (A, C, D, F, G, H, CRF01 AE, CRF02 AG, CRF12 BF) with EC_{50} values ranging from 28 to 116 nM with the mean EC_{50} values for each clade shown in Table 3. The mean fold change in TPV susceptibility for each clade compared to the reference strain never exceeded 1.6-fold. Group O and HIV-2 isolates have reduced susceptibility in vitro to TPV with EC_{50} values ranging from 164 -1,000 nM and 233-522 nM, respectively (Table 4).

Table 3. Mean TPV EC_{50} values for HIV-1 Clades

HIV-1 Clade	Mean TPV EC_{50} Value (nM)
Ref NL4-3 (clade B)	62
A/A2	57
B	52
C	62
D	80
F/F1	97
G	41
H	81
CRF01 AE	61
CRF02 AG	77
CRF12 BF	33

Table 4. TPV EC_{50} Values for HIV-2 and Group O Viruses

Strain	HIV-1 Clade	TPV EC_{50} value (nM)
5512	B	125
BCF01 (+Y181C)	O	>333 (100% inhibition at 1000 nM)
BCF11	O	164
MVP5180	O	>333 (50% inhibition at 1000 nM)
CBL-20	HIV-2	548
CBL-23	HIV-2	246
MVP 15132	HIV-2	45

Serum Binding

Most protease inhibitors are highly protein bound, and this binding can limit antiviral activity. To determine a target TPV trough concentration to be achieved during clinical trials, the degree of change in the IC_{90} value caused by protein binding was determined (serum shift). The target trough TPV concentration was assumed to represent the product of the average TPV IC_{90} value for resistant HIV-1 isolates (approximately 600 nM) multiplied by the serum shift and a "safety factor" of 10. In equilibrium dialysis experiments using whole human plasma, the fraction of TPV (assayed at 20 μM) bound to plasma protein was 99.97%. In cell culture medium (60% fetal bovine serum), the fraction of TPV bound to proteins was dependent on the concentration of TPV used, with saturation above 2 μM. In antiviral activity assays, it was determined that the addition of 33% or 75% human plasma resulted in a 1.6-fold and 4-fold shift in the in vitro antiviral activity of TPV. A serum shift of 3.75 was used. The estimated target TPV trough concentration would be: 0.6 μM X 3.75 X 10, giving a result of 22.5 μM. Therefore, an initial TPV target trough of 20 μM was chosen.

In vitro Anti-HIV Activity of Drug Combinations

Because TPV will be administered to HIV-positive patients as part of a HAART regimen comprising several antiretroviral agents, the activity of TPV in combination with other agents was determined in cell culture. A panel of antiviral agents including seven NRTIs (3TC, ABC, AZT, d4T, ddI, FTC, TNF), three NNRTIs (DLV, EFV, NVP), seven PIs (APV, ATV, IDV, LPV, NFV, RTV, SQV), one fusion inhibitor enfuvirtide (T20), the anti-HCV drug ribavirin (RBV), and the anti-HBV drug adefovir (PMEA) were tested alone or in combination with TPV to assess the impact of potential in vitro drug interactions on the overall antiviral activity against wild-type HIV-1 in cell culture. The degree of drug interactions was determined by the median-effect principle using the combination index (CI) calculation and the "mutually exclusive" drug interaction condition.

The majority of combinations showed less than 5% toxicity. Only the combinations TPV:TNF and TPV:FTC showed toxicity levels between 5% to 14% at the highest

concentration of the ratios tested while TPV:RBV showed 26% toxicity at the highest concentration of the 1:100 ratio only. The toxicity levels greater than 5% observed when TPV was combined with TNF, FTC, or RBV are consistent with the observed toxicities of these agents when tested alone.

The combination index (CI) values at the EC_{50} and EC_{75} values for the various TPV:drug combination ratios corresponding to equipotent amounts of both drugs were determined. The interpretation of the CI values was based on the system recommended by Chou and Hayball (Calcusyn Windows software, User manual, Biosoft 1996) but with fewer descriptive levels in order to be consistent with the observed intrinsic variability of the antiviral replication assay used. Thus a CI value of 0.8-1.2 indicates an additive effect. CI values incrementally larger than 1.2 suggest increasing level of antagonism while CI values incrementally smaller than 0.8 suggest increasing level of synergy.

The AZT:ddI combination at a ratio 1:100 (i.e., closely corresponding to equipotent amounts of both drugs) has been reported to show synergistic interactions in cell culture and was selected as a control for synergy. When AZT was combined with ddI at the ratio 1:100, the CI values at the EC_{50} and EC_{75} values ranged from 0.12 to 0.42 consistent with synergistic combination of the two drugs. Other drug combinations have been reported to act antagonistically such as AZT:RBV, LPV:APV, IDV:SQV and AZT:d4T. The combination of AZT:d4T was used as a control for antagonism. The antagonism between AZT:d4T is mechanistically interpreted in terms of a competition between these two structurally related nucleosides for the same phosphorylation pathway in cells and has been confirmed in the clinic. However, conflicting results showing evidence of synergy, additivity or slight antagonism for the AZT:d4T combination have also been reported in cell culture against wild type HIV-1. In the experiments in this report, the AZT:d4T combination showed a level of synergy even after expanding the range to high drug ratios. None of the combinations cited above as antagonistic showed clear evidence of antagonism in this report. The sponsor states that they are trying to identify a clear positive control for antagonism in their laboratory.

Eqipotent combinations of TPV with PIs generally ranged from additive to antagonistic at the 50% and 75% inhibition endpoint (Table 5). The highest level of antagonism (CI = 1.47) was observed with TPV:ATV at a 10:1 ratio and at 75% inhibition. This ratio approaches the ratio of 11:1 calculated based on the C_{max} values of 80 μM and 7 μM independently observed in patients treated with TPV and ATV respectively (at the recommended doses of 500 mg TPV/200 mg RTV bid and at 300 mg ATV/100 mg RTV qd). The combinations of TPV with NRTIs were generally additive at the 50% inhibition and the 75% inhibition level (Table 5). The pairs TPV:3TC and TPV:ddI showed additive to antagonistic effects (at 50% and 75% inhibition) depending on the ratio tested. A mean CI of 1.38 (antagonism) was observed at the TPV:ddI ratio of 10:1 and mean CI of 1.31 (antagonism) was observed at the TPV:3TC ratio of 3:1. The combinations of TPV with the two NNRTIs were additive with NVP and additive to antagonistic with EFV.

The combination of TPV with the HIV fusion inhibitor enfuvirtide was clearly indicative of synergy at all ratios at both 50% and 75% inhibition. This combination is the most synergistic observed in this study. The combination of TPV with ribavirin was additive to synergistic at both 50% and 75% inhibition while the combination with PMEA (adefovir) was additive.

Table 5. Summary of In Vitro Drug Combination Studies with TPV

Drugs	Ratio (Equipotent of both drugs)	Mean CI at 50% inhibition	Mean CI at 75% inhibition	Assessment
TPV:APV	3:1	1.13	1.33	Additive/antagonistic
TPV:ATV	10:1	0.84	1.47	Additive/antagonistic
TPV:IDV	3:1	0.82	1.13	Additive/antagonistic
TPV:LPV	10:1	1.24	0.76	Additive/antagonistic
TPV:NFV	10:1	0.88	0.77	Additive
TPV:RTV	3:1	1.06	0.89	Additive/antagonistic
TPV:SQV	10:1	1.01	1.34	Additive/antagonistic
TPV:3TC	3:1	1.00	1.31	Additive/antagonistic
TPV:ABC	1:10	0.53	0.59	Additive
TPV:AZT	3:1	0.98	0.91	Additive
TPV:d4T	1:10	0.76	0.96	Additive
TPV:ddI	1:10	0.98	0.72	Additive/antagonistic
TPV:FTC	3:1	1.01	0.84	Additive
TPV:TNF	1:10	0.70	0.85	Additive
TPV:EFV	100:1	1.09	1.19	Additive/antagonistic
TPV:NVP	5:1	0.78	0.88	Additive
TPV:DLV	10:1	0.56	0.65	Additive/synergistic
TPV:T20	1:3	0.46	0.62	synergistic
TPV:RBV	1:100	0.54	0.85	Additive/synergistic
TPV:PMEA	1:10	1.20	0.78	Additive

Range of CI	
<0.3	strongly synergistic
0.3-0.8	synergistic
0.8-1.2	additive
1.2-1.7	antagonistic
>1.7	strongly antagonistic

Overall, the results of in vitro combination activity assessments suggest that additive to antagonistic relationships were seen with combinations of TPV with the PIs. The combinations of TPV with the NRTIs were generally additive and additive to antagonistic for TPV in combination with 3TC and ddI. The combinations of TPV with the NNRTIs DLV and NVP were additive and additive to antagonistic with EFV. The combination of

TPV with the HIV fusion inhibitor enfuvirtide was synergistic. The combination of TPV with ribavirin was additive to synergistic and the combination with adefovir was additive.

After discussions with the applicant, the following was concluded for the in vitro combinations with TPV. The combination of tipranavir was additive to antagonistic with the PIs (amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) and generally additive with the NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, and zidovudine), and the NNRTIs (delavirdine, efavirenz, and nevirapine). The combination of TPV was synergistic with the HIV fusion inhibitor enfuvirtide and additive to synergistic with the two compounds used in the treatment of viral hepatitis, adefovir and ribavirin.

Development of Resistance In Vitro

HIV- 1 isolates NL4-3 (WT) and P37 (drug resistant) were evaluated in serial passage in MT-2 cells in the presence of TPV. The NL4-3 virus showed a 3.5-fold decrease in TPV susceptibility at passages 14 and 26. The P37 virus showed a decrease in TPV susceptibility of up to 2.9-fold at passages 7, 13, and 22 in another in vitro study. No viruses with >10-fold decrease in TPV susceptibility were detected in this study.

HIV-l isolate NL4-3 (WT) was passaged in C8166 cells in the presence of increasing concentrations of TPV in tissue culture for 9 months. Following each viral breakthrough, the HIV-l protease gene and adjacent cleavage sites were sequenced. Viral breakthroughs were detected as sudden increases in the cytopathic effect. To determine the contribution of the mutations found to resistance, molecular clones containing emerging mutations were constructed and tested in antiviral activity assays against TPV (Table 6).

Table 6. Tipranavir susceptibility of molecularly cloned breakthrough viruses obtained by serial passage in the presence of increasing concentrations of TPV

Passage Number	Mutations	IC_{50} Value (nM)	Fold WT	TPV Conc (nM)
16	**L33F, I84V**	100	1.7	800
33	L33F, **K45I**, I84V	167	2.8	1000
39	**I13V, V32I**, L33F, K45I, I84V	407	6.9	2000
49	I13V, V32I, L33F, K45I, **V82L**, I84V	967	16.0	5000
68	I13V, V32I, L33F, **M36I**, K45I, **A71V**, V82L, I84V	1687	29.0	20000
70	**L10F**, I13V, V32I, L33F, M36I, K45I, **I54T/V**, A71V, V82L, I84V	4156	70.0	20000

New Mutations appearing at a given passage are in bold.