

Plasma trough levels correlate with distinct genetic mechanisms during the development of amprenavir resistance

8th Conference on Retroviruses and Opportunistic Infections
February 4–8, 2001, Chicago, IL, USA

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Introduction

Agenerase® (Amprenavir, APV), is a novel and potent inhibitor of the HIV-1 protease. Development of resistance has been studied to date in unboosted APV regimens. The principal mutation pathway associated with APV resistance, both *in vitro* and in protease inhibitor (PI) - naive subjects, involves development of an I50V mutation, a mutation not observed in the development of resistance to other PIs. Three alternative pathways leading to the development of APV resistance have been identified in the clinic, V321 + I47V, I54L/M and, less commonly the I84V. Although the I84V mutation was identified transiently during *in vitro* passage experiments, the V321 + I47V and I54L/M pathways were never observed *in vitro*.

The purpose of this analysis was to investigate whether the development of specific genetic mechanisms of resistance correlated with variations in observed plasma levels of APV. Genotyping and pharmacokinetic analysis was performed on clinical trial samples from previously PI-naïve subjects who, after having a therapeutic response, experienced viral rebound or inadequate virologic suppression while receiving APV/NRTI combination therapy. In this poster we present the first evidence that the resistance pathway that develops during virological rebound on APV-based unboosted regimens correlates with the APV plasma trough concentration (C_{min}). The possible significance of this in relation to boosted APV regimens is discussed.

Methods & Materials

Genotypic analysis was performed on virus from adult subjects experiencing virological rebound while participating in PRO2002, a Phase II APV-dose ranging trial. Subjects were randomized to receive ZDV (300 mg BID), 3TC (150 mg BID) and APV (900 mg, 1050 mg or 1200 mg BID). Subjects were PI and 3TC naïve at entry and had < 1 year previous NRTI treatment. Additional data was generated using virus from PI-naïve, NRTI-experienced subjects participating in clinical trials PRO3006 (adult) and PRO2004 (pediatric). Subjects were randomized to receive 2 NRTIs in combination with APV 1200 mg BID (PRO3006) or oral solution 20 mg/kg BID or 15 mg/kg TID (PRO2004).

Mutations in the protease gene of the virus were identified using automated sequencing (ABI PRISM Big Dye Terminator kit and ABI 377). Phenotyping was performed by Virco, Belgium. For the virus growth experiment, equivalent amounts of recombinant virus (10 ng of HIV-1 p24) was used to infect 1x10⁶ MT4 cells, and was incubated in growth medium (12 ml) for 8 days. At days 2, 4, 6 and 8 supernatant was collected and the concentration of HIV-1 p24 (Murex HIV Antigen Mab kit, Abbott Diagnostics) determined.

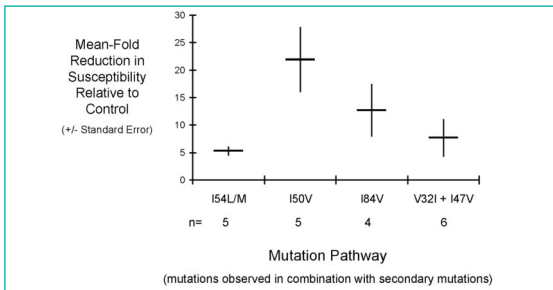
Pharmacokinetic analysis: Subjects in the trials had samples either drawn as part of a full pharmacokinetic profile (PRO2002 and PRO2004) or as trough samples at multiple visits during followup (PRO3006). A steady state trough (C_{min}) for those samples not part of a full profile was defined as being within 10–14h of the last documented dose. Samples were analyzed by cross-validated assays (either HPLC for part of PRO2002 or a LC/MS/MS for part of PRO2002 and all of PRO2004 and PRO3006). The assay accuracy was $\pm 12.1\%$, precision was $\pm 9.4\%$, and the linear range was 10–5000 ng/ml.

Statistical analysis: The C_{min} -association with the different mutational pathways was by use of the non-paired T-test assuming unequal variance. All p values are 2 sided.

Results

Viral isolates containing the I50V mutation have the greatest reduction in susceptibility to APV During clinical use resistance to APV develops through one of four characterized mutation pathways involving the amino acid changes, I50V, or V321 + I47V, or I54L/M or, less commonly, the I84V. Phenotypic analysis of samples from previously PI-naïve subjects failing an APV-based regimen (PRO3006) demonstrated that clinical isolates containing the I50V mutation have the greatest reduction in susceptibility to APV (Figure 1). Two of the mutation pathways not observed *in vitro*, V321 + I47V and the I54L/M, conferred the lowest resistance to APV (7.7 and 5.3 fold, respectively). Thus the presence of high selection pressure, as was applied during the *in vitro* experiments, may reduce the ability to detect mutations that confer lower levels of resistance.

Figure 1 • The I50V mutation pathway confers the greatest reduction in susceptibility to APV



APV drug dosage influences APV mutation pathway

To determine if drug concentration could influence the resistance pathway selected, retrospective resistance analysis was performed on the dose-ranging clinical trial PRO2002. Resistance analysis was performed on samples from subjects who received either 900 mg APV BID or 1050 mg APV BID (Table 1). The resistance pathway developed by the virus appeared to correlate with the drug dosage received. For example, I50V mutations only developed in the 1050 mg cohort whereas I54L/M mutations predominated in the 900 mg cohort.

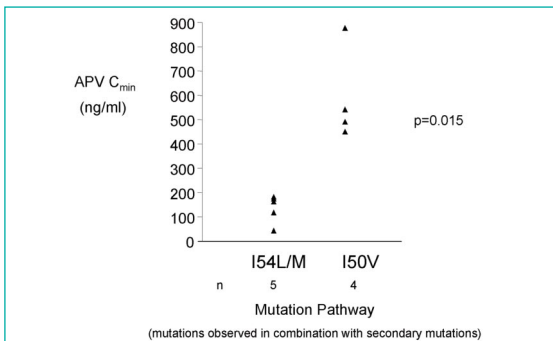
Table 1 • Resistance pathway correlates with APV dosage

Mutation Pathway	Amprenavir Dosage (BID)	
	900mg	1050mg
I50V	0	5
I54L or M	5	2
V321 + I47V	1	0
I84V	0	1

APV mutation pathway correlates with APV C_{min} plasma concentration

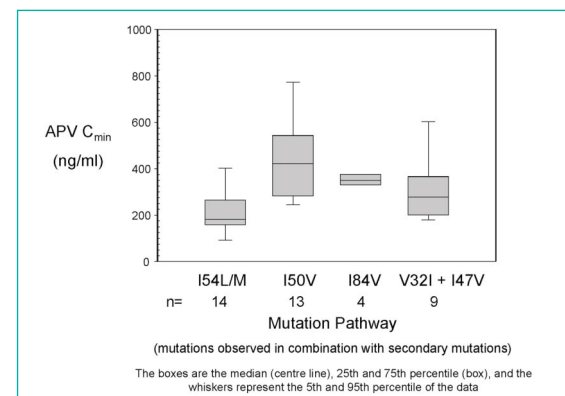
As the same dose of a drug gives a range of C_{min} secondary to inter-individual variability, the observation that different APV dosages were associated with the predominance of a specific resistance mechanism was further explored by analysis of APV C_{min} concentrations, where available (n=9) (Figure 2). This analysis revealed a significant difference ($p=0.015$) in APV trough exposure according to viral genotype. The development of the I50V mutation only occurred in subjects with higher C_{min} plasma concentrations of APV (median=517 ng/ml, range 451–877 ng/ml) whereas the I54L/M mutation only developed in subjects where the C_{min} levels were lower (median=164 ng/ml, range 43–183 ng/ml).

Figure 2 • Different APV C_{min} Concentrations correlate with the development of the I54L/M and the I50V mutation pathways



To further support the observations in PRO2002 and to gain additional data for the V321 + I47V and I84V pathways, analysis was performed on samples from a sub-set of subjects who participated in PRO2004 (n=9) and PRO3006 (n=21), who had both viral and pharmacokinetic data available. Figure 3 shows a box plot of the mean steady-state APV plasma C_{min} concentration according to APV resistance genotype. There was a significantly lower APV plasma C_{min} concentration in subjects whose virus developed the I54L/M mutation pathway (median=182.5 ng/ml, n=14) compared to subjects whose virus developed the I50V (median=421.2 ng/ml, n=13, $p=0.002$) or the I84V (median=349.5 ng/ml, n=4, $p=0.0006$). There was a trend for the C_{min} values to be lower for subjects who developed the I54L/M as compared to the V321 + I47V pathway (median 277 ng/ml, n=9, $p=0.09$). The C_{min} values among the I50V, I84V and V321 + I47V pathways were not significantly different.

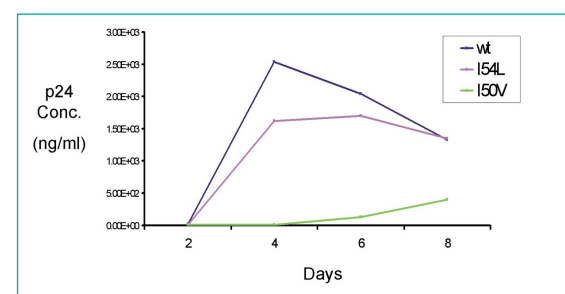
Figure 3 • Higher APV C_{min} concentrations are associated with development of the I50V and the I84V mutation pathways



Replicative capacity of virus containing the I50V and I54L mutations

Although the I50V pathway confers the greatest reduction in susceptibility to APV, low APV exposure does not appear to be sufficient to select for the I50V mutation. The presence of the I50V mutation has been shown to significantly reduce the growth rate of the virus when compared to wild-type. When APV trough concentrations are low the impact of the I50V mutation on viral fitness may mitigate against its development. In this situation other mutations conferring a lower reduction in APV susceptibility may predominate if the mutation has less of an impact on viral replication. Comparison of the growth rate of wild-type (HXB2) HIV-1 and point mutants containing the I50V or the I54L has been performed (Figure 4). As expected, the I50V-containing virus was severely compromised compared to the wild type virus. The I54L-containing virus was more fit than the I50V-containing virus, but was still growth compromised when compared to wild-type virus. Experiments are underway to determine the relative fitness of the APV-associated mutations in the presence and absence of accessory mutations.

Figure 4 • Virus containing an I50V mutation is severely growth compromised



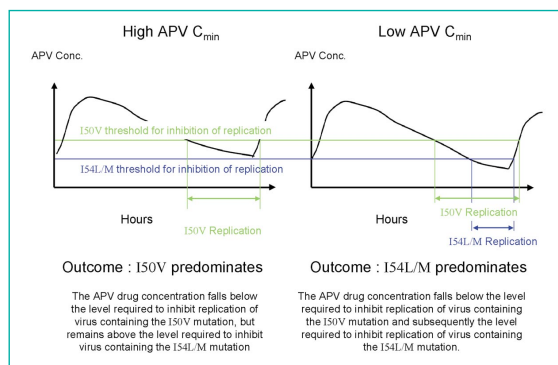
Discussion

In this poster we have shown that different APV C_{min} plasma concentrations correlate with the particular resistance pathway developed. Low APV C_{min} plasma concentrations were associated with the development of the I54L/M resistance pathway, whereas the highest APV C_{min} plasma concentrations were associated with the development of the I50V.

In early trials, PI-experienced adults receiving APV (1200 mg BID) co-administered with efavirenz (EFV) and abacavir (CNA2007), resistance to APV developed predominantly through acquisition of the I54L/M mutation (n=7) rather than the I50V resistance pathway (n=2). EFV is now known to significantly reduce the C_{min} plasma concentration of APV. Duval et al. recently reported median APV trough concentrations of 64 ng/ml (n=7, range 33–260 ng/ml) in subjects receiving APV (1200 mg BID) in the presence of EFV. Although no pharmacokinetic measurements were available for CNA2007, the reduction in APV C_{min} plasma concentrations would favor the development of the I54L/M mutation pathway. Interestingly, one subject in this study who failed initial therapy followed by therapy intensification including APV levels boosted by co-administration of RTV, subsequently developed the I50V mutation. More recent data have demonstrated that co-administration of low-dose RTV with APV can help offset the impact of EFV on APV C_{min} .

In the PI-experienced population it is unclear to what extent the genetic background present at the initiation of APV therapy impacts the resistance pathway observed. Virus from 2 subjects participating in CNA2007 developed a 54M mutation from a starting population containing the 54V. This required two nucleotide substitutions to change the valine codon (GUC) to methionine (AUG), rather than the single substitution required from wild-type isoleucine (AUC).

Why does the I54L/M pathway predominate at low APV C_{min} plasma concentrations? The pathway that predominates is dependent upon both the replicative capacity of the mutant virus and also the level of resistance to APV conferred by the mutation. In the absence of drug, an I50V-containing virus is very growth impaired compared to the I54L-containing virus, but the I50V mutation confers a greater reduction in susceptibility to APV (i.e. a greater concentration of drug is required to inhibit the replication of an I50V- than an I54L-containing virus). At high APV C_{min} plasma concentrations the APV level remains above that required to inhibit I54L/M-containing virus, but not the I50V-containing virus. At low APV C_{min} plasma concentrations, the I54L-containing virus would begin to replicate and, due to having greater replicative capacity than the I50V-containing virus, may predominate despite replicating for a shorter time period (an example is shown below).



APV C_{min} plasma concentrations can be significantly raised with co-administration of low dose RTV. Pacı-Bonaventure et al. reported that APV C_{min} concentrations were raised from a median of 230 ng/ml with 1200 mg APV BID to a median of 1798 ng/ml with 600/100 mg APV/rtv BID. In this study, 50% (11/22) of subjects on the standard APV regimen (i.e. 1200 mg BID) had C_{min} values below 220 ng/ml, the concentration below which the majority of subjects develop virus containing the I54L/M. In contrast only one subject (3%, 1/29) receiving 600/100 mg APV/rtv had C_{min} values below 220 ng/ml. Thus administration of APV/rtv (600/100) would be predicted to reduce the selection for the I54L/M mutation pathway and increase the selection for the I50V mutation pathway.

Conclusions

- ◆ Low APV C_{min} plasma concentrations correlate with the development of the I54L/M resistance pathway.
- ◆ The highest APV C_{min} plasma concentrations correlate with the development of the I50V resistance pathway.
- ◆ The I50V mutation pathway confers the greatest reduction in sensitivity to APV, but is very detrimental to viral fitness. The I54L/M mutation pathway confers limited resistance to APV, but has a lesser impact on replicative capacity.
- ◆ Boosted APV regimens (APV/rtv) achieve on average 5–6 fold higher APV C_{min} plasma concentrations and should reduce the proportion of isolates containing the I54L/M mutation in favor of an increase in the proportion containing the markedly less fit I50V mutation.
- ◆ Selection of the I50V mutation, which has not been reported during clinical use with other PIs, may preserve future treatment options following APV use.

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Acknowledgments

The authors would like to thank the APV study team and the subjects that participated in the clinical trials PRO2002, PRO2004, CNA2007 and PRO3006. We would also like to thank Josie Wolfram and Yu Lou for their assistance in providing the data.