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Introduction

Fosamprenavir (FPV) is an HIV-1 protease inhibitor, approved in both the USA (Lexiva™) and Europe (Telzir™) for use in PI-naïve and PI-experienced patients. In a 48 Week fosamprenavir/ritonavir (FPV/r) registration study (SOLO, APV30002) no PI resistance development was observed, and NRTI resistance to the concomitant abacavir/lamivudine (ABC/3TC) therapy, was substantially less common compared with the nelfinavir (NFV) comparator arm (MacManus et al., 2003). This study rolled over subsequently into APV30005 to continue monitoring long-term the response to boosted fosamprenavir. Results have now been reported out to Week 120 which confirmed the finding that no PI resistance developed during treatment with boosted fosamprenavir in subjects without baseline PI resistance (Gathe et al., 2004, Schürmann et al., 2005).

Subjects continue to be monitored in APV30005 for resistance development. Here we report the first observation of PI resistance development at Week 160 in a patient failing a boosted fosamprenavir first-line regimen, in the absence of baseline protease mutations.

Methods

The patient in this study was diagnosed HIV positive when he presented with *Pneumocystis pneumonia*, and refractory anogenital HSV-2. After these conditions were treated and he became clinically stable, he entered APV 30002. Viral load analysis was undertaken at Covance using the Roche ultrasensitive assay (limit of detection <50 copies/ml). Resistance analysis was performed at ViroLogic routinely on samples >1000copies/ml at Week 12 and beyond.

For the detailed analysis of this patient's samples, three laboratories were used in the resistance analysis. The first sample was sent by the investigator for genotypic analysis using TRUGENE® (Bayer HealthCare). Subsequently, further confirmatory genotypic (GENESEQ®) and phenotypic (PHENOSENSE®) analyses were undertaken by ViroLogic, Inc. Replication capacity was also determined by ViroLogic, Inc. in a single cycle cell-based assay.

Viral amplification was attempted on all samples that had viral load >50 copies/ml at GSK, Stevenage, UK. Standard guanidine based RNA extraction methods and nested RT-PCR were used, but for samples with viral load <400 copies/ml greater volumes (1ml) of plasma and high speed centrifugation to sediment virus were included prior to the extraction.

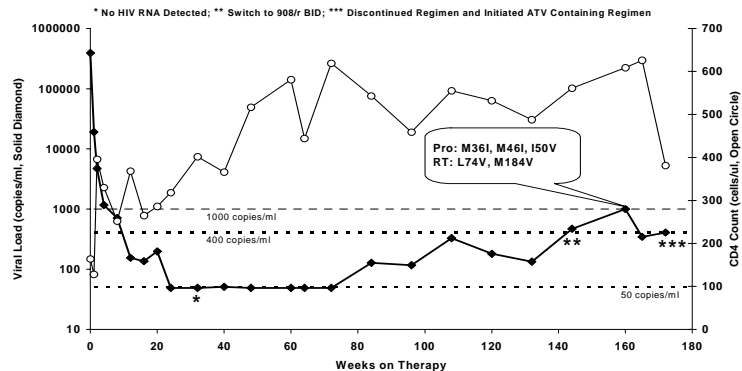
When the patient discontinued from the Clinical Study viral load was analyzed locally using VERSANT® Bayer bDNA assay (limit of detection, 50 copies/ml).

Results

1. Viral Load/CD4 Profile and Initial Failure Genotype, plus Regimen Changes.

The subject entered APV30002 with a baseline viral load of 392,183 copies/ml and a CD4 count of 164 cell/ul, and received a regimen of FPV/r (1400/200mg once daily) with ABC 300mg and 3TC 150mg BID. The subject had viral load <50 copies/mL from Week 24 to 72, with one exception (51 copies/mL (Week 40).

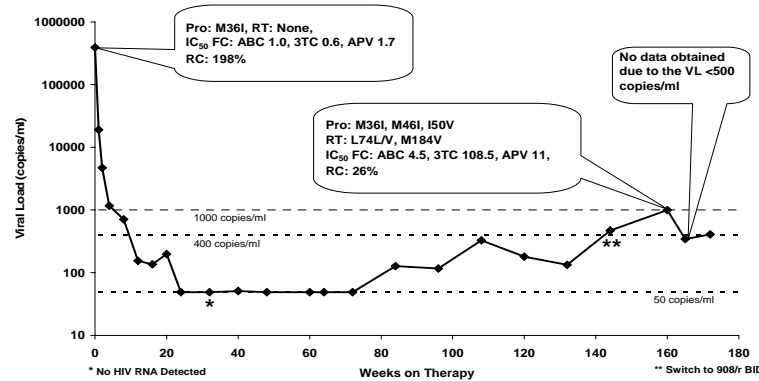
Figure 1. Viral Load /CD4 Profile, First Genotypic Result from TRUGENE®, plus Regimen Changes



- Viral load rebound to >50 copies/mL was first detected at Week 84 (133 copies/mL) and low level (<400 copies/mL) replication continued until Week 144 (472 copies/mL). At this time the subject switched to twice daily FPV/r, with no other change to the regimen.
- At Week 160, the viral load had increased to 998copies/mL and the investigator performed a genotypic resistance test (TRUGENE®, Bayer HealthCare).
- This genotype revealed the presence of the RT mutations L74V and M184V, and the protease mutations M36I, M46I and I50V, which would give resistance to the ABC/3TC/FPV/r regimen. However, despite this resistance, viral load dropped to 345 copies/mL at Week 165, and 407 copies/mL at Week 171.
- At Week 171 the subject discontinued the FPV/r BID regimen and started a regimen consisting of atazanavir ATV 300mg QD, RTV 100mg QD, tenofovir (TDF) 300mg QD, and fixed dose zidovudine/lamivudine (ZDV/3TC) BID.
- On this new regimen, he achieved virologic suppression, but became intolerant (malaise, fatigue, jaundice), and requested to resume FPV/r BID with ABC/3TC QD.
- After resumption of the original FPV/r BID, ABC, 3TC-containing regimen, he initially maintained an HIV RNA to <75 copies for 2 months. However, low-level virologic rebound subsequently occurred and 8 months after changing back to the FPV-containing regimen the HIV RNA was 802 copies/mL, with a CD4 cell count of 772 cells/ul. He remains clinically well with no signs or symptoms of HIV disease.

2. Confirmatory Resistance Analysis at ViroLogic.

Figure 2. Summary of Genotypic, Phenotypic Analysis Performed at ViroLogic, plus Replication Capacity of the Virus at Baseline and Week 160



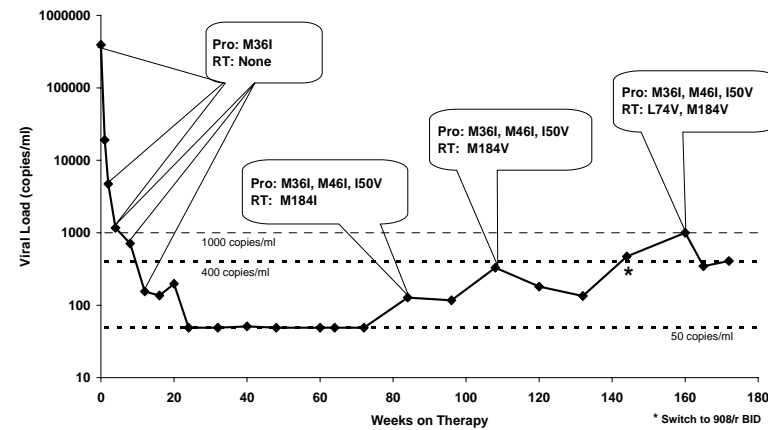
The ViroLogic assay showed :

- At Day 1, no resistance associated CS, PRO or RT mutation but a M36I natural polymorphism in PRO was observed. Consistent with this genotypic profile no phenotypic resistance was observed.
- At Week 160, the M46I and I50V mutations were detected in PRO, and the L449F mutation was observed in the p1/p6 cleavage site; the L74V and M184V mutations were detected in RT,
- At Week 160, phenotypic analysis showed that the virus had decreased susceptibility to ABC (4.5-fold), 3TC (>108-fold), didanosine (ddI) (1.7-fold), amprenavir (APV) (11-fold), and RTV (6.4-fold). The virus retained susceptibility to other drugs including lopinavir (LPV) (4.0-fold) and atazanavir (0.5-fold).

- Marginal increases in susceptibility to other drugs were observed at Week 160 compared to Day 1: TDF (0.9 to 0.3-fold), stavudine (d4T) (1.5 to 0.6-fold), ZDV (0.8 to 0.2-fold), indinavir (IDV) (1.1 to 0.4-fold), and saquinavir (SQV) (1.4 to 0.8-fold). These increases in susceptibility are consistent with presence of the M184V with or without the L74V in RT and with the M46I/I50V in PRO.
- Replicative Capacity decreased from 198% (Range 125-314) at Day 1 to 26% (Range 16-41) at Week 160.

3. Further Genotypic Analysis Undertaken by GSK

Figure 3. Summary of Genotypic Analysis Performed at GSK



- No resistance associated CS, PRO or RT mutations were detected prior to virological suppression at Day 1, Week 1, Week 4, Week 8 or Week 12 except for the PRO natural polymorphism M36I.
- Comparison of baseline genotype with reference HXB2; differences observed were: PRO - T12P, K14R, G16E, L19I, M36I, S37N, R41K, I62V, L63A, V82I, and I93L RT- V35M, E122K, K166R, D177E, G196E, Q197H, L214F, and V245E.
- Virus amplification was unsuccessful at Week 16 (136 copies/ml) and Week 20 (198 copies/ml) prior to suppression to <50copies/ml at Week 24.
- Resistance associated mutations PRO: M46I+I50V, RT: M184I and CS: L449F were detected at Week 84, the first time-point when the VL rebound to > 50copies/ml (128 copies/ml). At Week 108 the RT M184I mutation had changed to RT M184V and by Week 144 the RT L74V mutation was detected. There were no additional PRO mutations observed at these later time points.

4. Retrospective Analysis of Plasma Levels of Amprenavir (APV) and Ritonavir (RTV)

Ten plasma samples taken between Week 32 and Week 165 were analyzed for levels of APV and RTV using established methods. Since these samples were not taken for pharmacokinetic analysis there was no information on time relative to last dose taken. The data could not be quality controlled and therefore values have not been recorded. However, values observed were within the expected range for both APV and RTV after taking boosted FPV.

Discussion

In contrast to unboosted PIs, virological failure on boosted PIs generally has been associated with the absence of development of protease resistance (Walmsley, 2002; MacManus 2004). However, there have been reports of rare failures with development of PI resistance on lopinavir (Friend, 2004; Conradie, 2004; Wolf 2005) and on atazanavir (Coakley 2005).

Here, we report the first example of a failure on RTV-boosted fosamprenavir in the absence of baseline resistance. Self-reported adherence was excellent, and this was consistent with the plasma levels for boosted FPV. Virological failure was confirmed at Week 160 (>400copies/ml). Further ultrasensitive analysis showed that at Week 84, the first time point above 50 copies /ml, 4 mutations were present which would give rise to resistance to the 3 components of the triple regimen including boosted FPV. This suggests that viral resistance may have developed when the virus was suppressed below the 50 copies /ml, that is, at very low virus replication levels. Previously with ABC/3TC therapy it has been shown that the M184I/V may emerge during low level virus replication (Eron et al., 2002).

Despite the presence of resistance mutations to all three components of the treatment regimen, the viral load remained more than 3 logs below the pretreatment baseline for more than 22 months (most recent viral load measurement in June 2005). This viral load reduction has been associated with substantial CD4 recovery (Deeks et al 2002). One possible explanation is the reduction in replication capacity suggesting that the mutated virus is less virulent. Possibly elevated levels of boosted FPV may be able to at least partially control the replication of HIV despite the presence of fosamprenavir-selected resistance mutations. Finally, studies suggest that 3TC continues to exert some antiviral activity even after the development of the M184V (Campbell et al., 2005). No compensatory mutations were detected despite ongoing low-level viral replication.

Using more sensitive analysis of genotypes, this patient highlights two points - the development of resistance at low viral load, and the control of virus replication by a boosted protease inhibitor regimen, despite the presence of resistance.

Conclusions

- Development of PI resistance during long-term treatment with boosted FPV/r is a rare event in previously treatment-naïve subjects.
- Additional analysis of this rare FPV/r virological failure provided the first evidence that resistance may develop even at a low level viral load.
- Boosted FPV/r combination therapy may reduce virus replication even in the presence of the M46I/I50V amprenavir resistance mutations.

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