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# Insertions at positions 33 and 35 of the HIV-1 protease: prevalence and role in virologic failure in darunavir-treated patients

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## Introduction

- The protease inhibitor (PI) darunavir (DRV; TMC114) has shown significant, broad-spectrum in-vitro antiretroviral activity against both wild-type and multidrug-resistant HIV-1 strains.[1] DRV has a high genetic barrier, which reduces the development of resistance and allows the retention of antiviral activity, despite the occurrence of mutations within the target viral protein.[2]
- DRV with low-dose ritonavir (DRV/r) has shown significant efficacy and tolerability in a wide range of adult patients – from treatment-naïve to highly treatment-experienced patients.[3–5]
- Primary and secondary protease mutations, as well as protease cleavage-site mutations, are involved in HIV resistance to PIs. More recently, amino acid insertions in the protease have been associated with increased levels of PI resistance. In-vitro and structural analysis of amino acid insertions in the vicinity of the binding site such as insertions at positions 33 (ins33) and 35 (ins35) suggested a novel mechanism of HIV resistance development to most PIs.[6,7]
- The role of amino acid ins33 and ins35 of the protease on virologic failure (VF) was investigated in highly PI-experienced (POWER and DUET trials) and treatment-naïve (ARTEMIS trial) patients receiving DRV/r plus a background regimen.

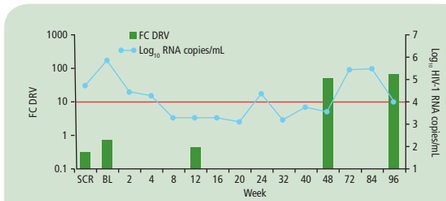
## Methods

- These analyses were performed on 1) patients who initiated treatment with DRV/r 600/100mg bid in POWER 1, 2 and 3, and with patients from the placebo (non-etravirine [ETR; TMC125]) groups of DUET-1 and DUET-2 (POWER/DUET dataset), and 2) patients from the DRV/r 800/100mg qd treatment arm of the ARTEMIS trial
  - the POWER studies were Phase IIb trials designed to investigate the efficacy and safety of DRV/r plus an optimized background regimen in highly treatment-experienced patients[5]
  - DUET-1 and DUET-2 are two, randomized, placebo-controlled, double-blind, Phase III trials investigating the efficacy and safety of the NNRTI ETR as part of a regimen including DRV/r, investigator-selected NRTIs and optional enfuvirtide in highly treatment-experienced patients[8,9]
  - ARTEMIS is an ongoing, randomized, controlled, Phase III trial to compare the efficacy, safety and tolerability of DRV/r versus lopinavir/ritonavir (LPV/r) in treatment-naïve, HIV-1-infected patients.[10]
- All patients included at the time of the database lock for the Week-96 analysis of the POWER trials and for the primary Week-24 analysis of the DUET trials were included in the first analyses.[8,9] ARTEMIS patients from the DRV/r treatment arm of the Week-96 analysis were included in the second analysis.[10]
- Genotypes and phenotypes of plasma viruses were determined using population-based sequencing and Antivirogram®, respectively (Virco BVBA, Mechelen, Belgium).
- DRV resistance-associated mutations (RAMs; V11I, V32I, L33F, I47V, I50V, I54L/M, T74P, L76V, I84V, L89V) were based on the IAS-USA list (December 2008).[11]
- Phenotypic resistance to PIs was defined as having a fold-change in 50% effective concentration (FC) above the biologic/clinical cut-off (Antivirogram®). A clinical cut-off of 10 was used for both DRV[12] and LPV,[13] and a clinical cut-off of 3 was used for tipranavir (TPV).[14] The biologic cut-offs 2.2, 2.4, 2.4, 2.2, and 1.8 were used for amprenavir (APV), atazanavir (ATV), indinavir (IDV), nelfinavir (NFV) and saquinavir (SQV), respectively.
- The prevalence of ins33 and ins35 was investigated in baseline samples. Baseline genotype data were available for 1060 patients from POWER 1–3 and DUET-1 and DUET-2 trials, and for 342 patients from the ARTEMIS trial.
- Virologic response was assessed as patients achieving HIV-1 RNA <50 copies/mL at Week 24 (POWER 1–3 and DUET) or Week 96 (ARTEMIS) (time-to-loss of virologic response [TLOVR; intent-to-treat] analysis).
- Development of ins33 and ins35 was investigated in samples from VFs from POWER 1–3 and DUET-1 and DUET-2, and from ARTEMIS.
- VFs were defined as patients that at least reached Week 16 (POWER and DUET) or Week 12 (ARTEMIS) and who lost or did not achieve HIV-1 RNA <50 copies/mL.
- The TLOVR (non-VF censored) imputation method was used for the identification of VFs, meaning that data were not imputed as non-response at timepoints after discontinuation for patients who discontinued for reasons other than VF (non-VF).
- A developing insertion was defined as one present at endpoint (i.e. last available genotype within the treatment period), but not at baseline.
- Paired baseline and endpoint genotype data were available for 456 out of 572 VFs from POWER 1–3 and DUET-1 and DUET-2 trials and for 31 out of 40 VFs in the DRV/r group from the ARTEMIS trial.

## Results

### Prevalence and development of ins33 and ins35 of the HIV-1 protease in highly PI-experienced patients (POWER 1–3 and DUET-1 and DUET-2)

- Ins33 and ins35 were present in 11/1060 (1.0%) and 9/1060 (0.8%) baseline samples from highly PI-experienced patients, respectively.
- Table 1 presents an overview of the resistance data of the baseline samples from these highly PI-experienced patients (patients 1–20) who were harboring ins33 and ins35 of the HIV-1 protease. Baseline DRV RAMs, FC values for different PIs, and response (HIV-1 RNA <50 copies/mL) at Week 24 are shown.
- Ins33 and ins35 were associated with the presence of ≥3 DRV RAMs in 9/11 and 7/9 patients, respectively (Table 1).
- Based on phenotypic susceptibility, 14/20 samples were resistant to DRV, 20/20 to APV, ATV, IDV, LPV, NFV and SQV, and 12/20 to TPV (Table 1).
- Among patients with baseline insertions, 10/11 with an ins33 and 8/9 with an ins35 failed virologically.
- A total of 572 (53.4%) patients in POWER 1–3 and from the placebo groups of DUET-1 and DUET-2 experienced VF.
- Ins33 of the HIV-1 protease developed at endpoint in two (patients 21 and 22) out of 456 (0.4%) VFs with paired baseline and endpoint genotypes, and zero out of 456 developed ins35. In these two cases, the patient was already failing virologically and already had a DRV FC >40 before the ins33 emerged. Viral load, phenotypic and genotypic profiles are presented in Figures 1 and 2, and Tables 2 and 3.

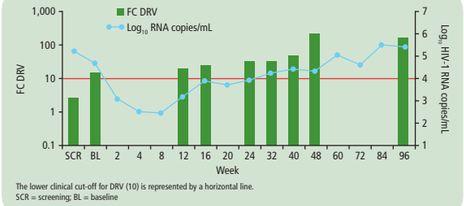



The lower clinical cut-off for DRV (10) is represented by a horizontal line. FC data at Wk 24 is missing.
SCR = screening; BL = baseline

**Figure 1. Viral load and phenotypic profile at different timepoints: patient 21.**

**Table 2. Genotypes (ins33 and DRV RAMs) at different timepoints: patient 21.**

| Visit | Number of DRV RAMs | V11 | V32 | L33 | L76 | I84 |
|---|---|---|---|---|---|---|
| Screening | 3 | I | – | F | – | V |
| Week 12 | 2 | I | – | – | – | V |
| Week 24 | 4 | I | I | F | – | V |
| Week 48 | 5 | I | I | F | V | V |
| Week 96 | 5 | I | I | F+**E** | V | V |




The lower clinical cut-off for DRV (10) is represented by a horizontal line.
SCR = screening; BL = baseline

**Figure 2. Viral load and phenotypic profile at different timepoints: patient 22.**

**Table 3. Genotypes (ins33 and DRV RAMs) at different timepoints: patient 22.**

| Visit | Number of DRV RAMs | V32 | L33 | I54 | L76 | I84 | L89 |
|---|---|---|---|---|---|---|---|
| Screening | 1 | – | – | – | V | – | – |
| Baseline | 1 | – | – | – | V | – | – |
| Week 12 | 2 | I | – | – | V | – | – |
| Week 16 | 2 | I | – | – | V | – | – |
| Week 24 | 3 | I | – | – | V | V | – |
| Week 32 | 4 | I | – | – | V | V | V |
| Week 40 | 3 | I | – | – | V | V | – |
| Week 48 | 3 | I | +**E** | – | V | V | – |
| Week 96 | 4 | I | +**E** | L | V | V | – |

**Table 4. Baseline samples from PI-naïve patients (ARTEMIS) harboring ins35 of the HIV-1 protease (patients 23 and 24).**

| Patient | Number of DRV RAMs at baseline | Ins35 E35 | FC (Antivirogram®) DRV | APV | ATV | IDV | LPV | NFV | SQV | TPV | Confirmed response at Week 96 (TLOVR; <50 copies/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 0 | D+**I+N** | 0.4 | 0.2 | 0.7 | 0.4 | 0.6 | 0.2 | 0.7 | 0.5 | Yes |
| 24 | 0 | +**D** | 0.4 | 0.5 | 0.4 | 0.4 | 0.6 | 0.4 | 0.5 | 0.7 | Yes |

'+' indicates an insertion at the respective position

### Prevalence and development of ins33 and ins35 of the HIV-1 protease in PI-naïve patients (ARTEMIS)

- The prevalence of ins33 and ins35 at baseline in these PI-naïve patients was zero out of 342 (0%) and two out of 342 (0.6%), respectively.
- Table 4 presents an overview of the resistance data of the baseline samples from these two PI-naïve patients (patients 23/24) who harbored ins35 of the HIV-1 protease. Number of baseline DRV RAMs, FC values of PIs, and response (HIV-1 RNA <50 copies/mL) at Week 96 are shown.
- The two samples were susceptible to all PIs and the patients achieved HIV-1 RNA <50 copies/mL.
- A total of 40 (11.7%) patients in the DRV/r treatment arm of the ARTEMIS trial experienced VF, of whom 31 had paired baseline and endpoint genotypes. None developed ins33 or ins35 of the HIV-1 protease.

## Conclusions

- The prevalence of ins33 and ins35 of the HIV-1 protease is low among both highly PI-experienced and PI-naïve patients.
- Although highly PI-experienced patients with protease ins33 and ins35 exhibited high rates of VF, the relationship with this outcome could not be attributed to the presence of insertions since these patients also had a high level of baseline DRV resistance.
- Two PI-naïve patients with protease ins35 were susceptible to all PIs and both patients achieved HIV-1 RNA <50 copies/mL.
- Protease ins33 and ins35 are rarely selected upon DRV failure.

**Table 1. Baseline samples from highly PI-experienced patients (POWER and DUET) harboring ins33 and ins35 of the HIV-1 protease (patients 1 to 20).**

| Patient | Number of DRV RAMs at baseline | Ins33/ins35 and DRV RAMs V11 | V32 | L33 | E35 | I47 | I54 | T74 | L76 | I84 | L89 | FC (Antivirogram®) DRV | APV | ATV | IDV | LPV | NFV | SQV | TPV | Confirmed response at Week 24 (TLOVR <50 copies/mL) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | I | – | +**V** | – | – | L | – | – | V | – | 6.5 | 17.9 | >112.8 | 9.4 | >87 | >52.3 | >54.6 | 0.2 | No |
| 2 | 4 | I | – | +**E** | – | – | L | – | – | V | V | 0.5 | 42.1 | 48.9 | 4 | 75.9 | 21.1 | >35.9 | <0.2 | No |
| 3 | 5 | I | I | +**E** | – | V | M | – | – | – | V | 147.9 | >97.8 | >141.4 | 55.6 | >43.3 | >107.3 | 25.2 | 28.7 | No |
| 4 | 3 | – | – | +**V** | – | – | L | – | – | V | V | 10.1 | 60 | >115.4 | 30.8 | >34.3 | >75.9 | >45.8 | 2.1 | No |
| 5 | 4 | – | – | +**Q** | – | V | M | – | – | V | V | 146.8 | >76.3 | >139.6 | 71.6 | >43.6 | 60.1 | 34.1 | 33.5 | No |
| 6 | 5 | I | I | +**E/V** | – | V | L | – | – | V | – | 518.5 | >75.5 | >115.4 | 35.6 | >34.3 | >75.9 | >45.8 | 1.3 | No |
| 7 | 4 | – | I | F+**Q** | – | V | M | – | – | – | – | 75.1 | >89.2 | 66.4 | 14.1 | >46.9 | 55.4 | 29.6 | 2.1 | No |
| 8 | 6 | I | I | F+**H/Y** | – | V | M | – | – | – | V | 14.4 | >99.4 | 22.5 | 24.4 | 142.9 | 39.5 | 3.2 | 5.6 | No |
| 9 | 2 | I | – | +**L** | – | – | – | – | – | – | V | 5.2 | 13.9 | >89.4 | 46 | >44.8 | >91.5 | 27.5 | 2.1 | Yes |
| 10 | 8 | I | I | F+**Q** | – | V | M | P | – | V | V | 312.1 | >86.2 | >135.2 | 33.1 | >42 | 62.1 | 44.4 | 16.1 | No |
| 11 | 2 | – | – | +**V** | – | – | L | – | – | V | – | 86.3 | >99.9 | >110.9 | 52.1 | >42.6 | >72 | >57.8 | 4.7 | No |
| 12 | 2 | – | – | – | +**T** | V | – | – | – | V | – | 0.8 | 10.5 | 59.3 | >87 | >88.5 | >46.6 | >45.4 | 5.8 | No |
| 13 | 3 | – | – | – | +**D+L+N** | – | M | – | V | V | – | 95.2 | >99 | 81.7 | 87 | >152.1 | 65.3 | >56.7 | 1.3 | No |
| 14 | 4 | – | – | – | +**E** | V | M | – | – | V | V | 94.3 | 63 | 88.9 | >79 | >43.9 | 75.2 | >73.7 | 3.4 | No |
| 15 | 4 | – | I | – | +**G** | V | M | – | – | – | V | 30.1 | >74.1 | 30.8 | >65.5 | >32.7 | >54.8 | 6.5 | 3.7 | No |
| 16 | 3 | I | – | – | +**Q+E** | – | – | – | – | V | V | 7.4 | 83.2 | >146.8 | 79 | >44.4 | >100.4 | >53.9 | 36 | Yes |
| 17 | 2 | – | – | F | +**D** | – | – | – | – | V | – | 7.5 | 37.6 | >107.7 | 65.5 | >32.7 | >57.9 | >54.9 | 10.6 | No |
| 18 | 4 | – | I | F | +**D/E** | – | – | – | – | V | V | 19.6 | 38 | >147.8 | 78.5 | 20.7 | >122.1 | 13.8 | 15.7 | No |
| 19 | 5 | I | I | – | +**Q+S** | V | L | – | – | V | – | 190.9 | >97.2 | 66.5 | 10.4 | >50.5 | 83.7 | >58.1 | 1.3 | No |
| 20 | 6 | I | I | F | +**H+E+N** | V | M | – | – | – | V | 154 | >62 | >121.4 | 26.4 | >32.5 | >50.3 | >32.9 | 42.2 | No |

'+' indicates an insertion at the respective position; the presence of ≥3 DRV RAMs (predictive for diminished response) at baseline is marked in red; FC values above the biologic/clinical cut-off (decreased susceptibility) are marked in red; no confirmed virologic response at Week 24 (TLOVR; <50 copies/mL) is marked in red

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