

Comparable in-vitro susceptibility and virological outcome to darunavir in patients infected with subtype B and non-subtype B HIV isolates participating in the ARTEMIS Phase III trial

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Introduction

- HIV-1 is characterised by extensive genetic heterogeneity. Subtype B is the predominant genetic form in Western and Central Europe, the Americas, and Australia. Subtype C is the predominant genetic form in India, Eastern and South Africa, and accounts for almost 50% of all HIV-1 infections worldwide. Circulating recombinant forms (CRFs) account for 18% of incident infections and represent the local predominant form in Southeast Asia (CRF01_AE).
- Limited data are available concerning clinical outcome of antiretroviral (ARV) therapy in patients infected with non-B subtypes, and the in-vitro susceptibility of these subtypes.
- Darunavir (DRV; TMC114) with low-dose ritonavir (DRV/r) at a dose of 600/100mg twice daily (bid) has been approved in the USA¹ and in other countries² for the treatment of HIV-1 infection in treatment-experienced adult patients.
- ARTEMIS (TMC114-C211; AntiRetroviral Therapy with TMC114 Examined In Naïve Subjects), is an ongoing Phase III trial assessing the efficacy and safety of once-daily (qd) DRV/r (800/100mg) versus lopinavir with low-dose ritonavir (LPV/r) 800/200mg total daily dose in treatment-naïve HIV-1-infected adult patients
 - in the primary Week 48 analysis of this study, 84% of DRV/r and 78% of LPV/r patients achieved HIV-1 RNA <50 copies/mL at Week 48 (p value for non-inferiority, p<0.001)³
 - a wide variation in HIV-1 viral subtypes was encountered in treatment-naïve patients participating in ARTEMIS.
- In this analysis, we studied the effect of HIV-1 subtype on
 - in-vitro susceptibility to DRV, using a broad panel of (primary) HIV-1 isolates
 - virological response to DRV/r in treatment-naïve patients participating in the ARTEMIS trial.

Methods

- In-vitro antiviral activity of DRV was assessed in freshly isolated human peripheral blood mononuclear cells (PBMCs) against a panel of 25 HIV-1 primary isolates, composed of at least three viruses from each of the Group M subtypes (A, B, C, D, E, F and G), as well as three isolates from HIV-1 Group O. Serial two-fold dilutions of DRV were added in triplicate to phytohaemagglutinin-stimulated PBMCs, pooled from at least two normal healthy donors, in 96-well plates containing 5.0 x 10⁶ cells/well in a final volume of 200µL. Test viruses were added to the cell cultures at a multiplicity of infection (MOI) of ± 0.1. After 6 days of culture, reverse transcriptase (RT) activity was quantified in the culture supernatants, as previously described.⁴ Fifty per cent effective concentration (EC₅₀) values were calculated from dose-response curves. The experiments were performed by the Southern Research Institute, Frederick, MD, USA.
- In ARTEMIS, treatment-naïve, HIV-1-infected adult patients with HIV-1 RNA >5,000 copies/mL were randomised to receive DRV/r 800/100mg qd (n=343) or LPV/r 800/200mg total daily dose (n=346), together with a fixed-dose combination of tenofovir disoproxil fumarate 300mg and emtricitabine 200mg qd (Figure 1).

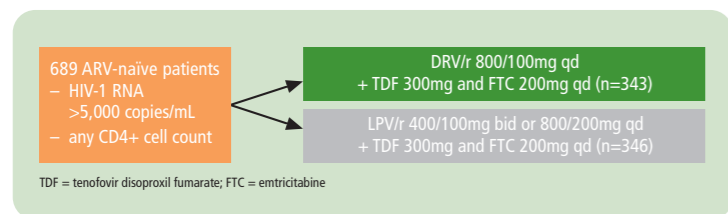


Figure 1. ARTEMIS study design.

- The HIV-1 subtype of baseline plasma samples from ARTEMIS was derived as the best match between the protease-RT nucleotide sequence (determined by Virco BVBA, Mechelen, Belgium) and the corresponding subtype consensus sequences from the Los Alamos National Laboratory subtype reference subset (2005).⁵
- Phenotypes of baseline plasma samples were determined using Antivirogram[®] (Virco BVBA).
- Virological response (defined as HIV-1 RNA <50 copies/mL) at Week 48 was determined using the time-to-loss of virological response (TLOVR) algorithm.
- For subgroup analysis, demographics were summarised at baseline, and virological (intent-to-treat [ITT], TLOVR) and immunological responses (ITT, non-completer status equals failure imputation algorithm [NC=F]) were summarised at Week 48 by HIV-1 subtype and by treatment arm.

Results

In-vitro antiviral activity against primary HIV-1 isolates from Group M and Group O

- DRV demonstrated high levels of in-vitro antiviral activity against all of the HIV-1 primary isolates evaluated in this study. The mean DRV EC₅₀ value was 1.04 nM (range: <0.10 to 4.28 nM) (Table 1).

Table 1. Antiviral activity of DRV against HIV-1 in PBMCs from Group M and Group O.

Subtype*	Number of isolates [†]	Median DRV EC ₅₀ nM (range)
Group M		
A	4	1.11 (0.20–2.66)
B	3	1.07 (0.39–4.28)
C	3	0.39 (0.38–0.47)
D	3	0.52 (0.27–1.14)
E	3	0.70 (0.21–0.83)
F	3	0.24 (0.13–1.21)
G	3	0.46 (<0.10–0.65)
Group O		
O	3	2.41 (1.59–2.54)

*Envelope sequences reported by the NAID AIDS Research and Reference Reagent Program (NARRRP); [†]All viral isolates were obtained from NARRRP, except for one subtype A isolate which was obtained from a paediatric patient attending the AIDS Clinic, University of Alabama, Birmingham, USA

Subtype distribution in ARTEMIS

- The majority (61%) of patients participating in ARTEMIS harboured HIV-1 subtype B; other prevalent subtypes found were subtype C (13%) and subtype CRF01_AE (17%); 9% of patients harboured other subtypes (Table 2).

Table 2. HIV-1 subtype distribution in ARTEMIS (n=687).

Subtype	n (%)
B	418 (60.8)
CRF01_AE	117 (17.0)
C	89 (13.0)
A1	15 (2.2)
CRF02_AG	14 (2.0)
CRF12_BF	11 (1.6)
CRF01_AE/A1	14 (2.0)
D	4 (0.6)
F1	4 (0.6)
BC	2 (0.3)
CRF03_AB	2 (0.3)
CX	2 (0.3)
K	2 (0.3)
CRF14_BG	1 (0.2)
F2	1 (0.2)
G	1 (0.2)

In-vitro protease inhibitor (PI) susceptibility by HIV-1 subtype in ARTEMIS

- The median EC₅₀ values for DRV were similar for different HIV-1 subtypes (B: 1.79 nM; C: 1.12 nM; CRF01_AE: 1.27 nM), (Table 3).
- For all PIs evaluated there was a trend for a higher susceptibility for samples harbouring subtype C or CRF01_AE compared with subtype B.

Baseline characteristics by HIV-1 subtype across treatment arms in ARTEMIS

- Subtype distribution was comparable between both treatment arms (Table 4).
- Baseline viral load and CD4+ cell counts were similar between patients with different viral subtypes in each treatment arm.

Virological and immunological responses by HIV-1 subtype in ARTEMIS

- Virological response according to HIV-1 subtype is shown in Figure 2
 - response rates in both treatment arms were comparable across patient groups harbouring subtypes B, C and CRF01_AE
 - higher numerical response rates were observed in all non-B subtype groups compared with the B subtype group in the DRV/r treatment arm
 - although the subgroup of patients with non-B subtypes was both limited in numbers and diverse (13 different subtypes), the differences in response rates observed between the DRV/r and LPV/r arms for each non-B subtype were consistent with that observed in the subgroup of patients with subtype B.

Table 3. In-vitro PI susceptibility of baseline samples in ARTEMIS.

Clade	N	Median EC ₅₀ nM (IQR)							
		DRV	APV	ATV	IDV	LPV	NFV	SQV	TPV
B	270	1.79 (1.26–2.63)	12.58 (9.61–20.61)	2.56 (2.07–3.39)	13.69 (10.04–22.19)	3.63 (2.44–4.66)	24.58 (14.22–35.42)	7.02 (4.17–8.72)	62.05 (40.75–100.45)
C	88	1.12 (0.82–1.38)	8.98 (7.31–12.16)	2.24 (1.68–2.68)	9.51 (8.07–12.36)	2.82 (1.69–3.70)	14.67 (10.94–23.02)	5.36 (3.91–7.17)	42.24 (35.76–63.26)
CRF01_AE	112	1.27 (1.03–1.68)	8.65 (6.48–10.84)	2.25 (1.92–2.81)	8.82 (7.35–10.71)	2.59 (1.93–3.43)	13.27 (9.26–21.18)	5.99 (4.06–7.20)	49.32 (37.10–64.83)
HIV-1/IIIB*	715	3.24 (2.54–3.31)	21.53 (14.08–23.86)	3.60 (2.44–4.50)	20.48 (16.36–23.45)	4.52 (3.96–5.28)	22.93 (15.26–27.69)	9.15 (5.84–10.18)	92.68 (40.81–108.57)

Clade	N	Median FC (IQR)							
		DRV	APV	ATV	IDV	LPV	NFV	SQV	TPV
B	270	0.60 (0.40–0.90)	0.70 (0.50–1.00)	0.70 (0.60–1.10)	0.70 (0.50–1.10)	0.80 (0.60–1.00)	1.10 (0.70–1.60)	0.75 (0.50–1.00)	0.80 (0.60–1.10)
C	88	0.30 (0.20–0.40)	0.40 (0.30–0.60)	0.50 (0.40–0.70)	0.40 (0.40–0.60)	0.60 (0.40–0.75)	0.60 (0.40–0.80)	0.60 (0.40–0.70)	0.40 (0.30–0.70)
CRF01_AE	112	0.40 (0.30–0.50)	0.40 (0.30–0.50)	0.50 (0.40–0.60)	0.40 (0.30–0.50)	0.50 (0.35–0.60)	0.50 (0.40–0.70)	0.50 (0.40–0.70)	0.50 (0.35–0.65)

*Subtype B HIV-1/IIIB reference strain
IQR = interquartile range; APV = amprenavir; ATV = atazanavir; DRV = darunavir; IDV = indinavir; LPV = lopinavir; NFV = nefinavir; SQV = saquinavir; TPV = tipranavir; FC = fold-change in EC₅₀ compared to subtype B HIV-1/IIIB reference strain

Table 4. Baseline disease characteristics by HIV-1 subtype and treatment arm in ARTEMIS.

	DRV/r	LPV/r
Number of patients		
Overall	343	346
Subtype B	210	208
Subtype C	39	50
Subtype CRF_AE	62	55
Other subtypes	31	32
Log₁₀ viral load (copies/mL), mean (SD)		
Overall	4.86 (0.64)	4.84 (0.60)
Subtype B	4.90 (0.61)	4.85 (0.58)
Subtype C	4.78 (0.62)	4.69 (0.60)
Subtype CRF_AE	4.83 (0.70)	4.93 (0.60)
Other subtypes	4.80 (0.75)	4.86 (0.75)
CD4+ cell count (x 10⁶/L), mean (SD)		
Overall	245 (148.8)	231 (132.6)
Subtype B	246 (134.8)	232 (134.6)
Subtype C	258 (170.5)	223 (120.5)
Subtype CRF_AE	238 (176.1)	240 (143.7)
Other subtypes	233 (157.7)	225 (118.9)

SD = standard deviation
Subtype determination was unavailable for one patient in the DRV/r arm and one patient in the LPV/r arm

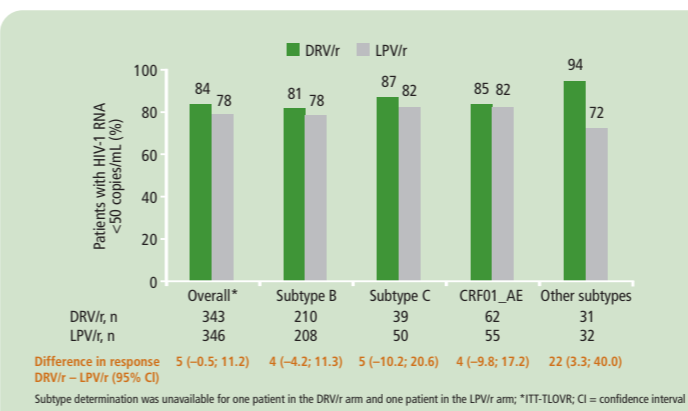


Figure 2. Proportion of patients achieving HIV-1 RNA <50 copies/mL at Week 48 by HIV-1 subtype in ARTEMIS.

- Mean changes in CD4+ cell count from baseline through Week 48 were comparable overall and across subtypes B, C, and CRF01_AE in both treatment arms. No clinically relevant impact of subtype on immunological response was seen in both treatment arms.⁶

Conclusions

- DRV is highly active *in vitro* against a selected set of Group M and Group O HIV-1 primary isolates from diverse origins.
- The in-vitro susceptibility of HIV-1 clinical isolates to DRV is comparable across all subtypes studied in the ARTEMIS trial.
- In ARV-naïve patients enrolled in the ARTEMIS study, DRV/r 800/100mg once daily was
 - highly effective, overall
 - equally effective irrespective of the HIV-1 subtype.
- The difference in response rates observed between the DRV/r and LPV/r arm in ARTEMIS were consistent across all HIV-1 subtypes.
- The results of this analysis confirm the broad spectrum activity of DRV/r against HIV-1.

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