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Darunavir Concentrations in Seminal Plasma in patients receiving Darunavir/ritonavir (DRV/r) monotherapy: a MONOI-ANRS 136 substudy

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anRS
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Abstract

Background: Concern remains on the efficacy of boosted-protease inhibitors (PI/r) monotherapy on viral sanctuaries such as male genital tract because of the poor penetration of most PI in semen and the subsequent risk of persistent viral replication and emergence of resistance

Objective: To evaluate the concentration of DRV and outcomes on HIV-1 shedding in the genital tract in patients receiving DRV/r

Methods: HIV-1 infected men enrolled in the MONOI randomized trial received DRV/r (600/100 mg bid) monotherapy or DRV/r + 2NRTIs after a 10 weeks run-in period of triple drugs therapy. Single paired samples of blood plasma (BP) and seminal plasma (SP) were collected at D0 and W48. The Cobas Taqman HIV-1 Assay was used to quantify HIV-1 RNA in BP and in SP (at D0 and W48) with limits of quantification of 40 and 200 c/mL respectively. Total and free fraction BP and Total SP DRV concentrations were determined at D0 and W48 using UPLC-MS/MS method (Acquity UPLC® - Acquity TQD®) after samples pretreatment (LOQ ~ 1ng/ml). DRV plasma protein binding was performed using an ultrafiltration assay with Centrifree® devices. Results are presented as median (IQR25%-75%).

Results: Among the 47 patients enrolled in the substudy, 23 received DRV/r alone and 24 the triple combination. Total and Free BP DRV concentrations determined 12 hours (10.8-13.5) after the last drug intake were 3,200ng/ml (2,127-4,179; n=70) and 212ng/mL (154-326; n=70), respectively. Total SP DRV concentrations determined 15.9 hours (13.3-17.3) after the last drug intake were 344ng/ml (149-652; n=95). The Free/Total BP and SP/BP ratio for DRV concentrations were 7.2% (5.9-9.0%) and 8.6% (5.7-22.2%), respectively. HIV-1 RNA was detectable in 6 SP samples in different patients (at D0: 1345; 345 and 385 c/ml and at W48: 270, 345 and 475 c/ml), although it was undetectable in the corresponding BP samples (3 at D0 while patients were all under triple combination). At W48, among the 3 discordant samples, 1 patient was receiving DRV/r monotherapy (5%) and 2 triple therapy (10.5%). Whatever the biological matrix, no relationship between DRV concentrations and HIV-RNA was evidenced.

Conclusion: Median DRV SP concentration is close to the BP Free fraction and approximately 6 fold higher than DRV EC50 corrected for protein binding of WT HIV-1 (~55ng/ml) demonstrating a good penetration of DRV in the genital tract

Background (1): MONOI Study

- MONOI is a prospective, open-label, non-inferiority, 96-week safety and efficacy trial in virologically suppressed patients on triple therapy who were randomized to a DRV/r triple drug regimen or DRV/r monotherapy
- In the Per Protocol analysis, DRV/r monotherapy showed non-inferior efficacy versus DRV/r + 2 NRTI at W48 in the primary analysis: **94.1% vs 99.0%** (δ = -4.9%, 90% confidence interval, -9.1 to -0.8)
- The efficacy rates in Intent to Treat analysis were very comparable and close to non-inferiority : **87.5% vs 92%** (δ = -4.5%, 90% confidence interval -11.2 to 2.1)
- Three virological failures (>400 cp/ml) were observed in DRV/r monotherapy with no induced resistance to DRV and subsequent viremia suppression after resuming 2 NRTIs
- Discordant Plasma/CNS symptomatic HIV replication in 2 patients on DRV/r with subsequent viral suppression

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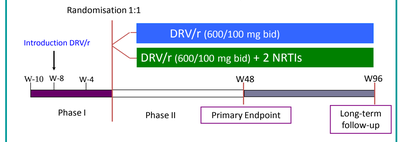
MONOI Study Design

- Multicenter open label randomized study



Main inclusion criteria
- cART ≥ 18 months
- CD4 count ≥ 200 cells/mm^3
- HIV RNA <400 copies/ml in the last 18 months and <50 copies/ml at entry
- No history of PI failure and naïve to darunavir

Background (2): MONOI PK Seminal Substudy

- Available information on antiretroviral drugs penetration into the male genital tract is sparse
- Concern remains on the antiviral activity of boosted-protease inhibitors (PI/r) monotherapy on viral sanctuaries such as male genital tract because of:
  - the poor penetration of most PI in semen
  - and the subsequent risk of persistent viral replication and emergence of resistance
- This is the first study demonstrating the DRV penetration into the seminal fluid and concomitantly the virological outcomes in Blood Plasma and Seminal Plasma compartments

Objectives

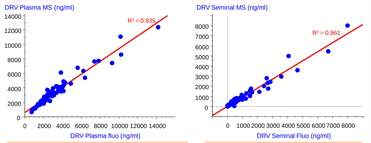
- **Pharmacokinetics:**
  - To determine the DRV concentrations in:
    - Blood plasma (free* and total protein fractions)
    - Seminal plasma (total protein fractions)
- **Virologicals:**
  - To evaluate the outcomes on HIV-1 shedding in the genital tract in patients receiving DRV/r

** Unbound fraction of drug is considered as the only effective fraction available for the diffusion/penetration in tissues*

Material and Methods

- HIV-1 infected men enrolled in the MONOI randomized trial received DRV/r (600/100 mg bid) monotherapy or DRV/r + 2NRTIs after a 10 weeks run-in period of triple drugs therapy
- Single paired samples of blood plasma (BP) and seminal plasma (SP) were collected at D0 and W48
- Total and free fraction BP and Total SP DRV concentrations were determined at D0 and W48 using UPLC-MS/MS method (Acquity UPLC® - Acquity TQD®) after samples pretreatment (LOQ ~ 1ng/ml)
- DRV plasma protein binding was performed using an ultrafiltration assay with Centrifree® devices. Duplicate determinations were performed and mean results were considered if CV < 20%. If not, re-analysis were performed
- Results of DRV concentrations were also determined using an HPLC-Fluorimetric method after samples extraction (SPE) to compare the results of the two methods (new Mass Spectrometry vs traditional fluorimetric)
- The Cobas Taqman HIV-1 Assay was used to quantify HIV-1 RNA in BP and in SP (at D0 and W48) with limits of quantification of 40 and 200 c/ml, respectively
- Results are presented as median (IQR25%-75%)

Determinations of DRV Concentrations : Comparison between UPLC-MS/MS and HPLC-Fluo methods

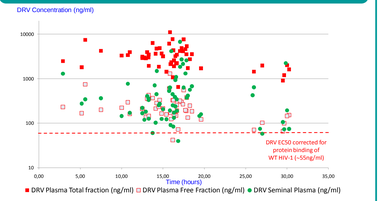


UPLC-MS/MS (Waters Corp. Milford MA, USA)
- 100 µL of Plasma (Blood/Seminal)
- Liquid Liquid Extraction
- Acquity UPLC® BEH C18 1.7µm (2.1 x 5.0 mm Column)
- Acquity TQD® (ESI+, at transition 548.5 → 392.2 uma)
- DRV LOQ ~ 1 ng/mL

HPLC-Fluo (Waters Corp. Milford MA, USA)
- 500 µL of Plasma (Blood/Seminal)
- Solid Phase Extraction (C18, 500 mg/3 mL)
- Column Beckman C18 5µm (4.6 x 250 mm)
- Fluo 475 Detector (λ_{ex} ~ 265 → λ_{em} ~ 340 nm)
- DRV LOQ ~ 5 ng/mL

- The 2 tested methods were appropriate to determine DRV concentrations (with highly correlation p<0.001) but the UPLC-MS/MS was more sensitive with lower sampling volume.

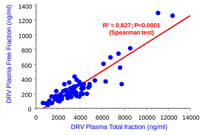
Distribution of DRV Concentrations in Blood Plasma and in Seminal Plasma over the sampling time



- The distribution of DRV free concentrations was close to the distribution of DRV seminal concentrations

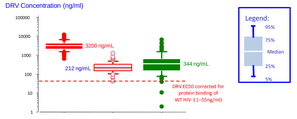
Relationships between DRV concentrations in Blood Plasma and in Seminal Plasma

- Free and total fractions of DRV Blood Plasma concentrations were statistically related:



- In contrast, neither relationship between BP free fraction nor total fraction with SP of DRV concentrations was found (role of membrane transporters?)

DRV Concentrations in Blood Plasma and in Seminal Plasma



	Interval last intake/Sampling		DRV Concentrations (ng/mL)			DRV Ratio (%)	
	Blood Plasma (Hours)	Seminal Plasma (Hours)	Blood Plasma Total Fraction	Blood Plasma Free Fraction	Seminal Plasma	Blood Plasma Free/ Total Fraction	Seminal Plasma/Blood Plasma Total fraction
N	69	87	70	70	95	70	70
Median	12.0	15.9	3200	212	344	7,2%	8,6%
IQR25%-75%	10.8-13.5	13.3-17.3	2127-4179	154-326	149-652	5.9-9.0%	5,7-22,2%

DRV results from triple and mono- therapy were merged for this analysis,

Virological results

- Among the 45 patients tested for HIV-1 RNA in both samples, HIV-1 RNA was detectable (> 200 c/mL) in 6 SP samples in 6 different patients:

Patients N°	D0*		W48		Arm after D0
	BP	SP	BP	SP	
1	<40	<200	<40	270	Mono
2	<40	1345	<40	<200	Mono
3	<40	345	<40	<200	Mono
4	<40	385	<40	NA	Triple
5	<40	<200	<40	345	Triple
6	<40	<200	<40	475	Triple

**At D0, all patients received a triple drug regimen containing DRV/r (Run-in period)*

- Whatever the biological matrix, no relationship between DRV concentrations and HIV-RNA was evidenced.

Summary (1)

- **Analytical results in BP and SP samples:**
- DRV concentrations determined using UPLC-MS/MS method (LOQ ~ 1 ng/ml) were statistically correlated with those obtained using HPLC-Fluorimetric method (LOQ ~ 5 ng/ml)
- Limit of quantification and sampling volume used with UPLC-MS/MS method was adapted with the necessity of the PK analysis in these compartments
- No matrix effect was expected using these methods

- **PK results in Blood Plasma:**
- Trough BP DRV ~ 3200 ng/mL (2127-4179) (median, IQR25-75%) determined in this study were close to those obtained from TMC114-C213 and TMC114-C202 studies (119 subjects receiving DRV/r 600/100 mg bid) ~ 3539 ng/mL (1255-7368) (median, range)
- DRV Plasma Free Fraction was ~ 7.2% (5.9-9.0%) was consistent with a 95% protein binding primarily to plasma alpha 1-acid glycoprotein*
- Good correlation between DRV Total and Free fractions of plasma proteins (r^2 = 0.827; p<0.0001)

**Prezista® Label Information, FDA, 2006*

Summary (2)

- **PK results in Seminal Plasma:**
- DRV Seminal plasma concentrations (median, IQR) was ~ 8.6% (5.7-22.2%) the Blood Plasma concentrations,
- Poor relationships between DRV Plasma Seminal concentrations and Total and Free fractions of plasma proteins
- DRV Seminal Plasma (Median ~ 344 ng/ml) was:
  - ~ 6 fold above the DRV EC_{50} (corrected for protein binding) of WT HIV-1 strains (~ 55 ng/ml),
  - Only 3 DRV SP concentrations were < 55 ng/mL

- **PK-PD relationship:**
- Whatever the biological matrix, no relationship between DRV concentrations and HIV-RNA was evidenced

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