Darunavir (DRV) concentrations exceed the Protein-corrected (PC) EC₅₀ for wild type HIV in the semen of HIV-1 infected Men

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Background

- Available information on ART drug penetration into the male and female genital tract is sparse. In part this is due to the difficulties with sample collection and analysis
- However, we have found that multiple sampling at specified time points post drug ingestion on the same or separate days can provide important insights into drug dynamics and concentrations achieved within these important body compartments
- With the continued interest in protease inhibitor monotherapy strategies, it is vital to have accurate data as to whether single drugs achieve therapeutic drug concentrations in all body compartments such as the central nervous system and the genital tract
- Furthermore, information on drug penetration and antiviral activity in the male and female genital tract has particular implications for the prevention of sexual and vertical transmission of HIV, as well as informing PEPSE and PREP strategies
- In this study we have evaluated the penetration of DRV into the semen of HIV +ve men and compared this to the published literature

Methods

- 18 HIV-1 positive men prospectively produced time matched semen and blood samples at designated time points post drug ingestion
- Samples were extracted by protein precipitation (acetonitrile) and analysed by HPLC-MS/MS (using a plasma calibration curve) with a lower limit of detection for [DRV] of 79ng/ml
- [DRV] concentrations were measured in seminal plasma and compared to published PC EC₅₀ values for WT HIV (55ng/ml) and the PC EC₅₀ for resistant HIV (550ng/ml)
- Time specific SP:BP ratios were calculated. When patients provided 4 or more time matched semen and blood samples at specific times post drug ingestion, seminal plasma and blood plasma area under the concentration time curves (AUC) were constructed using non-compartmental anlalysis; WinNonlin, version 5.2
- Blood plasma viral load (BPVL) was measured in all patients and seminal plasma RNA (SPVL) was measured when sufficient sample was available as using the Roche Cobas Taqman HIV-1 assay
- The lower limit of detection was < 40 c/ml for BPVL and <200c/ml for seminal plasma viral load
- In this study all patients were taking DRV 800mg OD with RTV 100mg OD
 Patients were questioned regarding symptoms of STIs and tested for chlamydia and gonorrhoea by PCR (Aptima Combo 2)

Results

- Patient Demographics (see Table 1)
- Drug concentration results (see Table 2 and Figure 1)
- 34 closely timed SP and BP samples were produced by 18 HIV+ individuals. 5 individuals provided 4 or more time matched SP and BP samples at designated time points post drug ingestion to allow construction of seminal plasma drug concentration time curves (see Figure 1)
- •RTV concentrations in semen were undetectable in all but 2 SP samples as previously described (1)
- In all patients who had semen available for viral load analysis, SPVL was undetectable in all samples with the exception of one patient (pt 9)
- Pt 9 had a detectable SPVL (1460 c/ml) which was confirmed on duplicate testing on 2 different semen samples produced 6 days apart (1310 c/ml). This patient declined to be tested for sexually transmitted infections as part of the study

Table 1: Patient demographics and status at the time of blood and semen collection

Patient	Age	Risk Group	ART at time of study	Time on DRV containing ART	CD4 cells/mm3	Blood Plasma Viral Load (cp/ml)	Seminal Plasma Viral load (cp/ml)
1.	40	MSM	TRV/DRV/RTV	2 Wks	833	<40	Not available
2.	42	MSM	TDF/CBV/DRV/RTV	60 Wks	439	<40	<200
3.	42	MSM	TRV/DRV/ RTV	59 Wks	615	146	<200
4.	59	MSM	TRV/DRV/ RTV	4 Wks	370	<40	<200
5.	46	HETEROSEXUAL	CBV/DRV/ RTV	3 Wks	466	<40	<200
6.	63	MSM	TRV/DRV/ RTV	18 Wks	456	242	Not available
7.	45	MSM	KVX/DRV RTV	35 Wks	850	102	<200
8.	46	MSM	TRV/DRV/ RTV	24 Wks	496	<40	<200
9.	53	MSM	TRV/DRV/ RTV	38 Wks	540	<40	1460*
10.	62	MSM	TDF/3TC/ DRV/RTV	38 Wks	484	<40	<200
11.	45	HETEROSEXUAL	TRV/DRV/ RTV	22 Wks	350	<40	Not available
12.	41	MSM	KVX/DRV/ RTV	2 Wks	633	<40	<200
13.	46	MSM	TRV/DRV/ RTV	2 Wks	379	87	<200
14.	46	HETEROSEXUAL	TRV/DRV/ RTV/MVC	18 Wks	87	71	<200
15.	46	HETEROSEXUAL	DRV/RTV/ MVC	21 Wks	608	70	<200
16.	26	HETEROSEXUAL	KVX/DRV/ RTV	22 Wks	536	<40	<200
17.	46	MSM	DRV/RTV/ 3TC/MVC	26 Wks	243	<40	<200
18.	35	MSM	TRV/DRV/ RTV	22 Wks	169	<40	<200

Table 2:

Median drug concentrations in semen and blood plasma and SP:BP penetration ratios for Darunavir calculated at time after last drug intake

Time post Drug Ingestion	1-3h	4-6h	22-24h	AUC ₀₋₂₄
[Darunavir] ng/ml				
Median BP [DRV] IQR	5579 (4639-7505)	3734 (2935-4586)	2445 (1365-3167)	
Median SP [DRV] IQR	588 (509-778)	490 (479-640)	217 (172-261)	
Median SP:BP ratio IQR	0.11 (0.09-0.15) n=8	0.13 (0.07-0.18) n=13	0.11 (0.09-0.15) n=14	SP:BP AUC _{0-24h} ratio 0.17 (range 0.07-0.19) n=5
Median multiple above PC EC ₅₀ for WT virus	11 fold	9 fold	4 fold	
55ng/ml	(6-45)	(3-21)	(2-16)	

Figure 1: Darunavir Semen vs Blood Concentrations in 18 HIV +ve men and Seminal Plasma DRV AUC vs Blood Plasma AUCs in 5 men

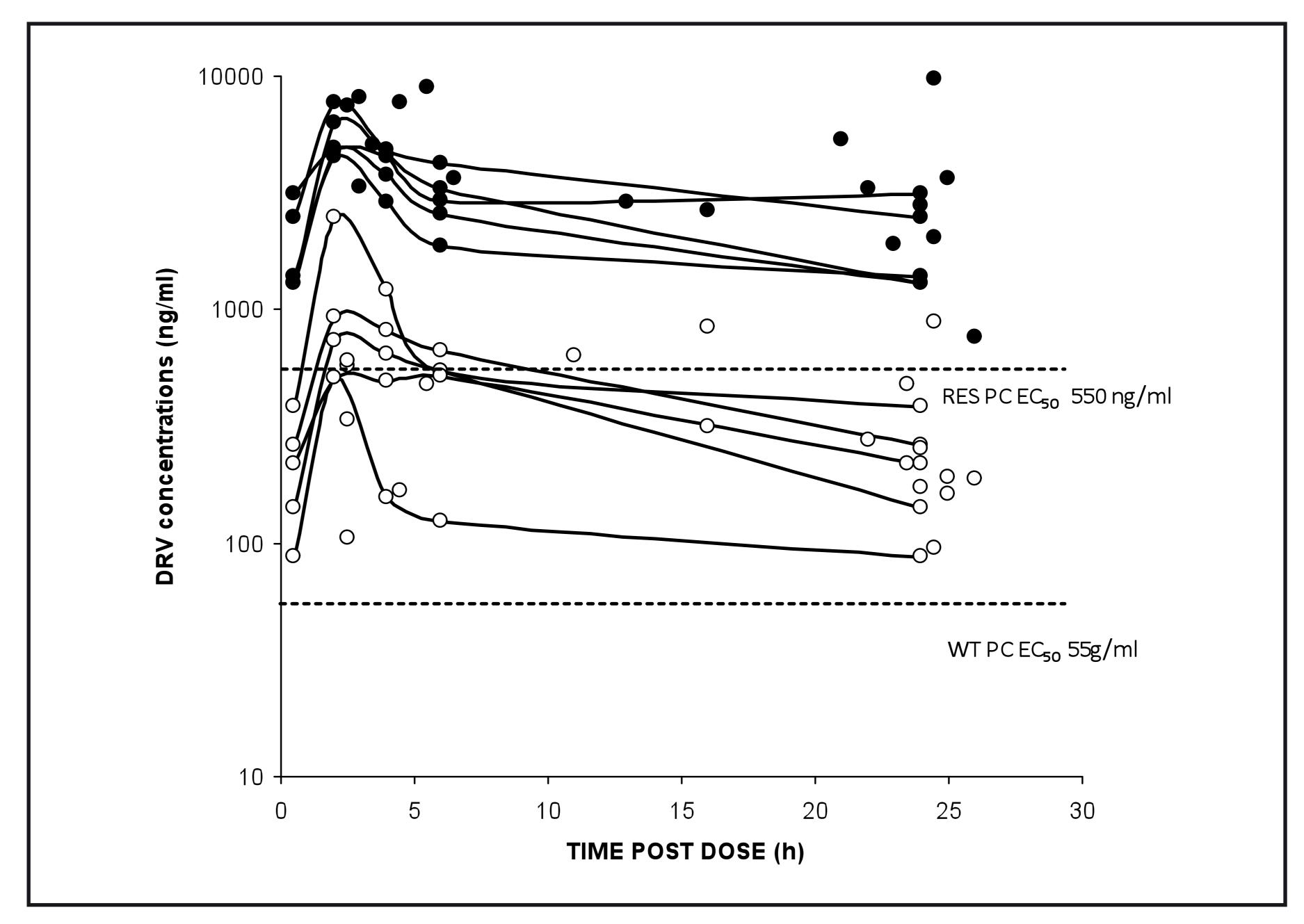


Figure 2. Comparison of DRV penetration into semen compared to other studies of ART in the genital tract

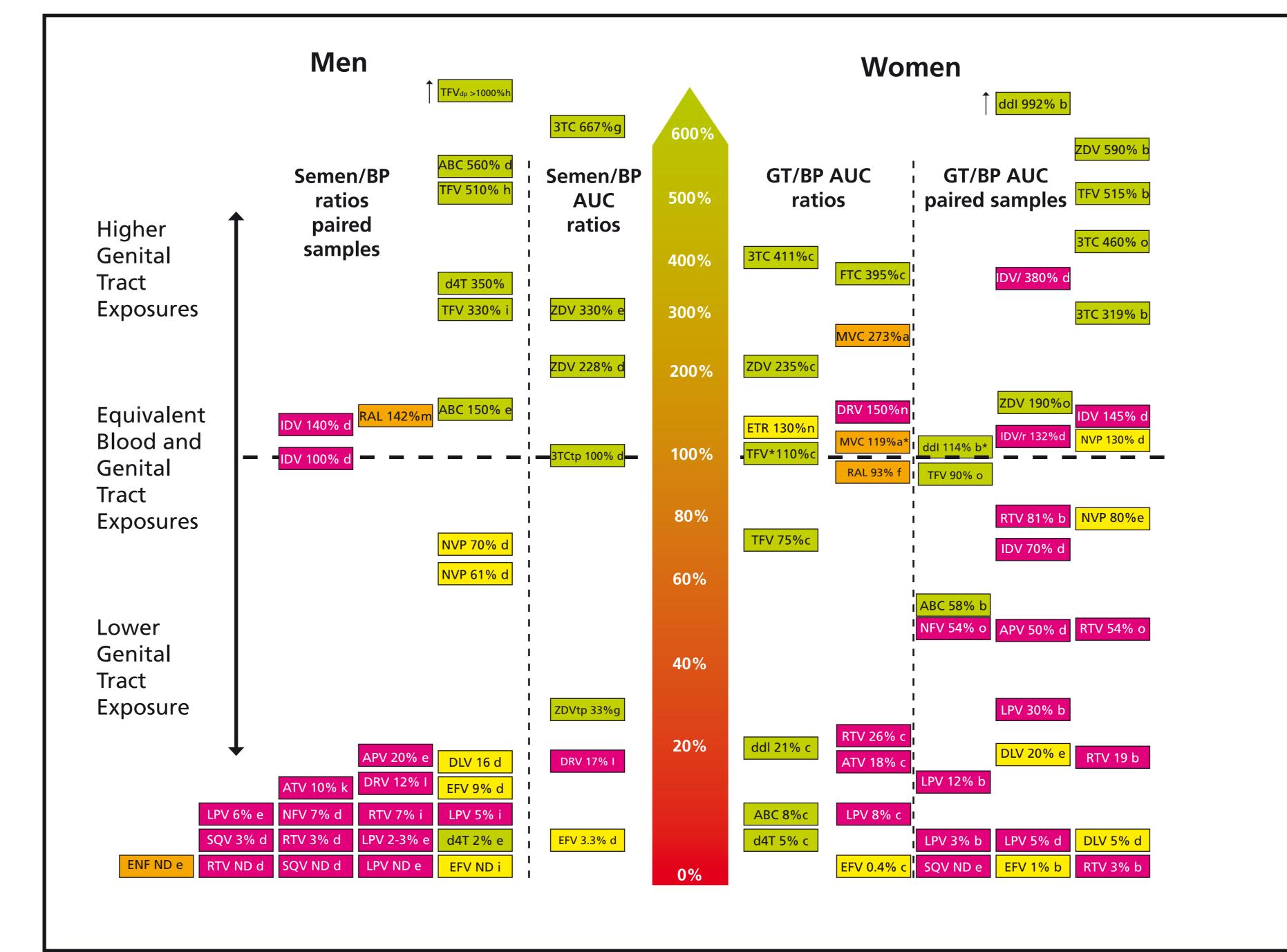


Figure 2: From Taylor and Davies 2010 adapted from Cohen, Gay and Kashuba 2007

Key: Green boxes; Nucleoside analogues, Pink Boxes; Protease inhibitors, Yellow boxes: NNRTIs, Orange boxes: Entry inhibitors, CCR5 and Integrase inhibitors, *indicates levels measured after single-dose of drug; all others steady-state levels

Discussion points

- This study is the first to systematically look at the penetration of DRV into the semen of HIV infected men using both time matched seminal plasma and blood plasma samples as well as SP:BP AUCs from individual patients
- The data suggest DRV has good penetration into the semen of HIV-1 infected men with concentrations approximating 10-20% of the concentrations achieved in the blood plasma at the same time points post drug ingestion
- More importantly all seminal plasma [DRV] were above the published protein corrected (PC) EC₅₀ values for WT HIV (55ng/ml
- Furthermore, 1/3rd of all SP [DRV] exceeded the PC EC50 required to inhibit PI resistant HIV
- The fact that 1 patient had low level detectable viraemia whilst on 3 drugs (at least 2 with good genital tract penetration) in the presence of an undetectable blood plasma viral load should raise caution about extrapolating "undetectable in blood = undetectable in the genital tract"
- This data may have important implications for protease inhibitor monotherapy studies, the evolution and transmission of resistant viruses, and the sexual transmission of HIV

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