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Does the E138A Mutation in ASPIRE Seroconverters Affect Susceptibility to Dapivirine?

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MTN microbicide trials network

ASPIRE A Study to Prevent Infection with a Ring for Extended Use

Background

- ASPIRE was a safety and effectiveness study of the non-nucleoside reverse transcriptase inhibitor (NNRTI) dapivirine (DPV)-containing vaginal ring for HIV-1 prevention conducted at 15 sites in South Africa, Zimbabwe, Malawi and Uganda.
- E138A is a polymorphism that occurs naturally in 5% of treatment-naïve HIV-1 subtype C-infected individuals.
- E138A has been shown *in vitro* to cause 3-fold resistance to other diarylpyrimidine (DAPY) class inhibitors like etravirine and rilpivirine. Mutations at codon 138 are frequently selected in therapy failures from DAPY class NNRTIs.
- The effect of E138A on DPV susceptibility is unknown.
- E138A was the most common NNRTI associated drug resistance mutation (DRM) found in ASPIRE. Prevalence of E138 mutations were not significantly different by arm *(Ref1)*.
- This study evaluates the prevalence and phenotypic effect of E138A in individuals who seroconverted in the ASPIRE study.

HIV-1 NNRTI resistance mutations found in the ASPIRE seroconverters

Mutation	PLB Ring N = 96	DPV Ring N = 68
V90I	1 (1%)	2 (3%)
L100I	0 (0%)	0 (0%)
K101E	1 (1%)	1 (1.5%)
K103N	1 (1%)	2 (3%)
K103S	0 (0%)	1 (1.5%)
V106M	0 (0%)	1 (1.5%)
V108I	0 (0%)	1 (1.5%)
E138A	**5 (5%)**	**3 (4%)**
E138G	0 (0%)	1 (1.5%)
E138K	0 (0%)	0 (0%)
V179D	2 (2%)	1 (1.5%)
V179I/T	0 (0%)	1 (1.5%)
Y181C	0 (0%)	0 (0%)
H221Y	1 (1%)	1 (1.5%)

*(Ref1)*Baeten JM, et al. 2016. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. N Engl J Med **375**:2121-2132.

Methods

Sample selection: Population sequencing was performed on plasma from 164 seroconverters from ASPIRE. Samples with mutations in RT codon 138 were selected for phenotypic testing. Matched samples (by study arm, viral load, and site) containing no HIV-1 DRM were also tested as controls.

	Description	Sequence Coverage	Plasma HIV-1 RNA Cut-Off	Mixture Cut-Off	Analysis
Genotyping Assay	In-house Sanger sequencing based population genotyping using primers optimized for non-B HIV-1 subtypes	Pro (aa 1 – 99) full-length RT (aa 1-560)	≥ 200 copies/ml	>20%	Stanford Genotypic Resistance Interpretation Algorithm v7.0
Phenotyping Assay	In-house population phenotyping using plasma-derived recombinant virus and TZM-bl cell line	full-length RT (aa 1-560)	≥ 200 copies/ml	Not established	Linear mixed-effects models were used with Satterthwaite approximations to determine significance, with Bonferroni corrections for multiple comparisons

Phenotyping Assay Workflow

Extract HIV-1 RNA from patient plasma

↓

Generate cDNA & PCR amplify RT full-length sequence (aa 1-560)

↓

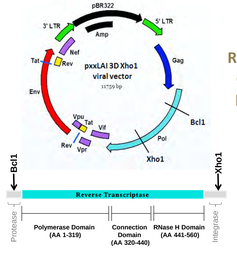
Clone full-length HIV-1 RT into viral vector

↓

Transfect cells with plasma-derived viral vector and prepare viral stocks

↓

Determine viral susceptibility to DPV, NVP and EFV and RPV using TZM-bl cells



Recombinant viruses contain full-length plasma-derived RT

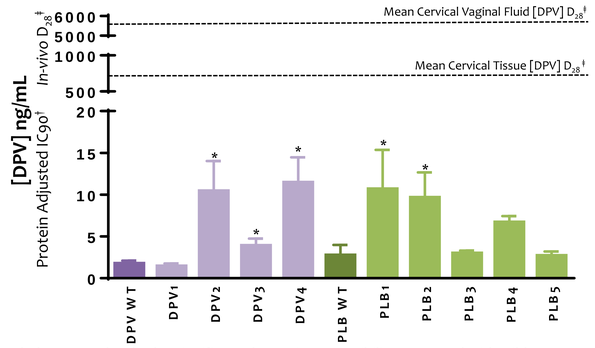
Results

1. Plasma-derived codon 138 mutations confer low level resistance to DPV in some genotypic backgrounds but not others.

		DPV Susceptibility			
Arm and Participant	**Genotype**	**IC_{50}(nM) ±SD§**	**Fold-Change¶**	**p-value**	**Plasma Drug levels (pg/mL)****
DPV WT‡	wild type	0.7 ± 0.1	---		206
DPV1	E138A, V179D	0.6 ± 0.1	0.9	1.00	508
DPV2*	**E138A, V179I/T**	**4.2 ± 1.4**	**6**	**<0.001**	**182**
DPV3*	**V108I/V, E138A**	**1.5 ± 0.3**	**2.1**	**<0.001**	**73.6**
DPV4*	**K101E, E138G**	**4.6±1.2**	**6.6**	**<0.001**	**477**
PLB WT‡	wild type	1.2± 0.5	---		---
PLB1*	**K101E, E138A**	**4.3 ± 1.9**	**3.6**	**<0.001**	---
PLB2*	**E138A**	**3.9 ±1.2**	**3.3**	**<0.001**	---
PLB3	E138A	1.2± 0.1	1	1.00	---
PLB4	E138A	2.7 ± 0.3	2.3	0.12	---
PLB5	E138A	1.1 ± 0.2	0.9	1.00	---

*These samples display a significant change in susceptibility compared with wild type samples from the same study arm.
‡DPV and PLB WT were generated by making a composite IC_{50} from plasma-derived recombinant viruses HIV-1 that had no NNRTI DRMs (DPV WT n=3, PLB WT n=5).
§IC_{50} values were generated for each plasma-derived recombinant virus in 3 independent experiments.
¶Fold-Change was calculated as IC_{50} of mutant/wildtype in each arm.
\\p-values were calculated using Linear mixed-effects models and used with Satterthwaite approximations to determine significance, with Bonferroni corrections for multiple comparisons .
**Plasma DPV levels were measured from the same blood draw collected for phenotypic testing. Plasma DPV levels of ≥95pg/mL indicate some level of adherence. The average DPV level is shown for the wild type samples.

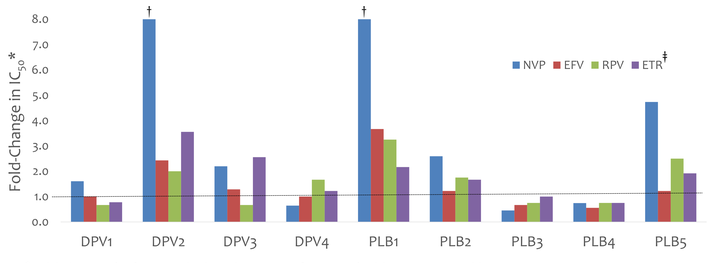
2. Cervical tissue and cervical vaginal fluid DPV concentrations from monthly ring use exceed low-level resistance conferred by codon 138 mutations by 40-400 fold.



* These samples display significant change in susceptibility compared with wild type samples from the same study arm.
†DPV *in- vivo* protein binding and estimated IC90 were calculated by multiplying IC50 (ng/mL) by a factor of 7.8 *(Ref2)*.
‡Mean cervical tissue (600 ng/mL) and cervical vaginal fluid (5,700 ng/mL) DPV concentrations found after 28 days (D28) of DPV ring use in the Phase 1 MTN-013 study *(Ref3)*.

(Ref 2) Penrose KJ, et al. 2016. Frequent Cross-Resistance to Dapivirine in HIV-1 Subtype C-Infected Individuals on Failing First-Line Antiretroviral Therapy in South Africa. Antimicrob Agents Chemother doi:10.1128/AAC.01805-16.
(Ref 3) Chen BA, et al. 2015. Phase 1 Safety, Pharmacokinetics, and Pharmacodynamics of Dapivirine and Maraviroc Vaginal Rings: A Double-Blind Randomized Trial. J Acquir Immune Defic Syndr **70**:242-249.

3. Plasma derived recombinant viruses display variable cross-resistance to other NNRTIs.



*Fold change (FC) was calculated as IC50 of mutant/wild type. Dotted line notates FC=1.
† FC values exceed the scale of this graph (see Table 3 for actual FC value)
‡ NVP (nevirapine), EFV (efavirenz), RPV (rilpivirine), ETR (etravirine)

4. Plasma-derived recombinant viruses display generally low but variable cross-resistance to other NNRTIs.

		NVP		EFV		RPV		ETR	
Arm and participant	**Genotype**	**IC_{50} (nM)**	**Fold-Change**	**IC_{50} (nM)**	**Fold-Change**	**IC_{50} (nM)**	**Fold-Change**	**IC_{50} (nM)**	**Fold-Change**
DPV WT	**wild type**	**36 ± 13**	**---**	**0.7 ± 0.1**	**---**	**0.3 ±0.03**	**---**	**0.9 ± 0.2**	**---**
DPV1	E138A, V179D	58	1.6	0.7	1.0	0.2	0.7	0.7	0.8
DPV2	E138A, V179I/T	376	11	1.7	2.4	0.6	2.0	3.2	3.6
DPV3	V108I/V, E138A	79	2.2	0.9	1.3	0.2	0.7	2.3	2.6
DPV4	K101E, E138G	23	0.6	0.7	1.0	0.5	1.7	1.1	1.2
PLB WT	**wild type**	**46 ± 23**	**---**	**0.9 ± 0.4**	**---**	**0.4 ± 0.1**	**---**	**1.2 ± 0.2**	**---**
PLB1	K101E, E138A	864	19	3.3	3.7	1.3	3.3	2.6	2.2
PLB2	E138A	119	2.6	1.1	1.2	0.7	1.8	2.0	1.7
PLB3	E138A	21	0.5	0.6	0.7	0.3	0.8	1.2	1.0
PLB4	E138A	34	0.7	0.5	0.6	0.3	0.8	0.9	0.8
PLB5	E138A	217	4.7	1.1	1.2	1.0	2.5	2.3	1.9

Conclusions

- E138A is a naturally occurring polymorphism in HIV-1 subtype C that is associated with modest reductions in DPV susceptibility in some RT backgrounds but not others. Cervical tissue and cervical vaginal fluid DPV concentrations from regular ring use exceed the highest DPV IC50s of isolates containing E138A by 40 to 400-fold.
- The frequency and extent of reduced susceptibility to DPV associated with E138A as the major variant was independent of the ASPIRE study arm.
- Although the low frequency of E138A limited the sample size, these phenotypic data provide reassurance that the E138A mutation was not selected by the DPV vaginal ring and is unlikely to reduce efficacy of the DPV vaginal ring for HIV-1 prevention.

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