MTN
microbicide trials network

Sensitive Next-Generation Sequencing of HIV-1 in ASPIRE

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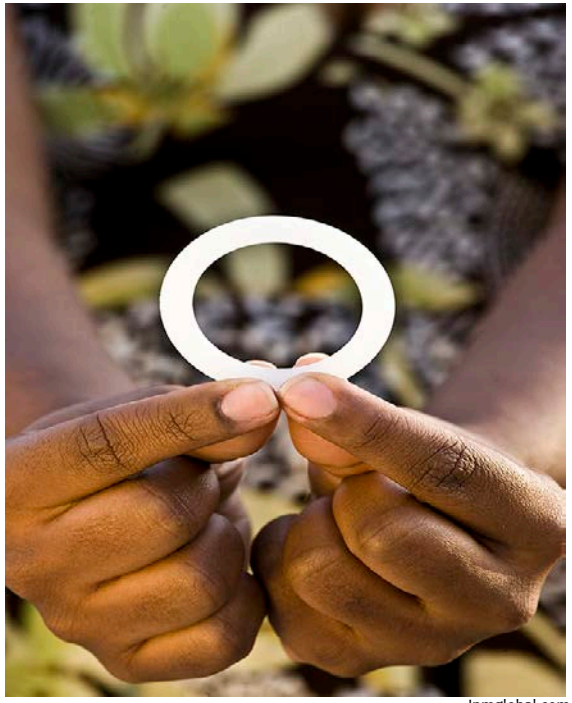
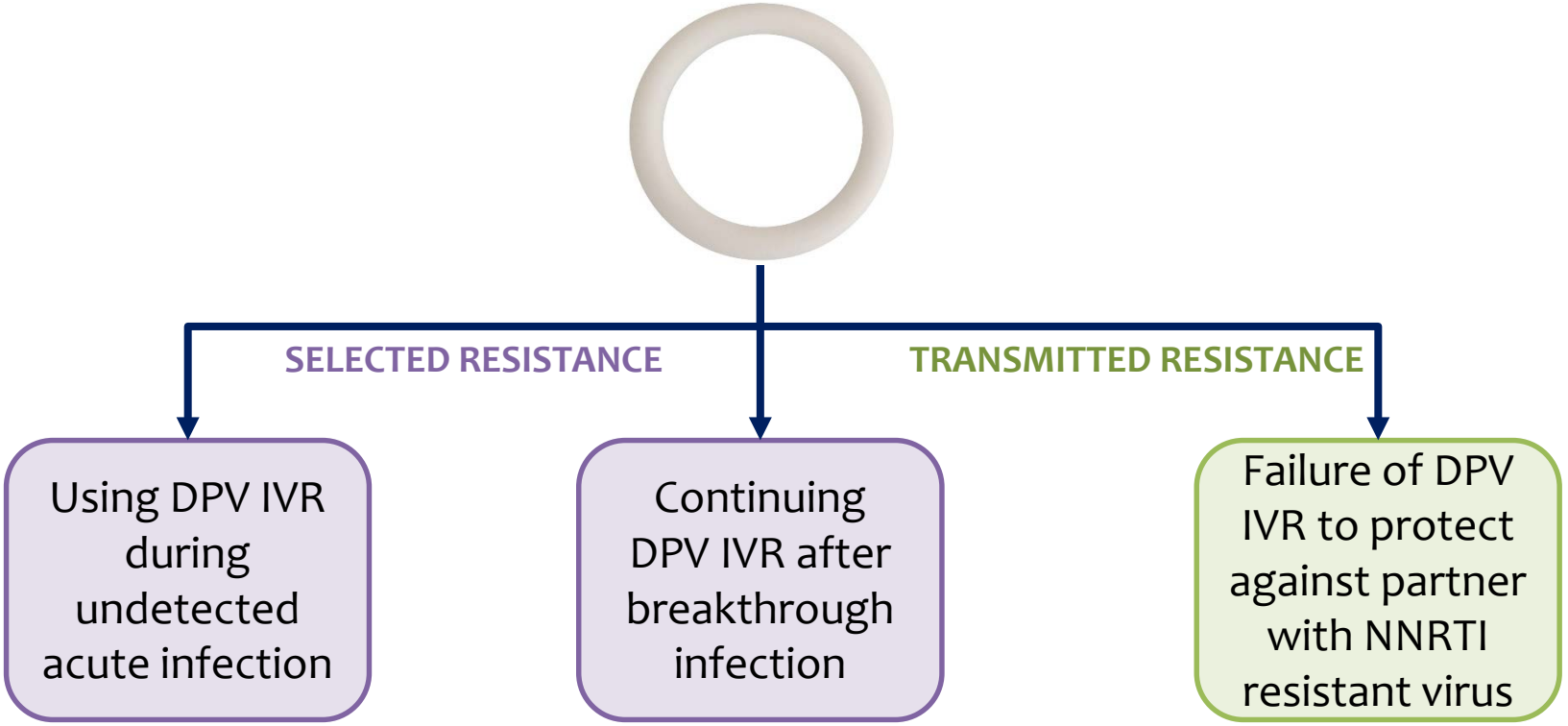
Dapivirine Intravaginal Ring (DPV IVR)

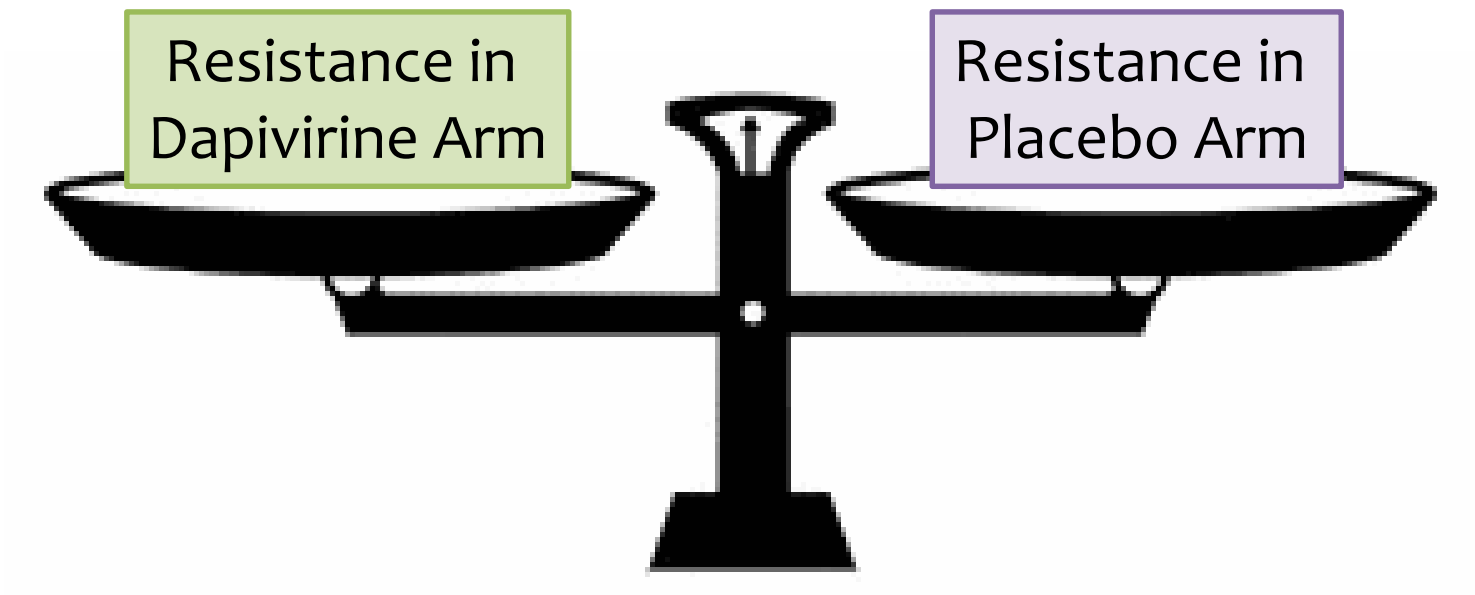
- Safe and effective to prevent HIV-1 infection in women
- Unlike tenofovir and FTC, DPV never used therapeutically
- Part of NNRTI class of drugs (same as NVP and EFV)



Risk of Resistance from DPV IVR Unknown



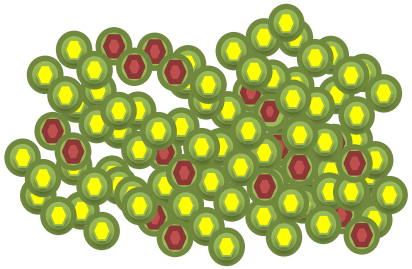
Look Carefully for Imbalance between Arms



Outline

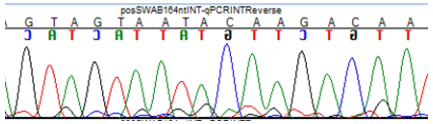
- Methods used to detect HIV drug resistance
- Resistance objective in ASPIRE
- Preliminary Results

How HIV Drug Resistance is Measured



Sample of HIV virions from plasma

Sanger "Standard" Sequencing

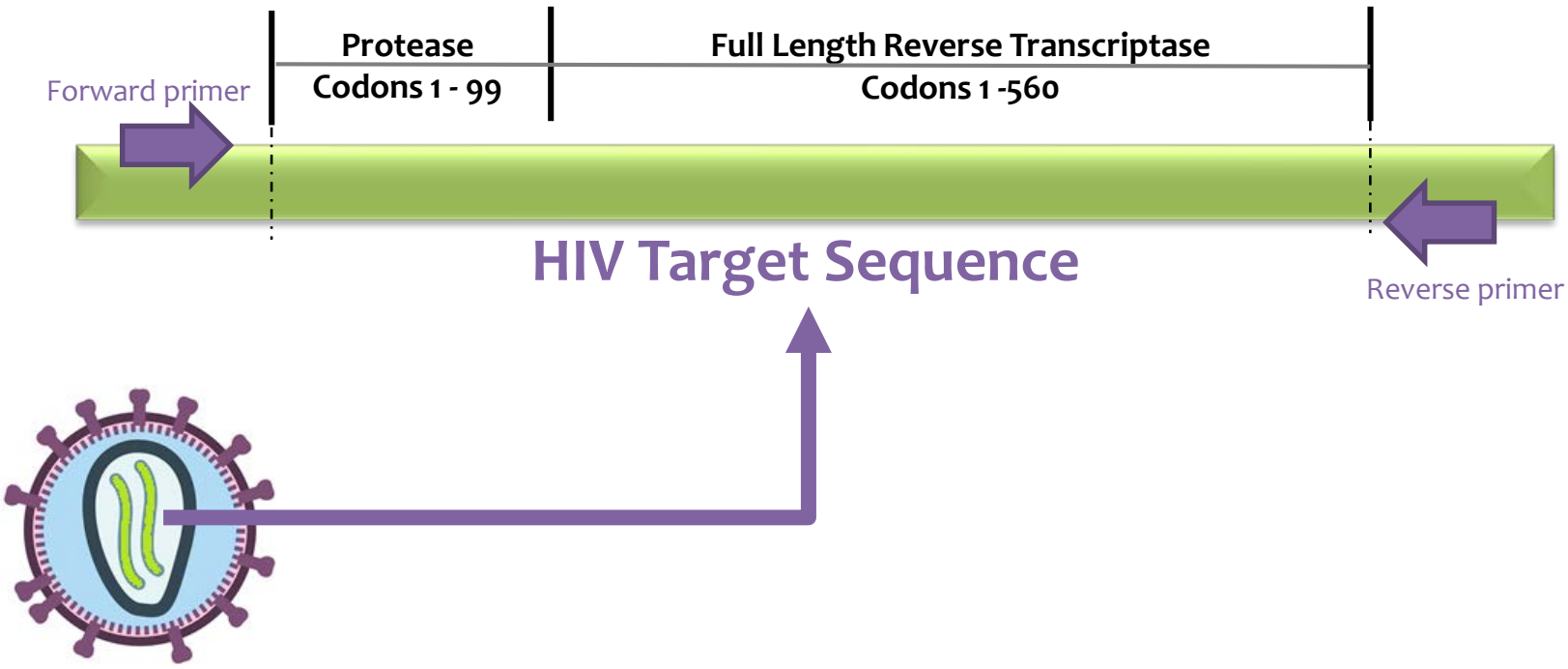


Consensus of all HIV quasispecies in sample
≥20% detected

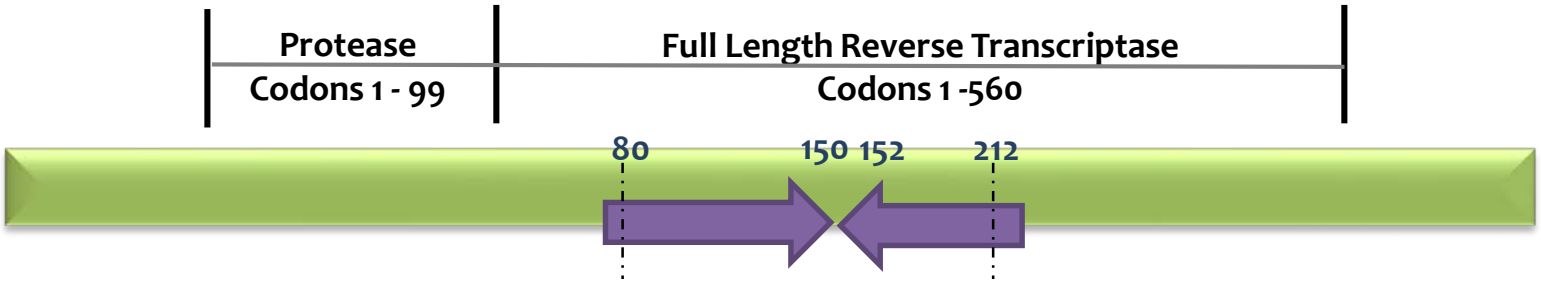
Next-Generation Sequencing (NGS)

Each virion individually sequenced
Thousands of sequences per sample
≥1% detected

Standard Sequencing Region



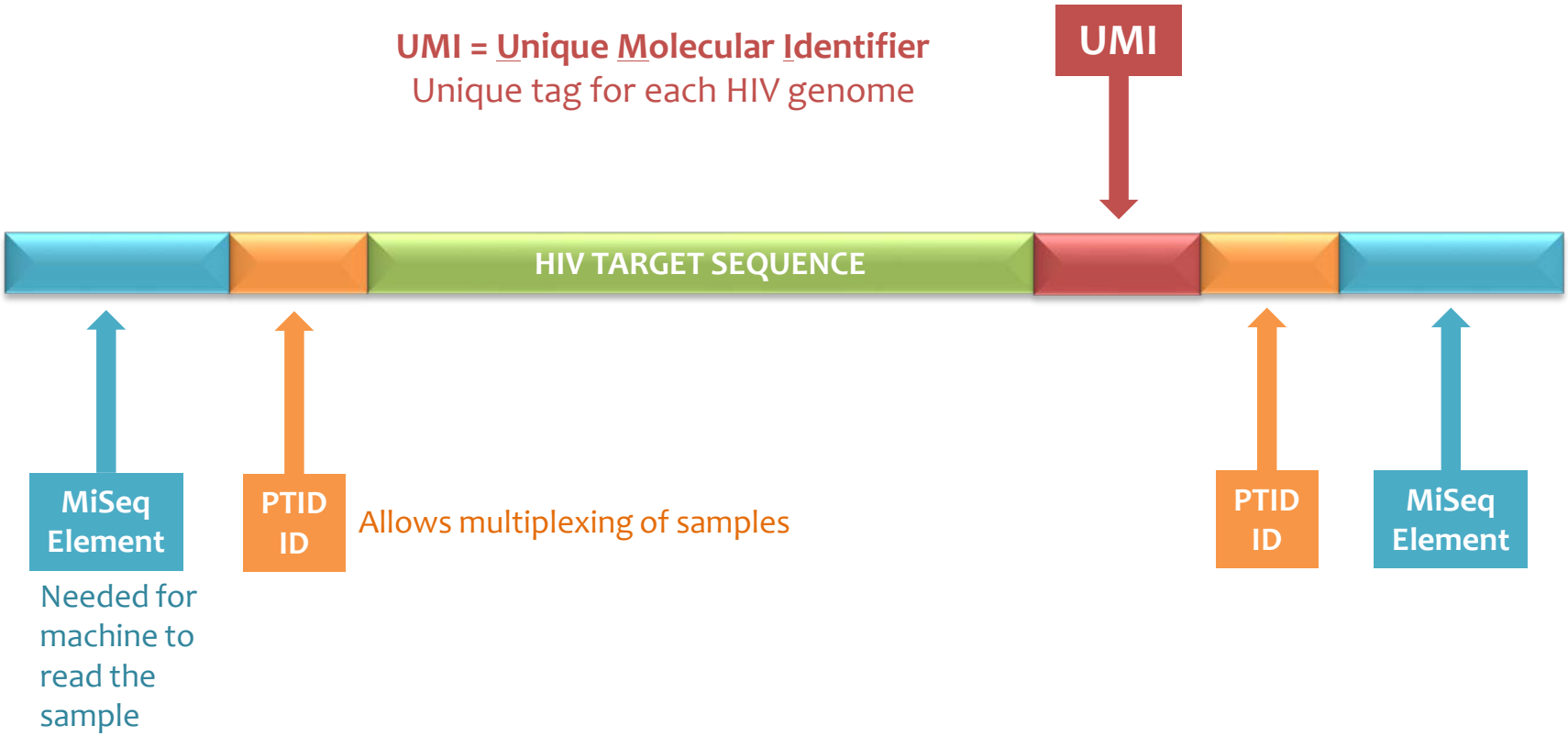
NGS Sequencing Region



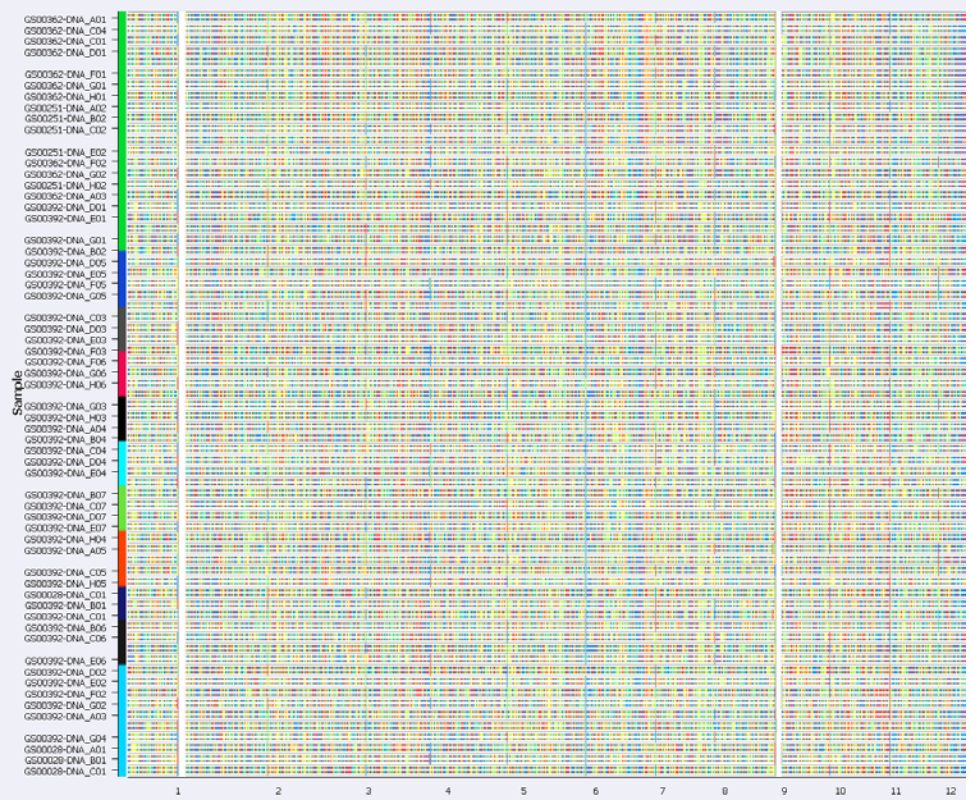
Mutations important for NNRTI resistance covered in this region

HIV Target Sequence

Principles of Sensitive NGS Assay



Principles of Sensitive NGS cont.



Thousands of sequences are generated per sample

Sensitivity of resistance detection can be determined individually for each sample and depends on HIV recovery from sample

Standard Genotyping vs NGS

Standard Genotyping	NGS
Gene Region includes protease and full-length RT	Targeted gene region include part of RT important for NNRTI resistance
Long & shallow sequence read length	Short & deep sequence read length
One sequence per sample	Thousands of sequences per sample with each virus genome individually tagged
Detect mutations at 20% or greater	Detect mutations at 1% or greater

Objective

- To evaluate seroconverters in MTN-020 (ASPIRE) for evidence of HIV drug resistance associated with DPV ring use using standard genotyping and NGS.

ASPIRE

A Study to Prevent Infection with a Ring for Extended Use

HIV Diagnosis in ASPIRE

2 Rapid Tests

Western blot

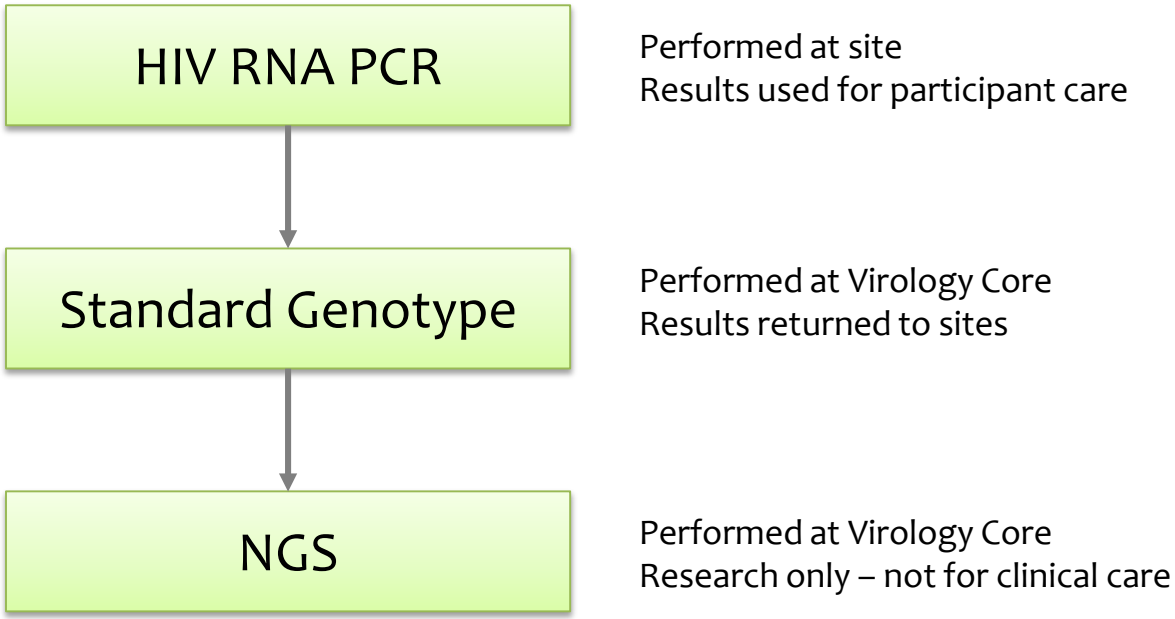
HIV Diagnosis and Confirmation

One or two positive

- **Product discontinuation**
- **Plasma collection for resistance testing**

Post-Seroconversion Testing

Plasma collected and stored after 1st positive rapid test.
If confirmed positive…



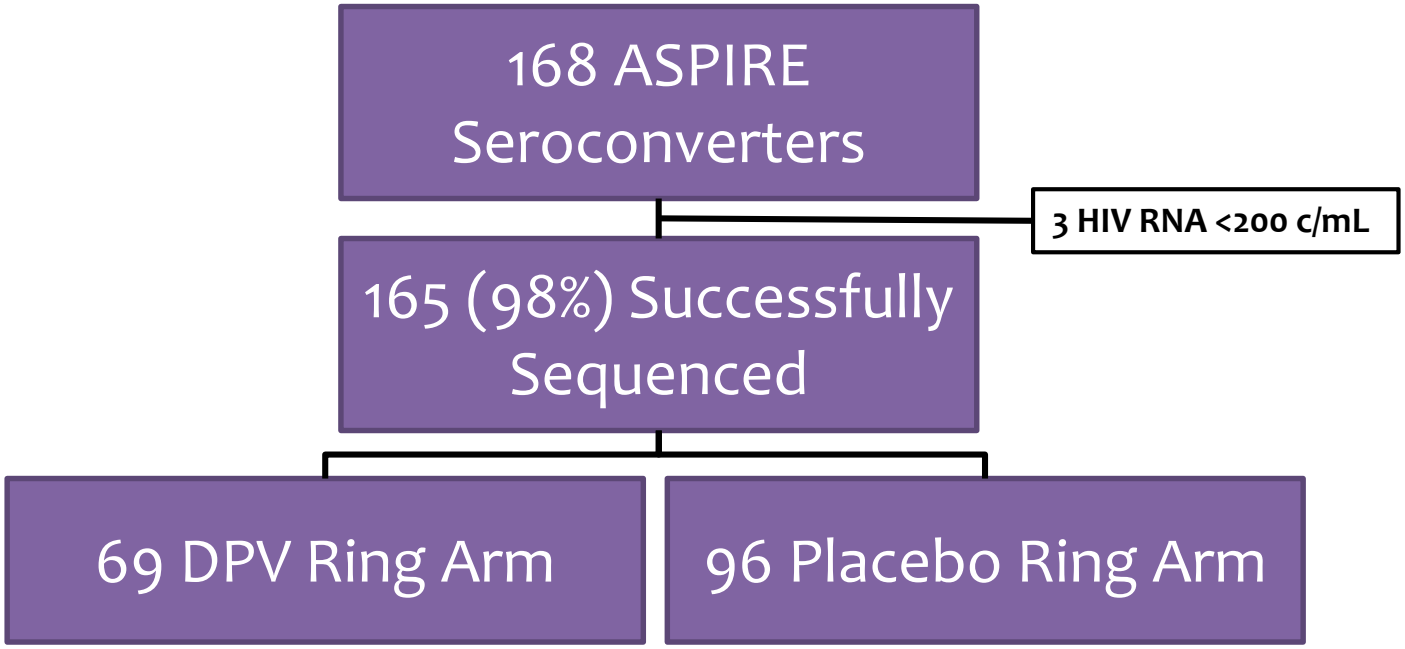
Methods

Standard Genotyping	NGS	
All seroconverters from both arms tested	PHASE I	**DPV ARM** • >95 pg/ml plasma DPV • residual drug levels of <23 per 5 mg • at any follow up visit **PLB ARM:** 1:1 random match
	PHASE II	Remaining specimens both arms

Sample stored after first positive rapid tested

Results

Standard Genotyping



DPV-Associated NNRTI Mutations: Standard

Frequency among participants who acquired HIV-1 infection after enrollment while on study product

Mutation*	PLB Ring N = 96	DPV Ring N = 68
L100I	0	0
K103N	1	2
E138K	0	0
Y181C	0	0

All differences were not significant between arms, $p > 0.05$

*Based on *in vitro* selection and cross-resistance data from Schader SM *et al. AAC* 2012 and Fletcher P *et al. AAC* 2009

Other NNRTI Mutations: Standard

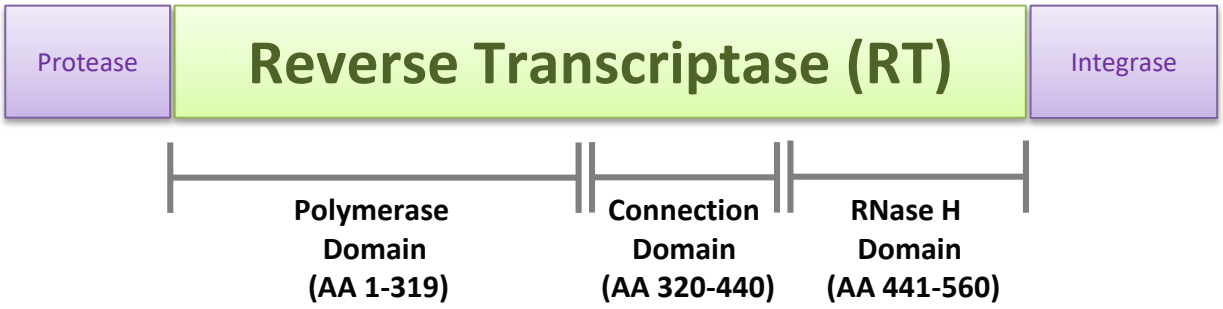
Among participants who acquired HIV-1 after enrollment while on study product

Mutation	PLB Ring (N = 96)	DPV Ring (N = 68)
V90I	1	2
K101E	1	1
K103S	0	1
V106M	0	1
V108I	0	1
E138A	**5**	**3**
E138G	0	1
V179D	2	1
V179T	0	1
H221Y	1	1

All differences were not significant between arms, $p > 0.05$

Full-length Analysis of RT

- No novel amino acid changes across all of RT were associated with seroconversion in the DPV arm



Standard Genotyping

NO DIFFERENCE

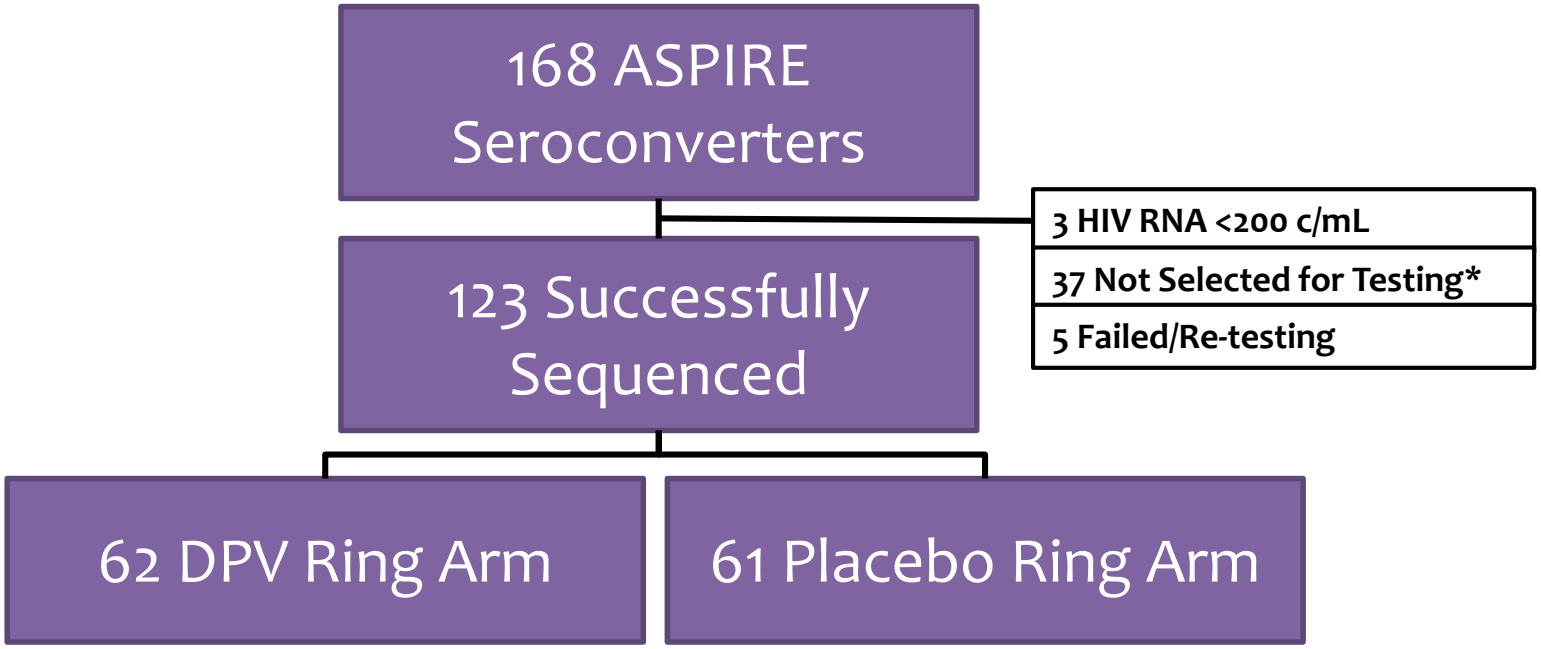
DPV RING ARM

8 of 69 (11.6%) with NNRTI mutations

PLB RING ARM

10 of 96 (10.4%) with NNRTI mutations

NGS



*32 Placebo + 5 DPV ring non-adherent defined by low plasma drug levels or high residual ring levels

DPV-Associated NNRTI Mutations: NGS

Frequency among participants who acquired HIV-1 infection after enrollment while on study product

Mutation	PLB Ring N = 61	DPV Ring N = 62
L100I	0	0
K103N	0*	2
E138K	0	0
Y181C	0	0

*1 PTID with K103N identified by standard genotyping not yet sequenced by NGS

K103N at 100% for both PTIDs same as standard genotype

No new low frequency DPV-associated mutations detected.

Other NNRTI Mutations: NGS

Among participants who acquired HIV-1 after enrollment while on study product

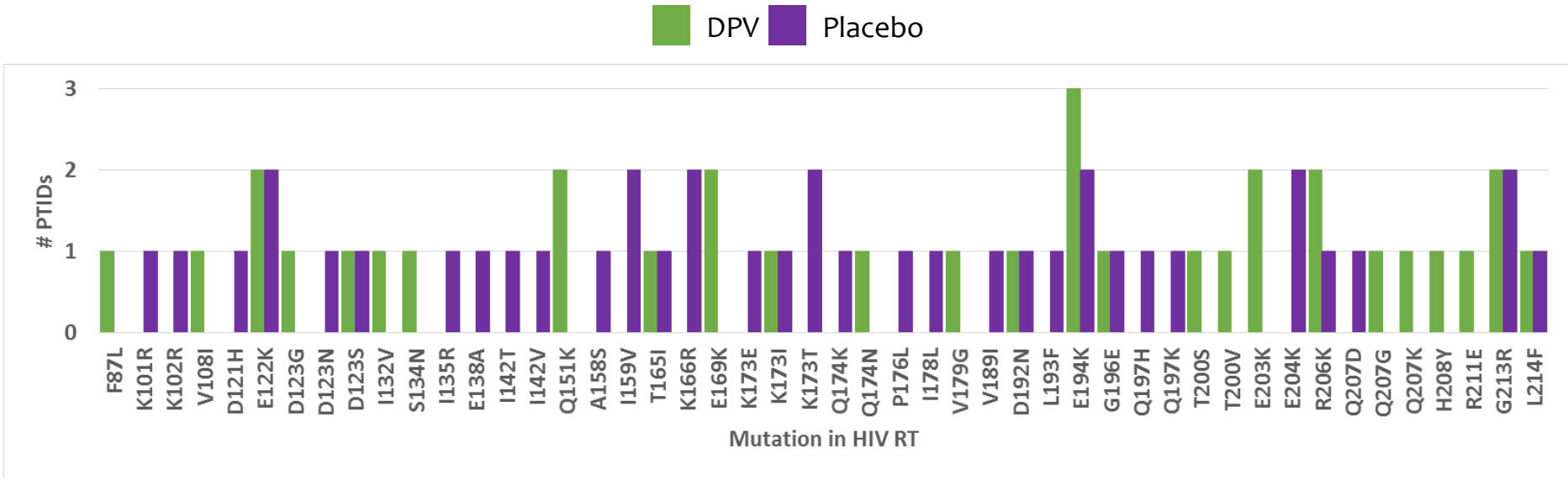
Mutation	PLB Ring (N = 61)	DPV Ring (N = 62)
V90I	No difference	
K101E	No difference	
K103S	No difference	
V106M	No difference	
V108I	No difference	
E138A	Note*	
E138G	No difference	
V179D	No difference	
V179T	No difference	

***1 new E138A at 9% frequency detected in 1 PTID from PLB Ring Arm**

Any Amino Acid Differences in RT? (NGS)

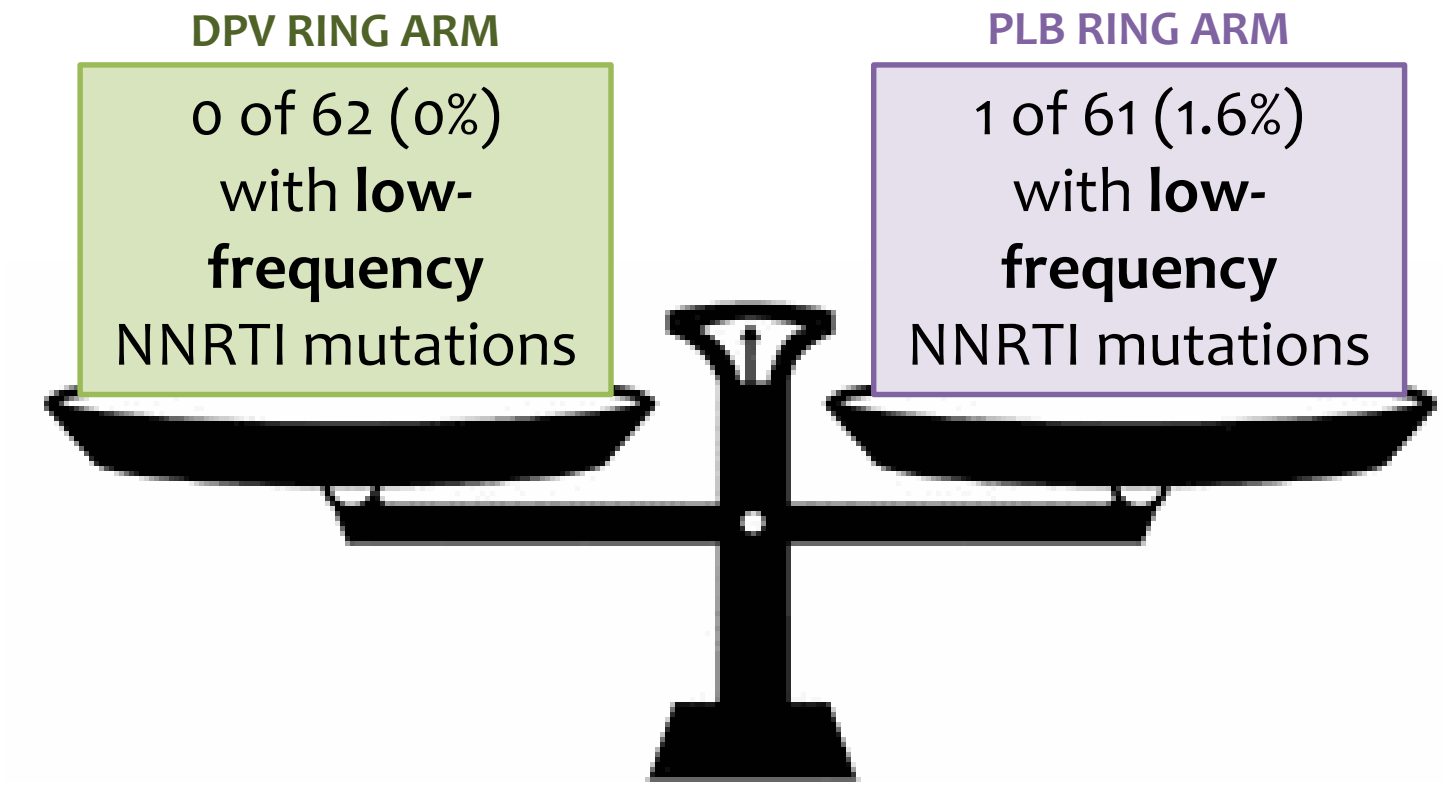
1. Evaluate full gene region amino acids 80 – 212 in RT
2. Compare number of low-frequency mutants (1 - 20% frequency) at every position
3. Chi-square test to compare placebo vs dapivirine arm

Other Amino Acid Differences: NGS



No significant differences between arms

NGS


DPV RING ARM
0 of 62 (0%) with **low-frequency** NNRTI mutations
PLB RING ARM
1 of 61 (1.6%) with **low-frequency** NNRTI mutations

Summary

- NNRTI mutation frequency did not differ by arm ($p > 0.05$)
- DPV-associated mutations E138K, L100I or Y181C were not detected in ASPIRE by standard or sensitive sequencing.
- The polymorphism E138A was the most common mutation amongst seroconverters but its frequency did not differ by arm.

Conclusion

- DPV-associated resistance mutations were **not** detected in ASPIRE by **standard or sensitive** resistance analysis
- The frequency of NNRTI mutations in seroconverters from ASPIRE **did not differ by arm** indicating that the NNRTI resistance was likely **transmitted** and **not selected** by DPV ring use.

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- **Malawi: Blantyre site (Malawi College of Medicine-John Hopkins University Research Project):** Bonus Makanani, Taha E. Taha
- **Malawi: Lilongwe site (University of North Carolina Project):** Francis Martinson
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