

# Rare HIV-1 drug resistance mutations exert subtle synergistic and antagonistic effects in the context of the genetic background

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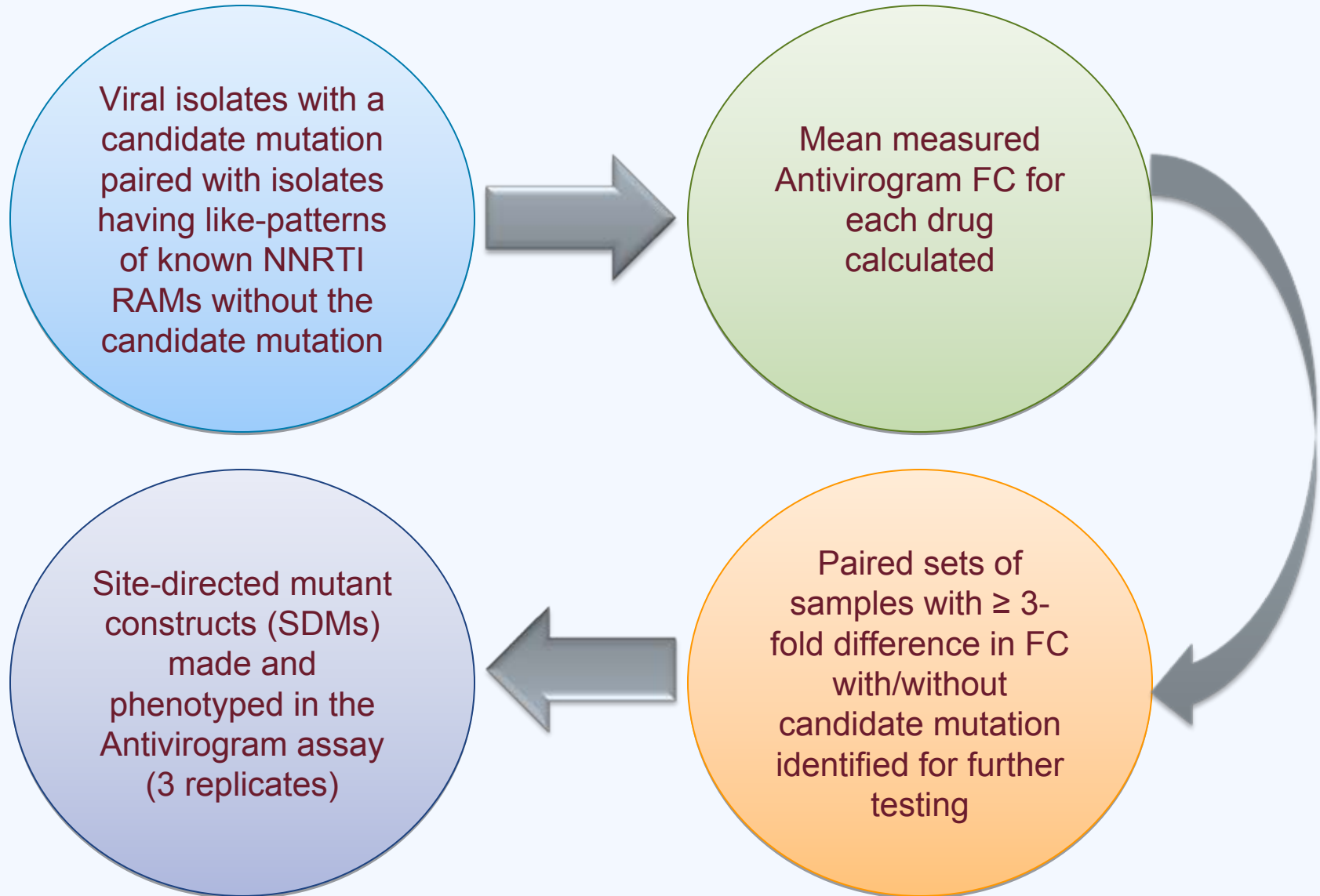
# Search for rare mutations

- Rare resistance-associated mutations (RAMs) are seldom characterized:
  - Often have small effect
  - Sometimes contribute to resistance only when co-occurring with other mutations
- Goal of the study:
  - Identify a number of such mutations associated with NNRTI resistance
  - Evaluate their contribution to NNRTI resistance as single mutations and in select genetic backgrounds

# Multi-pronged approach for identifying mutations

- 3-Fold cross-validation procedure for generating linear models that identify mutations/mutation pairs associated with drug resistance.
  - New/rare mutations at positions with changes known be associated with drug resistance.
  - Amino acids chemically similar to known RAMs.
  - Level of FC of *in vitro* phenotype not explained by genotype.
- ➔ A number of candidate mutations retained for analysis.

# Choice of genetic background for testing candidate mutations



# Mutations identified have low prevalence (Geno Database, N = 404 207)

Mutation	Prevalence (%)
K102L	63 (0.02)
E138Q	1 198 (0.30)
T139R	1 305 (0.32)
V179L	215 (0.05)
V179Y	96 (0.02)
Y181F	44 (0.01)
H221L	114 (0.03)
K219D	624 (0.15)
K219H	635 (0.16)
K103N	74 615 (18.46)
Y181C	34 162 (8.45)



Comparison with known  
RAMS

# Mutation 139R affects resistance in 103N+181C and 181C+188L background: clinical isolates

Genotype	Nevirapine (BCO = 6.0)		Efavirenz (BCO = 3.3)		Etravirine (BCO = 3.2)	
	FC*	N**	FC	N	FC	N
	wild-type virus	1.1	3065	1.0	3093	0.9
<b>139R</b>	2.7	20	0.8	19	1.2	9
103N+181C	>69.6	870	50.8	836	3.6	312
139R+103N+181C	>85.4	5	99.3	5	20.3	4
181C+188L	>75.4	32	541.7	34	23.7	24
139R+181C+188L	>81.3	11	458.2	11	136.4	6

\* Mean fold-change values; \*\* Number of viral isolates

# Contribution of mutation 139R to NNRTI resistance confirmed in SDMs

Genotype	Nevirapine	Efavirenz	Etravirine
	(BCO = 6.0)	(BCO = 3.3)	(BCO = 3.2)
<b>139R</b>	7	2.1	1.1
103N+181C	>22.7 (6)	19.8 (6)	1.8(6)
139R+103N+181C	>22.7	65.7	6.6
181C+188L	>22.7	144.5	5.4
139R+181C+188L	>22.7	420.1 (2)	26.6

\* FC values based on 3 observations, except where indicated within round brackets

# 219D and H affect resistance in 103N+181C background: clinical isolates

Genotype	Nevirapine (BCO = 6.0)		Efavirenz (BCO = 3.3)		Etravirine (BCO = 3.2)	
	FC*	N**	FC	N	FC	N
	wild-type virus	1.1	3065	1.0	3093	0.9
103N+181C	>69.6	870	50.8	836	3.6	312
<b>219D</b>	1.2	6	0.7	5	1.1	2
103N+181C+219D	>105.2	3	28	3	8.8	2
<b>219H</b>	0.6	13	0.6	12	0.6	5
103N+181C+219H	>83.6	4	199.9	4	13.8	2

\* Mean fold-change values; \*\* Number of viral isolates with the mutation combination.

# Contribution of mutations 219D/H to NNRTI resistance in SDMs

Genotype	Nevirapine (BCO = 6.0)	Efavirenz (BCO = 3.3)	Etravirine (BCO = 3.2)
103N+181C	>22.7 (6)	19.8 (6)	1.8(6)
<b>219D</b>	2.7	1.5	1
103N+181C+219D	>79.6	74.4	18.9
<b>219H</b>	1.8	1.3	1.5
103N+181C+219H	>79.6	47.2	14

\* FC values based on 3 observations, except where indicated within round brackets

Changes at position 219 of RT are typically associated with NRTI resistance: yet some variants affect NNRTI resistance.

# Some mutations have antagonistic effect on other mutations: 179Y

## Site-directed mutants:

Genotype	Nevirapine (BCO = 6.0)	Efavirenz (BCO = 3.3)	Etravirine (BCO = 3.2)
<b>179Y</b>	0.3	0.2	<0.1
190A	43.8 (6)	4.1 (6)	0.7 (6)
179Y+190A	16.5	1	<0.1

\* FC values based on 3 observations, except where indicated within round brackets

# Prevalence of mutations among isolates with/without evidence of drug exposure\*

Mutation	Evidence of Drug Exposure	
	No (N = 13 232)	Yes (N = 354 077)
Y181F	none	0.01
K102L	none	0.02
E138Q	0.05	0.31**
T139R	0.008	0.33**
V179L	0.008	0.06**
V179Y	none	0.03
H221L	none	0.03**
K219D	none	0.17**
K219H	none	0.17**

Virco Database (N = 367 309)

\* WHO Drug Surveillance List (Bennett et al. 2009); \*\* P<0.05

# Successfully identified new mutations contributing to NNRTI resistance

- The mutations analyzed here:
  - Exert their effect in clinical isolates with certain genetic backgrounds, and in themselves do not cause resistance
  - May act synergistically or antagonistically with the genetic background.
- SDM testing:
  - Broadly confirms the observations made on clinical isolates
  - But magnitude of the effects cannot be directly compared (heterogeneous genetic background in clinical isolates!).
- Mutations identified are rare and remain rare upon drug exposure.

# Special thanks to

**Virco Laboratory Operations** teams who helped build the Virco geno/pheno databases and who tested all the SDMs

and

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