

The Impact of Minor Populations of Wild Type HIV on the Replication Capacity and Phenotype of Mutant Variants in a Single-cycle HIV Resistance Assay

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INTRODUCTION

HIV resistance testing is recommended to guide the choice of new drug regimens after first or multiple treatment failures. Two commercially available phenotypic assays, Antivirogram and PhenoSense HIV assays are available to aid the clinician on the selection of regimens. Both assays are based on direct amplification of the viral gene of interest from plasma viral RNA. The “pool” of DNA generated from patient plasma is inserted into a modified HIV vector that lacks the analogous sequence. The resulting resistance test vectors, prepared as libraries, are transfected into target cells in order to capture and preserve the protease (PR) and reverse transcriptase (RT) sequence heterogeneity of the plasma virus. However, cotransfection of different viral variants into the same cell might provide the opportunity for genetic recombination and/or complementation. We undertook a study to elucidate the impact of cotransfection of mixtures of mutant and wild-type (WT) virus on the observed phenotype and replication capacity (RC) in a single-cycle resistance testing assay.

OBJECTIVE

- To elucidate the effect of cotransfected WT virus on the observed phenotype and RC of mutant variants.

MATERIALS AND METHODS

- A WT HIV clone was engineered to contain a Renilla luciferase gene in the *nef* coding region and a frameshift in envelope.
- A WT shuttle vector pNL4-3-FLuc-S was similarly constructed to contain a Firefly luciferase gene instead of Renilla luciferase gene. In addition, a *Sma* I cut site was introduced downstream of the PR coding region for convenient cloning.
- The PR coding region and cleavage sites were amplified by RT-PCR from plasma derived from PI-experienced subjects receiving lopinavir/ritonavir (LPV/r) therapy.
- Mutant clones were constructed by inserting the Apal-Smal fragment from the PCR products into a pNL4-3-FLuc-S shuttle vector that expresses Firefly luciferase. Individual clones were sequenced and used for further studies.
- Firefly and Renilla luciferase activities were subsequently measured from a single sample after cotransfecting the mutant constructs alone or as a mixtures of WT and mutant construct at a ratio of 10:1, 2:1 and 1:1.
- Both RC and drug susceptibility of the mutant and WT variants were calculated using Firefly and Renilla luciferase activity, respectively.

RESULTS

- Eleven mutant clones were used in this study, each contains a variety of primary and secondary mutations (Table 1).
- The RC values of four mutant clones (clone 1-4) ranged from 0.07 to 0.7% when transfected alone (Table 2).
- Cotransfection of as little as 9% of the WT clone resulted in an 18- to 33-fold increase in RC (to 1.6-12.3%) (Table 2).
- Cotransfection of 33% and 50% of the WT clone further enhanced the RC to >43% in all cases except clone 1 (11 and 22%, respectively). The incremental increase in RC of those four mutants ranged from 68- to 690-fold (Table 2).
- The RC values of the other 7 mutant clones (clone 5-11) were modest to high when transfected alone (15-68%), and did not change significantly upon cotransfection with up to 50% of the WT clone (1- to 4-fold change) (Table 2).

RESULTS *continued*

- In contrast to the results of the cotransfection experiment, coculture of cells containing individually transfected mutants (clone no. 3, 4, 5 and 6) and WT clones at a ratio of 1:1 did not affect the RC of mutant variants (Figure 1).
- Similarly, coinfection of a 1:1 ratio of mutant and WT virus did not change the RC of the mutant variants (Figure 1).
- The RC of the WT clone remained unchanged when cotransfected, cocultured or coinfecting with any of the four mutant clones at a 1:1 ratio (clone no. 3, 4, 5 and 6) (Figure 2).
- The LPV susceptibility of seven mutant clones (clones 5-11) with sufficient RC (transfected alone) for phenotypic analysis ranged from 21- to 277-fold, compared to the WT clone (Table 3).
- Cotransfection of as little as 9% of WT clone decreased the LPV IC₅₀ of all seven mutants by more than 40% (range 41-63%) compared to the corresponding mutants when transfected alone with the exception of clone 11 (Table 3 and Figure 3).
- Cotransfection of 33% and 50% of the WT clone further decreased the LPV IC₅₀ of all mutants by at least 72% (median 80% and 91% respectively).
- In contrast, the susceptibility of the WT variant did not change upon cotransfection with 50 to 67% of mutant strains (Table 4).
- Sequence analysis of the protease coding region and the cleavage sites of 16-20 clones derived from each cotransfection showed that the ratios of WT/mutant sequences detected in the supernatants corresponded to the WT/mutant ratio during cotransfection (Table 5).

Table 1. Genotype of the Mutant Clones

Mutant Clone	Genotype
1	L10I, L24I, E35D, M36I, M46L, I54V, L63P, I64V, V82A
2	L10F, L24I, E35D, M36V, N37S, R41K, M46I, I54V, D60E, Q61E, I62V, L63P, I64V, V82A
3	L10F, I15T, E35D, M36V, N37S, R41K, I54V, D60E, Q61E, I62V, L63P, I64V, V82A
4	L10V, V32I, N37S, M46I, I47V, I62V, L63P, V77I, P79S, Q92K, I93L, C95F
5	L10F, V32I, E35D, M36I, M46L, I54V, L63P, I64V, V82A
6	L10I, E35D, N37D, L63P, A71V, T74P, I84V, L90M, I93L
7	L10I, E35D, N37D, M46I, I54V, L63P, A71V, T74P, I84V, L90M, I93L
8	L10I, V32I, M46I, I47A, I62V, L63P, V77I, Q92K, I93L, C95F
9	L10I, G48V, I54V, L63P, A71V, I72M, V77I, V82A, L90M, I93L
10	L10I, V32F, G48V, I54V, V56K, L63P, A71V, I72M, V77I, V82A, L90M, I93L
11	L10V, I15V, G16E, K20R, E35D, M36I, R41K, M46I, I50V, I54V, K55R, R57K, Q61G, I64L, A71V, I72R, V82A, L89I, L90M, Q92K

Table 2. Effect of Cotransfection with WT Virus on the Replication Capacity of Mutant Isolates

Mutants	% of RC* (fold increase in RC)			
	Percentage of WT DNA Cotransfected			
	0%	9%	33%	50%
1	0.07 (1)	1.6 (22)	10.9 (155)	22.1 (315)
2	0.08 (1)	1.7 (21)	43.7 (546)	55.2 (690)
3	0.16 (1)	5.3 (33)	48.2 (301)	64.3 (402)
4	0.7 (1)	12.3 (18)	47.7 (68)	75.9 (108)
5	14.7 (1)	19.8 (1.3)	46.8 (3.2)	57.0 (3.9)
6	67.6 (1)	83.5 (1.2)	146.8 (2.2)	160 (2.4)
7	32.5 (1)	32.9 (1)	34.3 (1.1)	50.2 (1.5)
8	32.7 (1)	41.8 (1.3)	52.5 (1.6)	56.0 (1.7)
9	47.9 (1)	47.2 (1)	57.3 (1.2)	97.4 (2)
10	20.4 (1)	21.5 (1.1)	27.3 (1.3)	22.6 (1.1)
11	38.6 (1)	31.6 (0.8)	44.1 (1.1)	40.5 (1.1)

*RC = Firefly luciferase activity of mutant alone or in mixture/Firefly luciferase activity of WT pNL4-3-FLuc-S alone x 100.

Figure 1. The RC of Mutant Clones Is Affected by Cotransfection but Not by Coculture or Coinfection

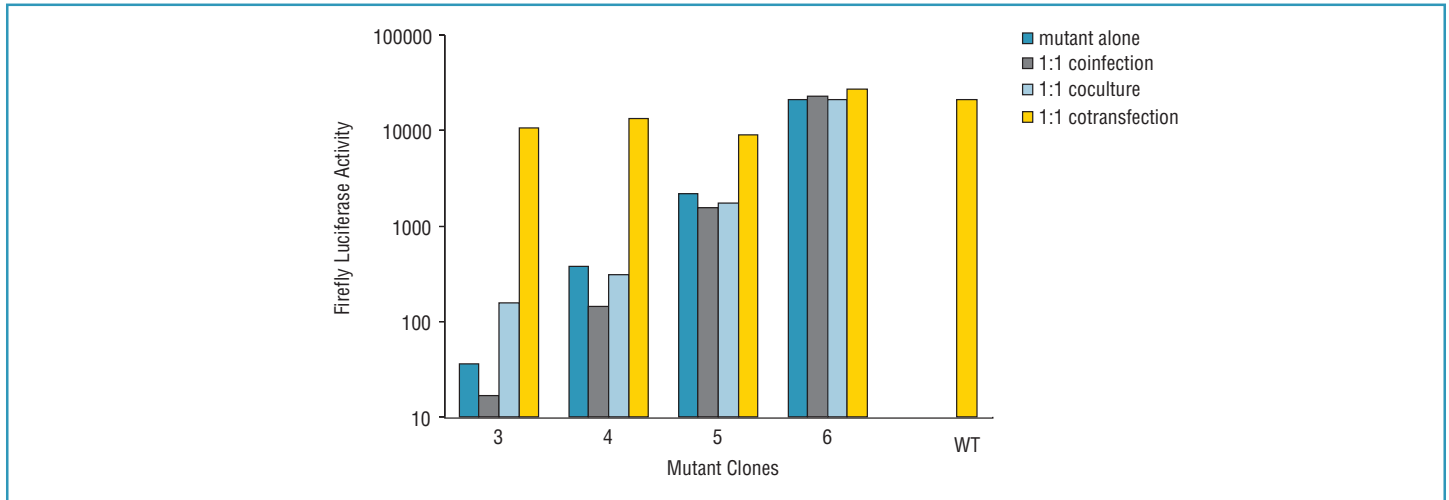


Figure 2. The RC of Wild-type Virus Is Not Affected by Cotransfection, Coculture or Coinfection with Mutant Clones

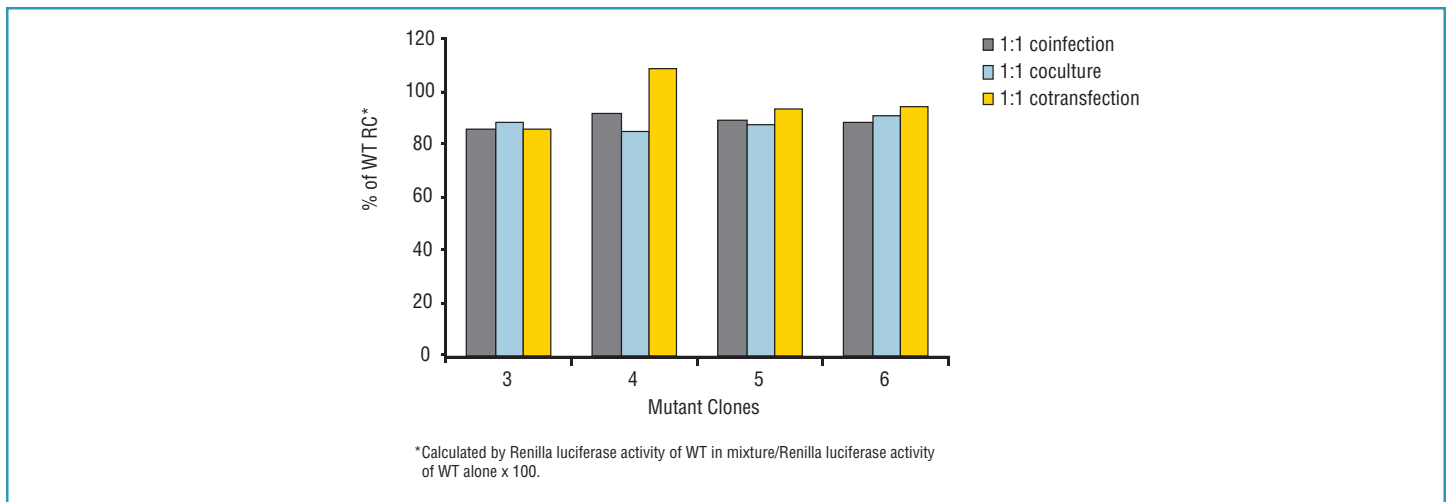


Table 3. Effect of Cotransfection with the WT Clone on the Phenotype of Mutant Isolates

Mutants	Fold Change in IC ₅₀ to LPV Compared to WT (Fold decrease in IC ₅₀ compared to that of each mutant when transfected alone)			
	Percentage of Wild Type DNA Cotransfected			
	0%	9%	33%	50%
5	25.8	13.5 (1.9)	7.2 (3.6)	6.7 (5.3)
6	21.2	7.9 (2.7)	4.5 (4.7)	2.8 (7.6)
7	66.2	29.6 (2.2)	6.1 (10.9)	3.0 (22)
8	276.9	140.9 (2)	29.5 (9.4)	9.0 (30.8)
9	35.8	21.1 (1.7)	7.2 (5.0)	3.1 (11.5)
10	54.5	24.4 (2.2)	5.0 (10.9)	3.0 (18.2)
11	150.1	132.6 (1.1)	31.3 (4.8)	16.7 (9.0)

RESULTS *continued*

Figure 3. Effect on Drug Susceptibility of Mutant Clones to LPV by Cotransfected WT DNA

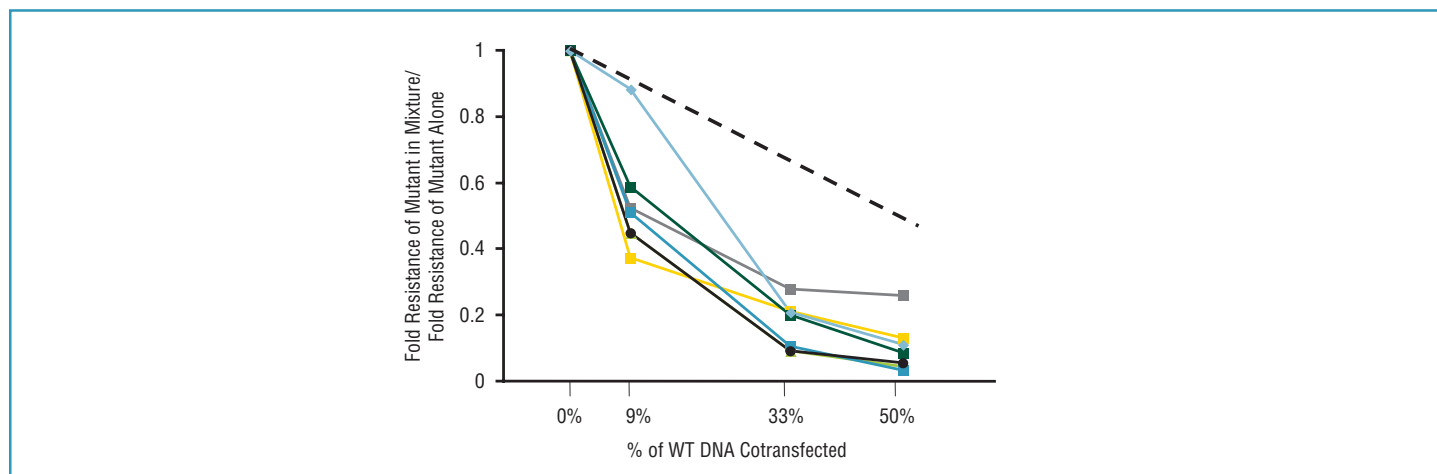


Table 4. Effect of the Cotransfection of Mutant Clones on the LPV Susceptibility of WT Virus

Mutants Cotransfected	IC ₅₀ of WT HIV-RLuc to LPV (μM)	
	% of Mutant DNA Cotransfected	
	50%	67%
4	0.011	0.021
8	0.013	0.013
9	0.014	0.024
10	0.017	0.025
HIV-RLuc (WT)	0.014	0.013

Table 5. Frequency of Mutant and Wild-type Sequences in the Supernatant from the Cotransfection

Mutants	% of WT Cotransfected	Number of Clones (% of Total)		
		Mutant	WT	Total
4	50%	9 (45%)	11 (55%)	20
	33%	14 (73%)	5 (27%)	19
	9%	20 (100%)	0 (0%)	20
6	50%	8 (45%)	10 (55%)	18
	33%	11 (68%)	5 (32%)	16
	9%	16 (84%)	3 (16%)	19

CONCLUSIONS

- Because of the unique cotransfection step inherent to single-cycle HIV phenotypic assays, even relatively small amounts of WT virus within a viral population can significant impact the apparent RC and phenotype of mutants strains.
- The enhancement of RC and decrease in IC₅₀ of mutant variants following cotransfection with the WT variant appear to be due to complementation rather than genetic recombination.
- The RC and susceptibility of plasma isolates from patients who are off therapy or not adherent to treatment, in which WT virus may expand to significant levels, should be interpreted with caution.