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Evolution of Reverse Transcriptase and Protease Resistance Mutations in HIV-1 Infected Patients who Continue a Failing Drug Regimen

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I. Background and Objectives

- It is accepted by several published guidelines that in patients failing a highly active antiretroviral treatment (HAART) regimen, further decisions regarding optional therapy should be made with the aid of reverse transcriptase (RT) and protease (PR) genotypic testing.
- There are few clinical data to support specific strategies for changing therapy in patients who have failed a HAART regimen. Thus, it is not clear whether it's more beneficial to change a failing regimen as early as possible or to wait until the viral load (VL) or CD4 counts reach a certain threshold.
- In choosing a treatment strategy for these patients, it is important to know if ongoing virus replication during continued therapy with a failing regimen allows evolution of mutations that might limit future treatment options.
- It is conceivable that in patients failing a treatment regimen with high rates of viral replication, more drug resistant mutations would develop over time, thereby creating more fit and more resistant viruses.
- To assess the effect of a persisting failing HAART regimen, we examined the evolution of drug resistance mutations in patients continuing therapy despite ongoing virus replication.

II. Methods

- Between 7/1/97 – 2/4/01, 771 patients from one institute had their genotypic testing done at Stanford University Hospital. Of these, 193 had ≥2 sequences performed. Of these 193 we identified 24 patients who had 2 genotypic resistance tests separated by ≥3 months without undergoing any change in their HAART regimen.
- Patient charts were reviewed and treatment histories, CD4 counts and VL were collected.
- For each patient, sequences from 2 time-points were analyzed for the existence and evolution of RT and PR drug resistance mutations.
- Changes in susceptibility to the different drugs were assessed by a computer program that interprets drug resistance (http://hivdb.stanford.edu/hiv). In this program sequences of RT and PR are analyzed for the existence of drug resistance mutations by comparing the user submitted sequence to a commonly used reference sequence (the subtype B consensus sequence). The program reports five levels of inferred drug resistance: susceptible, potential low - level resistance, low-level resistance, intermediate resistance and high-level resistance, according to publicly available mutation scoring tables, derived from published literature.

Table 1: Viral Load, CD4 and Time Data

Patient ID	Months of Rx Before 1st Sequence	VL1 (Copies/ml)	CD4-1 (Cells/fL)	Months Between Sequences	VL2 (Copies/ml)	CD4-2 (Cells/fL)
1	5	8813	254	24	40406	89
2	4	52270	266	9	55246	180
3	6	77390	48	3	NA	61
4	13	90830	785	4	95505	280
5	5	102880	288	16	3205	420
6	9	400	470	25	90	346
7	9	16710	223	11	12394	193
8	8	2702	238	5	7472	316
9	11	6346	309	13	2013	189
10	30	2851	166	4	4481	272
11	19	11135	76	6	1019	77
12	15	3013	280	5	133599	161
13	12	8098	238	7	7310	250
14	6	10358	368	6	40	484
15	6	3434	97	8	67180	50
16	8	163818	223	3	NA	NA
17	11	21497	172	7	12490	240
18	26	1049	130	8	4842	193
19	11	455	394	7	7151	524
20	28	4326	223	9	9334	186
21	11	18647	209	11	15005	152
22	14	1786	628	10	1458	611
23	4	29870	447	9	16127	516
24	10	21002	522	5	19646	234

Table 2 – PI Therapy and Related Mutations

PIs	PR Mutations	Mutations Gained	Mutations Lost
NFV	M46L, V82A, L10I, L24I, L63P, I93L	M36I, A71V	
NFV	M46I, L10V, L63P, A71T, V77I, N88S, I93L	K20KR	
RTV+SQV	L63P, V77I		
NFV	V82A, L63P, I93L	M46I, L90M, I93L	V82A
RTV+SQV	G48V, V82A, L10I, M36I, L63P, A71AT	I84V	
NFV	L63P, V77I	M46I, L90M, L10V, A71T, I93L	
NFV+SQV	M46I, I54V, V82A, L10I, L24I, M36I, F53L, L63P, G73GV		
IDV	L90M, L10FL, M36I, L63P, A71AITV, G73S	M46LM, I93L	
NFV+SQV	G48GV, I84V, L90M, L10I, M36V, L63P, A71V, G73GS, I93IL	V77IV	I84V, G73GS
IDV	I54A, V82F, L10I, K20M, M36V, A71V		
IDV	I54V, V82F, L90M, L10I, L63P, A71V, G73S, V77I, I93L		
IDV	M36I		
NFV+SQV	M46L, G48V, I54V, L90M, L63P, V77I, I93L	L10I	
RTV+IDV	L63P, V77I, I93L	M46L	
RTV+IDV	I54V, L90M, L10F, L63P, V77I, N88D, I93L	V82C	
RTV+SQV		I93IL	
NFV	L63PS	D30N, N88D	
NFV	M46I, L90M, L63P, A71AV, V77I, I93L		
NFV	M46LM, I54V, V82A, L63P	L10F, A71V	M46LM
NFV	M46I, L90M, K20I, M36I, A71V, G73S, I93L		
IDV	M46L, I54V, V82T, L10I, K20M, M36I, L63P, A71V		
NFV	M46L, L90LM, L10I, K20T, M36IM, L63P, N88DN, I93L	D30N, A71V	M46L, L90LM, K20T
NFV	L90M, L10I, M36IM, L63P, A71AV, V77I, I93L	I54IL	
APV	M46L, L63P	L10IL	

Table 3 – NRTI Therapy and Related Mutations

NRTIs	NRTI Mutations	Mutations Gained	Mutations Lost
3TC+D4T	M41L, M184V, L210W, T215Y	L74LV, V118I	
3TC+DDI	D67N, Q151M, M184V	K70E, F116Y	
DDI+D4T+ABC			
D4T+DDI	M41L, M184MV, T215CY		M184MV
3TC+D4T	M41L, E44DE, D67N, T69D, V75M, F77L, V118I, M184V, L210W, T215Y		
D4T+DDI	M184V	D67G, K219KR	M184V
D4T+DDI	M41L, D67N, K70R, T215Y, K219E	V118I	
3TC+D4T	M41L, M184V, T215F		
3TC+D4T	M41L, D67N, V75AV, M184V, T215F, K219Q	K70R	
3TC+D4T	M41L, E44D, D67N, T69D, V118I, M184V, L210W, T215Y, K219R		
3TC+D4T	M41L, E44D, D67N, V75M, V118I, M184V, L210W, T215Y, K219Q		
AZT+3TC			
AZT	M41L, D67N, V118I, T215Y		
DDI+D4T	M41L, T215Y		
AZT+3TC			
DDI+D4T			
DDI+D4T		D67N, K70R	
3TC+D4T	M41L, E44D, D67N, V118I, M184V, L210W, T215Y, K219E		
D4T+DDI	V118I, M184MV	Q151M	M184MV
3TC+D4T	M41L, Y115NY, V118IV, M184V, L210LW, T215Y		Y115NY, V118IV
AZT+3TC	M41L, D67N, V118I, M184V, L210W, T215Y	E44DE	
AZT+3TC	M41L, V118I, M184V, L210W, T215Y		
AZT+3TC	T69N, K70R, M184V, L210W, T215D	M41L	K70R
3TC+D4T	M41L, V118I, M184V, L210W, T215Y		

Table 4 – NNRTI Therapy and Related Mutations

NNRTIs	NNRTI Mutations	Mutations Gained	Mutations Lost
NVP	A98S, V108IV, V179E, Y181C		
DLV	Y188L		
	K101KQ, K103N, P225HP		K101KQ, P225HP
EFV		L100I, K103N	
	K103KN, V179I		K103KN
	V108I, M230LM		M230LM
EFV		K103N	
EFV	K101R	K103N	
	V179D		
	A98S		
	M230MV		M230MV

Figure 1 – Evolution of Drug Resistance Mutations

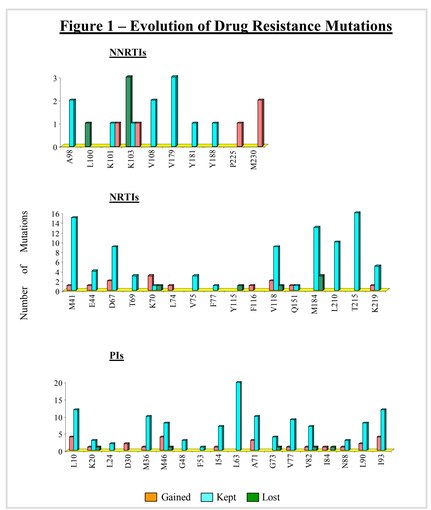

Figure 2 – Evolution of Drug Resistance According to a Computer Interpretation Program

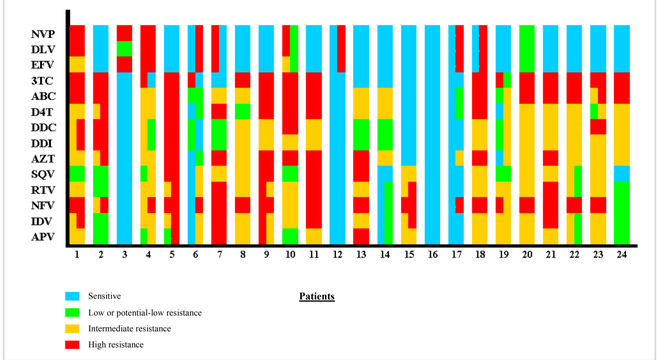

Table 5 - Summary of the evolution of drug resistance according to a computer interpretation program

Interpretation Category		PIs	NRTIs	NNRTIs	Total
New high-level resistance in the 2nd sequence	Drugs	10	5	12	27
	Patients	7	3	4	10
	Drugs per patient	1.43	1.67	3	2.7
Any new reduced susceptibility in the 2nd sequence	Drugs	20	16	12	48
	Patients	8	6	4	12
	Drugs per patient	2.5	2.67	3	4
Any new increased susceptibility in the 2nd sequence	Drugs	8	7	6	21
	Patients	3	3	2	7
	Drugs per patient	2.67	2.33	3	3

III. Results:

- Tables 1-4 show a breakdown of the 24 patients that had no change in drug therapy. Table 1 shows the period of time the patients have been receiving drug therapy prior to the first sequence, the VL and CD4 at the first sequence (VL1, CD4-1), the period of time (months) between the 2 sequences and the VL and CD4 at the second sequence (VL2, CD4-2).
- The median time period the patients were on drug therapy prior to the first sequence was 10.5 months (range 4-30). The median time between the 2 sequences was 7.5 months (range 3 - 25). The median VL (copies/ml) and CD4 (cells/fL) for the 1st and 2nd time-points were 9586, 246, and 8403, 234, respectively.
- Tables 2-4 show the specific drugs and the related mutations that were present on the 1st sequence and that were gained and/or lost in the 2nd sequence. The median number of drug-resistance mutations per patient was 10.5 and 11.5 at the 1st and 2nd time-points, respectively (p=0.02, Wilcoxon test).
- New resistance mutations developed in 19/24 (79%) patients. 16/24 patients (67%) developed new PI mutations (8/24 primary and 13/24 secondary mutations); 9/24 (38%) developed new NRTI mutations and 3/5 (60%) developed new NNRTI mutations. Only 9/24 patients lost drug resistance mutations between the 2 sequences (p=0.003, Chi-Square Test).
- Figure 1 shows a summary of mutations that were kept/gained/lost between the 2 time-points per position for each drug class. The most commonly gained mutations were primary PI mutations M46I (4/24) followed by L90M and D30N (2/24 each); secondary PI mutations L10I and I93L (4/24 each) followed by A71V (3/24); NRTI mutation K70R (3/24) followed by D67N, V118I and L74V (2/24 each); and NNRTI mutation K103N (3/5).
- Figure 2 shows the evolution of drug resistance between the 2 sequence time-points, according to a computer resistance-interpretation program. The 'X' axis denotes the 24 patients in the study and the 'Y' axis denotes the 14 drugs that the patients received. According to the mutations present in the sequence and to the rules of the program, each patient could be highly resistant (red squares of the figure), intermediate resistant (orange), low level/ potential-low level resistant (green) and sensitive (blue) to each of the drugs. The histogram bar for each patient is made of two columns: A left column for the first time-point sequence and a right column for the second time-point sequence.
- Table 5 gives a summary of the evolution of drug resistance between the 2 sequence time-points, according to a computer resistance-interpretation program. Isolates from 10/24 (42%) patients developed high-level resistance to a mean of 2.7 drugs during the interval between the two sequences. Isolates from 12/24 (50%) patients developed a higher level of resistance to a mean of 4.0 drugs during the interval between the two sequences.

IV. Summary

- 80% of sequences from patients staying on a failing multi-drug regimen accumulated either RT or PR drug resistance mutations.
- The evolution of resistance mutations in patients continuing a failing HAART regimen resulted in a significantly greater number of resistance mutations per patient in the second time-point compared to the first time-point.
- 50% of patients staying on a failing multi-drug regimen developed a higher level of resistance to a mean of 4.0 drugs during the interval between the two sequences according to a computer drug-resistance interpretation program. The majority of these became highly resistant to the different drugs according to the program.

V. Conclusions

- The observations presented in this study provide insight into how an uninterrupted regimen affects the natural history of mutation evolution and may limit future drug options.
- The continuation of viral replication under a failing drug regimen can lead to the evolution of viruses that are more fit, more resistant, and more pathogenic resulting in resistance to new treatment regimens.
- In patients with a median VL of <10000 and a median CD4 count of 250, it may be a disservice not to change drug treatment.