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ANTIRETROVIRAL THERAPY MANAGEMENT BASED ON THE RESULTS OF GENOTYPING RESISTANCE TESTS IN PATIENTS FAILING ANTIRETROVIRAL THERAPY

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Abstract. This study was **aimed** to investigate the efficiency of the ARV management based on plasma genotypic antiretroviral resistance testing in patients with failing ARV therapy. **Material and methods.** There were included 48 of patients in which ARV therapy was modulated according to the results of genotypic resistance which registered therapeutic failure in 43 of cases (89.6%) in 2000. In 2001 out of 42 plasma samples, viral load was undetectable in 23 (55%). The protease was amplified in 19/42 of cases, ten of which being wild type. **Results.** New resistance mutations comparing to 2000 were observed in proteases from 7 patients. Reverse-transcriptase (RT) was amplified in 19/42 patients and new resistance mutations were observed in 4. These mutations conferred resistance toward nucleozid inhibitors of RT (NNRTIs). Reverse-mutations occurred very rarely following therapeutic switch. **Conclusion**. Our study shows that monitoring the ARV therapy based on genotypic testing is highly effective in controlling the HIV infection evolution. The low rates of reverse mutations following the therapeutic switch stress the importance of the responsible treatment monitoring.

Key words: HIV-1, reverse-transcriptase, protease, resistance mutations, viral resistance, GART

Rezumat. Scopul acestui studiu a fost investigarea eficienței managementului terapiei antiretrovirale (ARV) în funcție de rezultatele testelor genotipice de rezistență la pacienții care tratamentul a eşuat. **Material şi metodă.** În acest studiu au fost incluşi 48 din pacienții la care terapia antiretrovirală a fost modulată în funcție de rezultatele testelor genotipice de rezistență efectuate în 2000, care înregistraseră eşec terapeutic în 43 (89,6%) din cazuri. În 2001, din 42 de pacienți, 23 (55%) prezentau viremii plasmatice nedetectabile. Proteaza a fost amplificată la 19/42 din pacienți; 10/19 proteaze prezentau încă un genotip sălbatic. **Rezultate.** La 7 pacienți se acumulaseră mutații de rezistență în raport cu secvențele amplificate în 2000. Revers-transcriptaza (RT) a putut fi amplificată la 19/42 pacienți; mutații noi de rezistență, comparativ cu anul 2000 au fost observate la 4 pacienți. Aceste mutații au conferit tulpinilor rezistența la inhibitori non-nucleozidici de revers-transcriptază (INNRT). Nu au fost observate mutații reverse decât foarte rar, consecutiv modificării schemelor terapeutice. **Concluzii.** Studiul nostru demonstrează că monitorizarea terapiei ARV în funcție de rezultatele testelor genotipice de rezistență este o metodă foarte eficace pentru controlul

evoluției infecției cu HIV. Rata redusă a mutațiilor reverse consecutiv eliminării din scheme a unui anumit compus subliniază necesitatea administrării terapiei ARV cu maximă responsabilitate.

Cuvinte cheie: HIV-1, revers-transcriptază, protează, mutații de rezistență, rezistență virală

INTRODUCTION

Until recently, the routine use of HIV-1 resistance testing towards antiretroviral drugs was considered unfeasible because of time consuming, needing qualified personnel and implying high costs (1). Therefore, surrogate markers were used to monitor the clinical efficacy of the antiretroviral therapy (ART), the most widely used being the dynamics of HIV-1 plasma viral load (VL) (2-4). This surrogate marker is currently widely used in patients receiving ART. However, monitoring of VL is not always useful for monitoring, due to the fact that patients with "undetectable" VL levels (under the limit of detection of untrasensitive techniques which is <20 viral RNA copies/ml) may still present viral replication, as demonstrated by the accumulation of viral mutations (5). Moreover, studies have shown that the occurrence of viral resistance to antiretroviral is a negative predictive factor, which is independent of VL levels, $CD4^+$ T cell counts or the history of antiretroviral treatments (6,7). Finally, numerous studies revealed an increasing prevalence and transmission worldwide of HIV-1 strains with antiretroviral resistance mutations (8-10). Al these aspects being considered, there are arguments for the introduction in clinical practice of testing of HIV-1 resistance to antiretroviral drugs.

In Romania, ARV was gradually introduced starting from 1995, when AZT monotherapy was initiated. In 1998 the systematic administration of thritherapy was assured for the majority of HIV-infected Romanian patients. Unfortunately, numerous accidental or incidental circumstances contributed to disfunctionalities in treatment administration, in particular treatment interruptions or alternating changes in drug associations. Both situations are prohibited by current guidelines (1). Although structured treatment interruptions (STI) are increasingly used as alternative to current schemes, being less expensive and more effective in stimulating the immune reactivity of the treated patients, data regarding this strategy are still preliminary, STI not being yet accepted in routine practice (11). The accidental and incidental circumstances contributed to high level of viral resistance and subsequent treatment failures, as observed in infectious diseases clinics in Romania (reflected in increasing of VL). In the Therapeutic Guidelines it is stated that viral resistance may interest over 50% of Romanian patients receiving ARVs (12). In our previous study (the first systematic genotypic testing of viral resistance in Romania) we have reported extremely high levels of viral resistance (in over 80% of patients) mainly due to the occurrence of

resistance mutations to non-nucleosidic reverse-transcriptase inhibitors (NNRTIs) (13). Following this gentotypic testing, all the tested patients received new associations of ARV drugs, based on the results of genotypic tests.

Here we present the results of the follow-up of VL and resistance pattern in genotype-driven therapies one year after the first genotypic studies.

MATERIAL AND METHODS

Study group was formed by 43 HIV-1-infected children aged between 11 and 13 years (12 ± 0.7 years), hospitalized in "Gulliver House" Chronic Nursing facility of Iasi and diagnosed between 1990 and 1995. All these patients were nosocomially infected with HIV-1 subtype F (13). They were receiving ARVs starting from 1996, in successive associations. These patients were under tritherapy, with associations or ARVs belonging to all three classes of drugs starting from 1999. In 2000, all these patients have been tested in order to evaluate the resistance to ARVs (13). Following these tests, all children received new drugs associations, based on the results of the genotypic tests. The new associations are presented in table 1. Previous treatments have been reported elsewhere (13). For 7 patients we also disposed of RT and protease sequences obtained in 1994-1996, which allowed us to define the wild genotypes and to compare the dynamics of antiretroviral mutations following drug administration (15).

Table 1. Comparative results of serial genotypic testing of VL dynamics

No	Age	CDC	Treatment	VL date	VL** (cp/ml)	Protease	Reverse transcriptase
1*	11	B2		06.00	24,800	V82A	D67N, K101L/E, K103N, Y181Y/C, K219Q
			ABC, 3TC, SAQ, NFV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
2	9	C3		06.00	419,000	salbatic	D67D/N, T69A/D, K70K/R, K103R, Y188L, K219K/Q
			D4T, ddI, IDV, RTV	20.06.01	50,800	**M46I, I54I/V, V82T, I84I/V**	D67N, T69D, K70R, K103R, **V179D,** Y188L, K219Q
3	9	C1		06.00	28,900	Wild type	K103N
			D4T, 3TC, IDV, RTV	20.06.01	257	Wild type	K103N, **P225P/H**
4	11	C3		06.00	<200	*Unamplifiable*	*Unamplifiable*
			EFV, 3TC, IDV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
5	11	C3		06.00	1,180,000	Wild type	K70R, K103N
			D4T, 3TC, IDV, RTV	20.06.01	205	Wild type	K103N, **V108I**
6	12	B3		06.00	8,670	Wild type	M41L, T69T/A, K103K/N, T215S/C
			ABC, 3TC, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
7	12	C1		06.00	1,390,000	Wild type	T69S, K103N
			ABC, ddI, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
8	10	B2		06.00	699	V82I	K70R, K219K/Q
			AZT, 3TC, IDV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
11	12	C2		06.00	226,000	I54V, V82A	D67N, K70R, K103N, Y181C, T215I, K219Q
			D4T, 3TC, SAQ, NFV	20.06.01	105,000	**M46M/L,** I54I/V, V82V/A	K70K/R, K103K/N, Y181C, T215I, K219Q/E

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No	Age	CDC	Treatment	VL date	VL** (cp/ml)	Protease	Reverse transcriptase
12	10	C1		06.00	27,400	Wild type	K103N
			ddI, 3TC, SAQ, NFV	20.06.01	1,110	Wild type	K103N, **P225P/H**
14	12	B1		06.00	4,300	Wild type	K103N
			ABC, 3TC, SAQ, NFV	20.06.01	<200	*Unamplifiable*	*Neamplificabil*
15	13	A1		06.00	240	*Unamplifiable*	K103N
			CBV, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
16	11	B1		06.00	46,600	Wild type	K103N
			ABC, ddI, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
17	10	B1		06.00	408	I54V, V82A	K70R, M184V
			D4T, ddI, SAQ, NFV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
19	11	C2		06.00	356	*Unamplifiable*	*Unamplifiable*
			AZT, NVP, IDV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
20	12	C3		06.00	17,000	*Unamplifiable*	*Unamplifiable*
			ABC, ddI, SAQ, NFV	20.06.01	QI	*Unamplifiable*	*Unamplifiable*
21	10	C2		06.00	2,090	Wild type	K70R, K103N
			AZT, 3TC, IDV	20.06.01	216	Wild type	K70K/R
22	12	A1		06.00	3,950	Wild type	K103N
			D4T, 3TC, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
23	11	B2		06.00	5,780	Wild type	K103N
			ABC, 3TC, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
24	12	C1		06.00	45,900	Wild type	K103N
			ABC, AZT, ddI	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
25	10	B3		06.00	<200	Wild type	*Unamplifiable*
			AZT, NVP, IDV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
26	11	C2		06.00	10,100	Wild type	T69T/I, K103N, Y181C
			ABC, 3TC, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
28	10	B3		06.00	<200	*Unamplifiable*	Wild type
			D4T, NVP, NFV	20.06.01	1,110	**K20T, L90M**	**K70R, Y181C**
29	13	C1		06.00	<200	Wild type	Wild type
			D4T, NVP, NFV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
30	11	B3		06.00	8,510	Wild type	K70K/R, Y188Y/C, G190G/A
			D4T, 3TC, IDV, RTV	20.06.01	2,090	Wild type	**V179D, M184M/V,** G190A
31	11	B2		06.00	3,270	Wild type	K103N
			D4T, 3TC, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
32	13	C3		06.00	213,000	M46I, I54V, V82A	M41L, T69D, L74V, L100I, K103N, M184V, L210W, T215C
			D4T, SAQ, NFV	20.06.01	39,700	**L24I,** M46I, **G48G/V, F53F/L,** I54V, V82A, **I84I/V**	M41L, T69G, L100I, K103N, L210W, T215C
33	13	C2		06.00	12,500	Wild type	K103N
			ABC, 3TC, IDV, RTV	20.06.01	216	**M46L, V82A**	K103N, **M184V, P225P/H**
34	14	B3		06.00	359,000	Wild type	K103N, T215D
			D4T, 3TC, NFV	20.06.01	308	Wild type	K103N, T215D

No	Age	CDC	Treatment	VL date	VL (cp/ml)	Protease	Reverse-transcriptase
35	11	B2		06.00	4,030	Wild type	T69S
			AZT, 3TC, IDV	20.06.01	253	Wild type	**K103K/N**
36	12	C2		06.00	19,200	Wild type	67D/A, K103N
			ABC, 3TC, IDV, RTV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
37	11	B1		06.00	357	Wild type	69T/I
			D4T, NVP, NFV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
38	12	C3		06.00	625,000	Wild type	D67G, Y188L, T215I, K219E
			ABC, ddI, SAQ, NFV	20.06.01	690	Wild type	D67G, Y188L, T215I, K219E
39	10	C		06.00	de diluat	Wild type	K101K/Q, K103N, G190G/A
			D4T, ddI, IDV	20.06.01	199,000	**G48V,V82A**	K101K/Q, K103N, **P225P/H**
40	11	C3		06.00	349,000	V82A	D67N, T69D, K70R, K103N, Y181C, K219Q
			ddI, 3TC, SAQ, NFV	20.06.01	5,200	V82A	D67N, T69D, K70R, K103N, Y181C, K219Q
41	12	C3		06.00	91,600	M46I, I54V, V82A	D67N, T69D, K70R, K103N, K219Q
			ABC, 3TC, SAQ, NFV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
42	11	C2		06.00	75,200	M46M/L, V82A	D67N, T69D, K70R, K103N, Y181C
			ddI, 3TC, SAQ, NFV	20.06.01	2,430	M46M/L, **I54V, A71A/V,** V82A	D67N, T69D, K70R, K103N, Y181C, **T215T/I, K219Q**
43	13	C2		06.00	40,800	Wild type	K103N
			ddI, 3TC, IDV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
44	11	C3		06.00	20,000	M46L, I54V, V82A	K70R, K103N
			ABC, 3TC, SAQ, NFV	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
45	13	C1		06.00	558	V82V/I	Salbatic
			D4T, NVP, NFV	20.06.01	559	V82V/I	**K103S/N, T215C**
46	10	C2		06.00	491,000	Wild type	T69T/A, K103K/N, K219Q
			non-traité	20.06.01	130,000	Wild type	K103N, K219K/Q
47	11	C2		06.00	20,000	I54V, V82A	Y181C
			ABC, D4T, 3TC	20.06.01	<200	*Unamplifiable*	*Unamplifiable*
48	12	C1		06.00	3,470	Wild type	T69S
			AZT, 3TC, IDV	20.06.01	1,120	Wild type	T69S

MATERIALS AND METHODS

Samples. Four ml of EDTA blood was sampled from all the patients in the study group. Plasma was obtained through blood centrifugation at 1700 RPM for 10 minutes. Plasma was then clarified by centrifugation at 3000 g for 10 minutes. Plasma samples were then stored in liquid nitrogen (-192°C) until tested. The time between samples thaw and testing was less than 5 hours. Plasma RNA was extracted using RNA lysis buffer from Amplicor HCV Specimen Preparation Kit (Roche Diagnostic Systems Inc. Branchburg, USA).

Viral load quantification was done by Monitor Roche 1.5 (Roche Diagnostic Systems Inc. Branchburg, USA), with detection limit of 200 copies/mL. Results were confirmed by bDNA (Chiron, Emeriville, CA, USA), which has a detection limit of 500 copies/ml. This method is highly sensitive in quantifying non-subtype B samples.

Genotypic resistance tests were done on plasma RNA based on the protocol of the *Agence Française pour la Recherche sur le SIDA* (ANRS), as previously described (13). RT and protease mutations were defined based on those described in the consensus paper of International AIDS Society (IAS)-USA (16).

RESULTS

Background on the ARV resistance testing in June 2000. Genotypic anti-retroviral testing (**GART**) performed in June 2000 revealed a high prevalence of therapeutic failures in this group of children. Thus, 43 out of the 48 included patients (90%), showed a detectable VL, which was associated with resistance mutations in both protease and RT genes. However, the majority of these mutations were conferring resistance towards NNRTI. Based on the genotyping results, ARV treatments were modified in all included patients.

Genotype-based ARV therapy management in patients previously with ARV therapy failure. In June 2001 we have retested 43 of the patients included in the original study (13). Results are shown in table 1. One sample was of low volume and it was excluded from this analysis. Out of the remaining 42 samples VL quantification showed an undetectable VL in 23 patients (55%). Six of the patients with detectable VLs showed VL ranging from 200 to 500 copies/ml. Therefore, we concluded that genotype-based ARV managements resulted in an increase of clinical efficacy from 10% to 55%.

Comparison of current results to those observed at the first testing revealed a different evolution of VL between different groups of patients. Thus, 3 out of the 4 patients with undetectable VLs in 2000, for which ARV treatment was maintained using the initial combinations, showed undetectable VLs at the new testing. Treatment failure was observed in only one of these patients (VL dynamics being $<200 \rightarrow 1110$ copies/ml) and it was due to occurrence of resistance mutations to nevirapine (NVP) (Y181C) and nelfinavir (NVF) (L90M). Four out of

the 5 patients with VLs ranging from 200 to 500 copies/ml in 2000 showed undetectable VLs in 2001. The last patient in this group showed a stable VL at the two testing (558→559), probably due to the replacement of the wild RT genotype with a mutant one containing the K103N substitution. This patient also presented the T215C substitution, which is not associated with AZT resistance. Finally, patients showing VLs ranging from 200 to 500 copies in 2001, had been shown to present high VLs during the first testing (the evolution of VLs in each of these patients being as follows: 29.000→253; 1.180.000→205; 2.090→216; 12.500→216; 359.000→308; 4.030→253 cp/ml). Altogether, these results indicate an extremely favorable evolution of VLs in those patients for which the management of ARV therapy was based on genotypic testing.

Dynamics of the occurrence of resistance mutations and of reverse mutations. Protease gene was amplified and sequenced in 19 out of the 43 included patients. Ten out of 19 gene sequences were still wild type. In the remaining patients, the protease had been amplified in 2000 in 20 out of 24 and the results were as follows: 14 had shown a wild genotype and 6 showed mutations to protease inhibitors (PI) (table 1). Dynamic testing of genotypic susceptibility revealed that between 2000 and 2001 resistance mutations to PI occurred in 7 patients. In three subjects, the wild type was replaced with a resistant one (patients #2, #33, #39) (table 1); in other three patients (#11, #32, #42), a mutant genotype was replaced with a new one showing more resistance mutations; finally in the last patient (#28), the protease was not amplified in 2000. In the remaining two patients (#40, #45), the resistant genotype was conserved between 2000 and 2001 (V82A and V82V/I, respectively). Major resistance mutations toward the PIs (V82A/T, I84V, L90M) were present in all 9 patients showing a resistant protease genotype. Accessory mutations associated with HIV resistance to PIs were observed in most of the patients, the most frequently present being M46I/L and I54V.

RTs were amplified in 19 out of the 43 patients. In patients for whom RT could not be amplified in 2001, results in 2000 were as follows: in 4 cases RT could not be amplified in 2000 too, whereas in the remaining cases RT showed a resistant genotype mainly containing NNRTI resistance mutations (table 1). New resistance mutations were observed in 4 patients. In 2001 testing, resistance mutations were present as follows: in 4 cases these mutations were associated with NNRTI resistance (#5-V108I; #28-Y181C; #35-K103K/N; #45-K103S/N), in 2 cases (#30; #33) they were associated with resistance to 3TC (M184V), whereas in the remaining patients (#42; #45) they were associated with resistance to AZT (T215C/I).

Following GART-managed therapy, reverse mutations were of very rare occurrence. In a single case (#32) reverse M184V mutation occurred. Reverse mutation to NNRTI seem to be a rare event, being observed in a

single patient (#30). One should note that in 2000 this strain was of showed mixed genotype Y188Y/C, therefore the reverse mutation in 2001 may be rather due to a random selection by PCR of the wild population than to a reverse mutation.

DISCUSSION

Numerous retrospective and prospective trials revealed the clinical usefulness of ARV therapy management based on the results of genotypic testing of viral resistance. A significant correlation between ARV resistance and virological response to a new therapeutic association administered following therapeutic failure was previously reported (6, 7, 17-21). Thus a randomized European trial designed to investigate the efficacy of therapy management based on genotypic testing (**VIRADAPT trial**) included 108 patients with VLs > 10.000 copies/ml which previously received PI for at least 3 months (22). Patients were randomized, the ARV associations being changed based on the standard monitoring (VL) or on the genotypic test results. During the 6 month follow-up better results were observed in the genotype-based study group, reflected in both a better control of VL (-1.15 *vs* -0.67 $\log_{10}$ copies/ml) and a higher level of undetectable VLs (32% *vs* 14% patients with VLs < 200 copies/ml) (22). During the next 6 months of follow-up all the patients were included in the genotype-based treatment group, the results monitored after 1 year of follow-up being similar for all the patients (30% of patients showing undetectable VLs). This clinical trial demonstrated the usefulness of treatment monitoring based on the results of genotyping resistance tests and also revealed that favorable results can be obtained in all the patients even in the context of delaying genotypic tests for different reasons.

In a second clinical trial conducted in the USA (**GART**-*Genotypic Antiviral Resistance Testing Trial*), changes in therapy based on the results of genotypic tests were carried out in the study group but not in the control group (20). Changes in ARV associations were recommended by an expert panel. Results were similar to those reported in the VIRADAPT Trial, after 4-8 weeks following treatment changes a better virological response being observed in the study group compared to the control group (-1.17 *vs* -0.62 $\log_{10}$ copies/ml).

However, sustained virological responses were not observed in GART trial: viral suppression was 50% lat 8 weeks and 33% at 12 weeks. However the expert panel explicitly presented the major circumstance of these failures: **"of note, only half of the patients for which genotypic tests were available received the therapy recommended by the expert panel"** (20).

All patients, in our study group, received new therapeutic associations based on the results of genotypic tests. The management of ARV therapy registered excellent results after 12 months, consisting in an increase of the treatment efficacy from 10% to 55%. Moreover, in patients showing detectable VLs, the reduction of VL

was of 1.24 log_{10} copies/ml, which is a supplementary virological argument for GART-based ARV treatment management. Three months after the second change in therapeutic associations following the second genotypic test, >80% of patients were showing an undetectable VL (data not shown).

The investigation of ARV resistance through direct sequencing gave us the opportunity to determine the occurrence of resistance mutations during the therapeutic failure. Moreover, the analyses reported here revealed that resistance mutations which determine the new associations do not revert following treatment changes, in spite of changes in the administered drugs or in ARV classes. In this study, the majority of patients were receiving NNRTI prior to the first test and therefore current treatments completely excluding this drug class. However, NNRTI mutations were conserved at the second testing, which suggests that the therapeutic alternance of these drugs is impossible in those patients who will develop resistance towards other ARV classes. This result stress the responsibility of the practitioner at the time of ARV initiation in a patient: ARV association should not only insure viral suppression in as many patients as possible but also should maintain this suppression over the longest period possible. Moreover, these results support the idea that initiation of ARV treatments should be done in such manner to allow the use of at least one class of ARVs as salvage therapy once the resistance is suggested by follow-up and proven by genotypic testing.

Finally, data presented here, as well as our previous results suggest that whether NNRTI failure is observed (K103R, Y181M), this entire class of drug is to be completely avoided. The administration of two NNRTI is medically and economically useless.

Unfortunately, Romanian experiences with ARV therapy suggest that our data reflect correctly the prevalence of resistance at a national level. It is extremely probable, as suggested in the Therapeutic Guideline, that more than 50% of patients are therapeutic failures (12). Our results call for extreme responsibility in prescribing ARV therapy in order to prevent reoccurrence of situations observed. The Government effort to provide necessary treatments and organize the monitoring of treatment efficacy as well as the efforts of the infectious diseases specialists to elaborate the guidelines for ARV prescription should be back-up by practitioners. Otherwise, the responsibility is individual.

CONCLUSIONS

The present study has investigated the efficiency of the ARV management based on plasma genotypic antiretroviral resistance testing in patients with failing ARV therapy.

The genotype ARV management registered an increase of the treatment efficiency from 10% to 55, in the 12 months of follow up. The reduction of VL was of 1.24 log_{10} copies/ml. This can be an argument for GART-bared ARV treatment management.

More than 80% of patients were showing

no detectable VL, after the second change of therapeutic associations following the second genotypic test.
The administration of two NNRTI is medically and economically useless.

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