Virological Factors Associated With Outcome of Dual MVC/RAL Therapy (ANRS-157 Trial)

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ABSTRACT

- **Background**: ROCnRAL ANRS-157 was a single-arm study designed to evaluate a switch to a maraviroc (MVC) 300 mg bid plus raltegravir (RAL) 400 mg bid regimen in virologically suppressed patients with lipohypertrophy, and R5-tropic B or CRF02_AG HIV-1 subtype determined on DNA by Sanger sequencing. In these long-term antiretroviral experienced patients (n=44), the combo MVC/RAL lacks virological robustness despite a good treatment adherence, adequate respective plasma pharmacokinetics and benefit in lipid profile: 7 patients failed MVC/RAL therapy with 5 virological failures (VF) defined as two consecutive plasma viral load (pVL) > 50 cp/mL and 2 treatment discontinuations due to serious adverse events. The aim of this work was to investigate the factors associated with VF.
- **Methods**: At baseline (BL), Ultra Deep Sequencing (UDS) of DNA gp120 V3 and integrase regions (454 Roche) and quantification of cellular HIV DNA were performed in PBMC. Viral tropism was interpreted using Geno2pheno algorithm (samples harboring >2% non-R5 variants were classified non-R5 viruses). Genotypic resistance mutations were evaluated according to the ANRS and IAS mutations list. Quantification of pVL was measured using an ultrasensitive assay (1 cp/mL). Tropism, BL ultrasensitive HIV RNA pVL, BL HIV DNA VL, subtype, age, ethnicity, transmission group, AIDS status, nadir CD4 and BL CD4 cell count, time since HIV diagnosis, duration of antiretroviral treatment, duration of suppressed viremia, pVL zenith, CD4/CD8 ratio, BL CD8 cell count were investigated as potential factors associated with VF.
- **Results**: The proportion of patients with pVL < 1 cp/mL did not evolve overtime: 72%, 63%, 72%, 68%, 57%, 47%, 68% and 69% at BL, W4, W8, W12, W16, W20, W24 and end of the study respectively. Median BL cell associated DNA in PBMC was similar in patients with BL pVL< 1 and > 1 cp/mL (2.77 and 2.96 log_{10} copies/10^6 PBMCs, respectively). Among the 44 patients, 3 patients had minority X4-tropic viruses determined by UDS at BL and one of them presented VF. Some BL resistance mutations in integrase gene were detected by UDS at two positions: E138 and G140. None of studied factor was associated with VF.
- **Conclusions**: Overall, the proportion of patients with a VL < 1 cp/mL was similar during all the follow-up, suggesting that VF is not related to a lack of efficacy of the MVC/RAL combination. Neither UDS, nor ultrasensitive viremia were an added value to predict VF in this context of MVC/RAL switch.

BACKGROUND

- Treatment simplification strategies could be envisaged in reason of toxicity/adverse events, treatment failure/resistance, decision of patients or physicians.
- A dual combination of maraviroc (MVC) /raltegravir (RAL) was evaluated: ROCnRAL ANRS-157 study was a pilot Phase II, single-arm, multicenter clinical trial designed to evaluate the capacity of the RAL plus MVC combination to maintain HIV viraemia below 50 copies/mL over 24 weeks in HIV-1-infected patients with controlled viral load under antiretroviral treatment and clinical lipohypertrophy (ClinicalTrials.gov: NCT01420523).
- MVC and RAL failed to maintain virological suppression in this population of HIV-1 infected patients (5 patients had VF defined as two consecutive measurements of HIV-1 RNA > 50 copies/mL and 2 patients developed adverse events). Then, the study was prematurely discontinued. In these long-term ARV experienced patients, the therapy seemed to lack virological robustness despite a benefit in lipid profile. [1]

OBJECTIVES

- **The aim of this study was to investigate the factors associated with VF and virological rebound at 50 copies/mL or 1 copy/mL in the clinical trial ROCnRAL ANRS 157 study.**

PATIENTS AND METHODS

Table 1: Baseline patient characteristics [1]

	Total n=44
Age, (year) (median [IQR])	55 (50–60)
Male sex – n (%)	38 (86)
Ethnic origins – n (%)	
- Caucasian	40 (91)
- Sub-Saharan Africans	4 (9)
Transmission group – n (%)	
- Men who have sex with men	28 (64)
- Heterosexual	11 (24)
Unknown/Other	5 (12)
Time post HIV diagnosis, year, median (IQR)	20 (14–24)
Time on suppressed HIV viremia (< 50 copies /mL), year, median (IQR)	5.2 (4.4–7.9)
Nadir CD4 cells count per mm3, median (IQR)	210 (150–276)
CD4 cells count per mm3, median (IQR)	635 (546–757)
CD8 cells count per mm3, median (IQR)	731 (557–987)
CD4/CD8 ratio, median (IQR)	0.9 (0.6–1.2)
Plasma viral load zenith, log10 copies/mL, median (IQR)	4.8 (4.4–5.3)
HIV DNA in PBMCs, log10 copies/10^6 PBMCs, median (IQR)	2.8 (2.5–3.1)
Viral tropism detection: false positive rate (FPR, %), median (IQR)	52.8 (39.6–74.3)
CDC Stage C – n (%)	9 (20)
Duration of antiretroviral treatment, year, median (IQR)	15 (13–19)
cART at screening – n (%)	
2NRTIs+PI/r	16 (36)
- 2NRTIs+PI	5 (11)
- PI/r	9 (20)
- 2NRTIs+NNRTI	7 (16)
- NNRTI+PI/r	2 (5)
Others	5 (11)
Daily dosing – n (%)	
- QD	27 (61)
- BID	17 (39)
Self reported adherence level	
- Low (0-79%)	6 (14)
- Medium (80-99%)	14 (32)
High (>99%)	24 (55)

- Failure: VF: two consecutive measurements of HIV-1 RNA > 50 copies/mL taken 2–4 weeks apart (n=5); or any treatment modification or discontinuation (n=2).
- Virological rebound > 50 copies/mL , n=12/43 (43 patients had effectively a follow up in the trial)
- Virological rebound > 1 copy/mL, n=19/31 (31 patients had a VL < 1 copy/mL at baseline)
- Viral tropism: Geno2Pheno algorithm [false positive rate (FPR) 20%].
- Genotypic resistance testing: interpretation with the last ANRS genotypic algorithm (http://www.hivfrenchresistance.org).
- Quantification of plasma viral load (pVL): Amplicor monitors assay (Cobas 2.0, Roche Diagnostics, Basel, Switzerland).
- Whole blood was collected at D0 and cellular HIV-1 DNA was quantified as previously described.[2]
- 454 sequencing technology (454 Life Sciences; Roche Diagnostics Corp., Branford, CT):
  - 334 and 573 base-pair fragment were PCR-amplified with primers as previously described for gp120 and integrase genes, respectively. [3,4]
  - Protocol was previously described for Ultra Deep Sequencing (UDS). [5]
  - Data generated with the UDS method were first analyzed with PyroClass.
  - Tropism was determined by Geno2pheno [454]. The patient was considered to have X4-tropic variants when the percentage of X4-tropic viruses > 2%.
  - Integrase mutations were searched with PyroMut according to the last ANRS mutation list and IAS list (http://www.hivfrenchresistance.org/).
- Statistical analysis: SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). All variables providing a P-value < 0.15 in the univariate analysis were selected for the multivariable model.
- Univariable and multivariable logistic regression models were used to identify factors predictive of loss of viral control (>50 copies/mL or >1 copy/mL), among the following variables: age, sex, ethnicity, CDC stage, transmission group, previous antiretroviral treatment, duration of antiretroviral treatment, baseline (BL) CD4 cell count, BL CD8 cell count, BL CD4/CD8 ratio, nadir CD4 cell count, time since HIV diagnosis, plasma VL zenith, BL plasma HIV RNA VL, duration of suppressed viraemia, BL HIV DNA in PBMCs, HIV-1 subtype, BL tropism UDS, FPR tropism, presence of integrase minority resistant variants (UDS method) at BL. [1]

References
1. Katlama C, Assoumou L, Valantin M-A, *et al*. Maraviroc plus raltegravir failed to maintain virological suppression in HIV-infected patients with lipohypertrophy: results from the ROCnRAL ANRS 157 study. *J Antimicrob Chemother* 2014; **69**: 1648–52.
2. Avettand-Fenoël V, Oaw M-L, Blanche S, *et al*. LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO.01). *J Med Virol* 2009; **81**: 217–23.
3. Malet I, Delelis O, Soulie C, *et al*. Quasispecies variant dynamics during emergence of resistance to raltegravir in HIV-1-infected patients. *J Antimicrob Chemother* 2009; **63**: 795–804.
4. Soulié C, Derache A, Aimé C, *et al*. Comparison of two genotypic algorithms to determine HIV-1 tropism. *HIV Med* 2008; **9**: 1–5.
5. Daigle D, Simen BB, Pochart P. High-throughput sequencing of PCR products tagged with universal primers using 454 life sciences systems. *Curr Protoc Mol Biol Ed Frederick M Ausubel Al* 2011; **Chapter 7**: Unit7.5.
6. Malet I, Gimferrer L, Artese A, *et al*. New raltegravir resistance pathways induce broad cross-resistance to all currently used integrase inhibitors. *J Antimicrob Chemother* 2014; **69**: 2118–22.

RESULTS

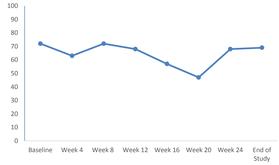
Table 2: Patients who discontinued the maraviroc/raltegravir combination due to virological failure

	W-4 Screening			Baseline				Virological failure				
Patient	cART prior entry	Self reported adherence level	Duration of suppressed viremia (years)	CD4 cell count (cells/mm³)	Subtype	HIV DNA tropism Sanger	HIV DNA X4-tropic viruses (%) UDS	Integrase mutations (%) UDS	Plasma HIV-1 viral load (copies/ml)	Time of failure	Drug concentrations Plasma C_{min} (ng/ml)*	Integrase mutation Sanger	HIV-RNA Tropism Sanger
1	TDF/FTC/EFV	High (>99%)	9.5	477	B	CCR5	3.1	0.0	105 2973	W4 W8	RAL: 21 MVC: 13	none	CCR5
2	DRV/r	High (>99%)	7.5	832	B	CCR5	0.0	0.0	8972	W16	RAL: 1960 MVC: 160	Y143C	CXCR4
3	TDF/FTC/DRV/r	Medium (80-99%)	9.8	893	B	CCR5	1.7	0.0	1453 69 8070	W20 W16 W20	RAL 56 MVC 104	N155H	CXCR4
4	TDF/FTC/DRV/r	Medium (80-99%)	3.6	601	CRF02_AG	CCR5	0.0	0.0	27434 259 375 2820	W12 W16 W20 W22	RAL: 121 MVC: 28 RAL: 87 MVC: 105	none	CCR5
5	TDF/FTC/EFV	High (>99%)	6.6	954	B	CCR5	0.0	0.0				F121Y	CCR5

*Raltegravir and maraviroc plasma Cmin 12h target value : 50 ng/ml

- **5 patients in virological failure (2 consecutive viral load > 50 copies/mL)**
- **2 patients had low concentrations of MVC and/or RAL, with no integrase mutations and no X4-tropic viruses.**
- **3 patients had integrase mutations:**
  - **1 with Y143C and 1 with N155H (resistant to RAL and EVG; susceptible to DTG) , both with X4 tropism.**
  - **1 with F121Y (resistant to RAL, EVG and DTG) [6] with R5 tropism.**

Figure 1: % of patients with < 1 copy/mL



- **Proportion of patients with VL < 1 copy/mL was stable overtime.**

- **No integrase minority variant was detected at baseline in HIV DNA for patients in VF**
- **Baseline minority X4-tropic variants were detected for 2 patients in VF:**
  - **1 with 3.1% of X4-tropic variants in DNA and R5-tropic viruses at failure**
  - **1 with 1.7% of X4-tropic variants in DNA and X4-tropic viruses at failure**

Table 3: Summary of HIV minority variants detected by UDS in DNA at baseline (X4-tropic variants > 2% and/or integrase resistant minority variants >1%)

Patient	Subtype	Baseline UDS X4-tropic viruses (%)	Baseline UDS Integrase mutations (%)
1	B	3.10	
6	B	3.50	
7	B	7.30	
8	B	0.00	**E138K** (4.83)
9	B	0.10	**G140S** (2.56)
10	B	0.00	**E138A/K** (1.04)

- **3 patients have X4-tropic variants > 2%:**
  - **1 in VF : 3.1% (patient 1 in the table 2)**
  - **2 in virological success: 3.5% and 7.3%**
- **Some baseline integrase minority resistant variants were detected for 3 patients in virological success (none of them in VF): E138K (4.8%); G140S (2.56%); E138A/K (1.0%).**

Table 4: Factors statistically associated or not with the virological rebound > 50 copies/mL to MVC/RAL treatment in univariate logistic regression analysis.

	Patients with VL <50 copies/mL (n=31)	Patients with VL >50 copies/mL (n=12)	Univariate analysis HR (95%CI, p)
Age, years, median (IQR)	54 (46-60)	57 (50-63)	1.04 (0.97-1.13, p=0.243)
Gender-sex, n (%)			
• **Male**	27 (73%)	10 (27%)	1
• **Female**	4 (67%)	2 (33%)	1.28 (0.28-5.87, p=0.747)
Origin-ethnicity, n (%)			
• **Caucasian**	29 (73%)	11 (28%)	1
• **Sub-saharan Africa**	2 (67%)	1 (33%)	1.71 (0.22-13.41, p=0.610)
CDC stage, n (%)			
• **A,B**	26 (74%)	9 (26%)	1
• **C**	5 (63%)	3 (38%)	1.55 (0.42-5.77, p=0.510)
Transmission group, n (%)			
• **Other**	11 (73%)	4 (27%)	1
• **Men who sex with men**	20 (71%)	8 (29%)	0.96 (0.53-1.75, p=0.897)
Previous antiretroviral treatment intake			
• **Once daily**	18 (67%)	9 (33%)	1
• **Twice daily**	13 (81%)	3 (19%)	0.63 (0.17-2.34, p=0.490)
Duration of antiretroviral treatment, years	15 (13-18)	15 (13-19)	1.03 (0.88-1.21, p=0.710)
Baseline CD4 cell count, cells/mm³	614 (555-799)	598 (491-867)	0.93 (0.33-2.62, p=0.886)
Baseline CD8 cell count, cells/mm³	799 (548-966)	584 (528-764)	0.70 (0.31-1.59, p=0.399)
CD4/CD8 ratio	0.81 (0.66-1.23)	0.95 (0.75-1.44)	1.77 (0.41-7.60, p=0.442)
Nadir CD4 cell count, cells/mm³	207 (149-277)	237 (154-306)	1.55 (0.30-8.12, p=0.603)
Time since HIV diagnosis, years	20 (14-24)	24 (19-26)	1.11 (0.98-1.26, p=0.113)
Plasma viral load zenith, log_{10} copies/mL	4.59 (4.27-5.09)	5.12 (4.62-5.33)	1.43 (0.67-3.02, p=0.354)
Baseline plasma HIV RNA, n (%)			
• **<1 copie/mL**	23 (74%)	8 (26%)	1
• **1-50 copies/mL**	8 (67%)	4 (33%)	1.49 (0.44-4.98, p=0.521)
Duration of suppressed vireamia, years	5 (4-9)	6 (5-10)	1.05 (0.89-1.24, p=0.541)
HIV DNA in PBMCs, copies/10^6 cells	629 (300-1238)	668 (516-1214)	1.36 (0.37-5.07, p=0.643)
Subtype, n (%)			
• **B**	28 (74%)	10 (26%)	1
• **CRF02_AG**	3 (60%)	2 (40%)	1.89 (0.41-8.74, p=0.418)
False Positive Rate of the tropism detection, %	51(42-74)	61 (34-75)	1.00 (0.97-1.03, p=0.916)
Tropism UDS	0.10 (0.00-0.67)	0.05 (0.00-0.35)	0.86 (0.50-1.48, p=0.592)
Presence of integrase mutations			
• **no**	25 (69%)	11 (31%)	1
• **yes**	5 (83%)	1 (17%)	0.56 (0.07-4.36, p=0.582)

- **None of studied factors were related to the virological rebound VL > 50 copies/mL in the univariate analysis.**

Table 5: Factors statistically associated or not with the virological rebound > 1 copy/mL to MVC/RAL treatment in univariate and multivariable logistic regression analysis.

	Patients with VL <1 copy/mL (n=12)	Patients with VL >1 copy/mL (n=19)	Univariable analysis HR (95%CI, p)	Multivariable analysis HR (95%CI, p)
Age, years, median (IQR)	53 (44-58)	56 (50-63)	1.06 (0.99-1.12, **p=0.065**)	1.02 (0.95-1.10, p=0.543)
Gender-sex, n (%)				
• **Male**	8 (30%)	19 (100%)	1	
• **Female**	4 (100%)	0 (0%)	0.03 (0.00-4.80, p=0.179)	
Origin-ethnicity, n (%)				
• **Caucasian**	10 (34%)	19 (66%)	1	
• **Sub-saharan Africa**	2 (100%)	0 (0%)	0.04 (0.00-59.24, p=0.393)	
CDC stage, n (%)				
• **A,B**	12 (46%)	13 (54%)	1	
• **C**	1 (14%)	6 (86%)	1.80 (0.67-4.82, p=0.243)	
Transmission group, n (%)				
• **Other**	6 (55%)	5 (45%)	1	
• **Men who sex with men**	6 (30%)	14 (70%)	2.24 (0.79-6.35, **p=0.130**)	1.46 (0.43-4.90, p=0.542)
Previous antiretroviral treatment intake				
• **Once daily**	9 (43%)	12 (57%)	1	
• **Twice daily**	3 (30%)	7 (70%)	1.56 (0.60-4.08, p=0.366)	
Duration of antiretroviral treatment, years	17 (15-19)	17 (14-19)	1.03 (0.89-1.18, p=0.714)	
Baseline CD4 cell count, cells/mm³	604 (442-761)	624 (548-878)	2.42 (0.52-11.23, p=0.260)	
Baseline CD8 cell count, cells/mm³	568 (458-837)	805 (597-966)	2.76 (0.65-11.74, p=0.169)	
CD4/CD8 ratio	0.90 (0.72-1.32)	0.94 (0.66-1.10)	0.84 (0.19-3.67, p=0.821)	
Nadir CD4 cell count, cells/mm³	176 (143-211)	208 (153-277)	2.14 (0.67-6.80, p=0.197)	
Time since HIV diagnosis, years	21 (16-23)	24 (17-24)	1.08 (0.97-1.20, p=0.184)	
Plasma viral load zenith, log_{10} copies/mL	4.35 (3.89-4.69)	4.77 (4.44-5.11)	1.51 (0.71-3.21, p=0.282)	
Duration of suppressed vireamia, years	5 (3-10)	6 (5-9)	0.97 (0.84-1.12, p=0.654)	
HIV DNA in PBMCs, copies/10^6 cells	589 (83-982)	583 (471-1238)	3.64 (0.92-14.38, **p=0.065**)	2.70 (0.64-11.43, p=0.117)
Subtype, n (%)				
• **B**	9 (33%)	18 (67%)	1	
• **CRF02_AG**	3 (75%)	1 (25%)	0.29 (0.04-2.17, p=0.227)	
False Positive Rate of the tropism detection, %	62(37-78)	51 (42-72)	1.00 (0.98-1.02, p=0.740)	
Tropism UDS	0.10 (0.00-1.10)	0.00 (0.00-0.30)	1.01 (0.60-1.71, p=0.976	
Presence of integrase mutations				
• **no**	8 (30%)	19 (70%)	1	
• **yes**	3 (100%)	0 (0%)	0.03 (0.00-5.72, p=0.193)	

- **31 patients had VL< 1 copy/mL at baseline and were included in this analysis.**
- **12 patients have a VL < 1 copy/mL overtime**
- **19 patients experienced a VL > 1 copy/mL during the follow up**
- **3 factors were associated with virological rebound > 1 copy/mL with a p-value <0.15 in the univariate analysis: HIV DNA in PBMCs, age and transmission group.**
- **None of these 3 factors were significant in the multivariable analysis .**

CONCLUSIONS

- **In a MVC/RAL treatment as a switch strategy, in case of virological failure, there is a risk of emergence of X4-tropic viruses and integrase resistance mutations.**
- **UDS in DNA was able to detect some minority X4-tropic or integrase resistance mutations at baseline. However, the presence of these variants in DNA did not explain the virological failure.**
- **No factor was found to be predictive of viral rebound at >50 copies/mL or at >1 copy/mL, probably owing to a lack of statistical power.**
- **In conclusion, the ROCnRAL-ANRS 157 study suggests that, in a population of long-term-treated ageing patients, MVC/RAL dual therapy lacks virological robustness, resulting in the emergence of resistance mutations, and therefore cannot be recommended for further evaluation on a larger scale.**
- **The results of a phase II pilot study of simplification to MVC/RAL dual therapy after 6 months of MVC-RAL- Tenofovir - Emtricitabine quadruple therapy in ARV treatment-naive HIV-1-infected patients with CCR5- tropic virus (NCT01291459) are expected and it is interesting to evaluate the efficacy of this dual therapy in a different population of patients.**
- **These results do not predict the efficacy of MVC in combination with antiretrovirals with higher genetic barrier.**

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