



HAL
open science

HIV-1 group P infection: towards a dead-end infection?

Elodie Alessandri-Gradt, Fabienne de Oliveira, Marie Leoz, Veronique Lemée,
David L Robertson, Felix Feyertag, Paul-Alain Ngoupo, Philippe Mauclère,
Francois Simon, Jean-Christophe Plantier

► **To cite this version:**

Elodie Alessandri-Gradt, Fabienne de Oliveira, Marie Leoz, Veronique Lemée, David L Robertson, et al.. HIV-1 group P infection: towards a dead-end infection?. AIDS. Official journal of the international AIDS Society, 2018, 32 (10), pp.1317-1322. <10.1097/QAD.0000000000001791>. <hal-02383299>

HAL Id: hal-02383299

<https://normandie-univ.hal.science/hal-02383299v1>

Submitted on 28 Apr 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Alessandri-Gradt, E., De Oliveira, F., Leoz, M., Lemee, V., Robertson, D. L., Feyertag, F., Ngoupo, P.-A., Mauclere, P., Simon, F. and Plantier, J.-C. (2018) HIV-1 group P infection: towards a dead-end infection? *AIDS*, 32(10), pp. 1317-1322. (doi:[10.1097/QAD.0000000000001791](https://doi.org/10.1097/QAD.0000000000001791))

This is the author's final accepted version.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

<http://eprints.gla.ac.uk/159689/>

Deposited on: 16 April 2018

Enlighten – Research publications by members of the University of Glasgow
<http://eprints.gla.ac.uk>

HIV-1 group P infection: towards a dead-end infection?

Elodie ALESSANDRI-GRADT¹, Fabienne DE OLIVEIRA¹, Marie LEOZ¹, Véronique LEMEE¹, David L ROBERTSON^{2,3}, Felix FEYERTAG^{2,4}, Paul-Alain NGOUPO⁵, Philippe MAUCLERE⁶, François SIMON⁷, Jean-Christophe PLANTIER¹

¹Normandie Univ, UNIROUEN, EA2656, GRAM, CHU de Rouen, Laboratoire de Virologie associé au CNR du VIH, F-76000 Rouen, France

²Evolution & Genomic Sciences, School of Biological Sciences, University of Manchester, Oxford Road, Manchester, United Kingdom

³MRC-University of Glasgow Centre for Virus Research, Garscube Campus, Glasgow, United Kingdom

⁴Department of Biology, University of Nevada, Reno, Nevada, United States of America

⁵Laboratoire de Virologie, Centre Pasteur du Cameroun, Yaoundé, Cameroun

⁶Ravinala Conseil, F-75012 Paris, France

⁷Département de microbiologie, Hôpital Saint Louis, Faculté de médecine Paris Diderot, F-75000 Paris, France

Corresponding author: JC Plantier

jc.plantier@chu-rouen.fr

Institut de Biologie Clinique, hôpital C. Nicolle, CHU Rouen

1 rue de Germont, 76031 Rouen cedex, France

Tél +33 2 32 88 14 62

Fax +33 2 32 88 04 30

Running head: new insights on HIV-1/P

Concise communication ; Total number of words: 1801

ACCEPTED

ABSTRACT

Objectives: HIV/1 group P (HIV-1/P) is the last HIV/1 group discovered and to date, comprises only two strains. To obtain new insights into this divergent group, we screened for new infections by developing specific tools, and analysed phenotypic and genotypic properties of the prototypic strain RBF168. In addition, the follow-up of the unique patient monitored so far, has raised the knowledge of the natural history of this infection and its therapeutic management.

Design/Methods: We developed an HIV-1/P specific sero-molecular strategy and screened over 29,498 specimen samples. Infectivity and evolution of the gag-30 position, considered as marker of adaptation to human, were explored by successive passages of RBF168 strain onto human PBMCs. Natural history and immuno-virological responses to combined antiretroviral therapy (cART) were analysed based on CD4 counts and plasmatic viral load evolution.

Results: No new infection was detected. Infectivity of RBF168 was found lower, relative to other main HIV groups and the conservative methionine found in the gag-30 position revealed a lack of adaptation to human. The follow-up of the patient during the five-year ART-free period, showed a relative stability of CD4 cell count with a mean of 326 mm^{-3} . Initiation of cART led to rapid RNA undetectability with a significant increase of CD4, reaching 687 mm^{-3} after 8 years.

Conclusions: Our results showed that HIV-1/P strains remain extremely rare and could be less adapted and pathogenic than other HIV strains. These data lead to the hypothesis that HIV-1/P infection could evolve towards, or even already correspond to, a dead-end infection.

Key words: HIV genetic diversity ; group P ; adaptation ; immuno-virological response to cART

INTRODUCTION

Human Immunodeficiency Virus type 1 (HIV-1) is characterized by high genetic diversity, classified into 4 groups - M to P [1, 2]; each group results from a separate cross species transmission to humans of simian immunodeficiency virus (SIV) found in chimpanzees (SIVcpz) or gorillas (SIVgor) [3, 4].

HIV-1 group P (HIV-1/P), the last group identified, is derived from gorillas [5, 6] and represented by only two cases to date. The prototypic strain, RBF168, was reported in a Cameroonian woman living in France [6], while the second strain, U14788, was identified in retrospective serological screening on specimens collected in Cameroon [7].

The scarcity of group P infection may be linked to a relatively recent emergence in humans, since the time of the most recent common ancestor (tMRCA) of HIV-1/P was estimated, based on only two sequences, between the 1950s and 1990s [8]. Another hypothesis is that HIV-1/P has distinct virological properties from the other groups, especially HIV-1/M, leading to a limited spread. It has been shown that residue replacements of methionine or leucine found at the gag-30 position in SIVcpz/SIVgor, by lysine or arginine in the inferred HIV-1 ancestral sequences of group M, N and O strains, represent a host-specific adaptation marker [9, 10]. For group P, the RBF168 strain harbours methionine, supporting a shared property with simian strains, whereas the second strain, U14788, harbours lysine [7].

Concerning *in vivo* features, only limited data from patient RBF168 have been reported [6]. Data on immuno-virological response to combined antiretroviral-treatment (cART) were not available, while these variants are phylogenetically close to HIV-1/O, harbouring natural polymorphism impacting therapeutic management [11].

In order to obtain new insights into HIV-1/P, we screened for new infections, and analysed phenotypic and genotypic properties of the prototypic strain RBF168. In addition, the follow-up of the unique HIV-1/P positive patient monitored so far, gave unique data on the natural history, and on the immuno-virological responses to cART.

METHODS

We first defined and validated specific tools for HIV-1/P detection. We adapted a serological immunoassay, initially designed to discriminate between HIV-1 groups M, N and O, HIV-2 and major SIV lineages [12], by including a group P specific antigen, selected among four candidate synthetic oligopeptides (supplementary methods, <http://links.lww.com/QAD/B242>). To confirm group P reactivity, we developed a specific nested RT-PCR, based on the amplification of a fragment of 1,110 pb in the gp120 region (supplementary methods, <http://links.lww.com/QAD/B242>).

We then screened for new HIV-1/P infections among 1,083 samples from collections, for which previous results using a serological immunoassay without a P specific antigen were inconclusive; they corresponded to:

- 609 serum/plasma samples received by our laboratory for exploration of atypical reactivities at diagnosis time or during the patient's follow-up.
- 405 serum/plasma samples from HIV collections from Cameroon and Central African Republic, constituted in the 90's.
- 69 simian samples

All samples with reactivity (OD) \geq 0.500 were first subjected to the group P specific RT-PCR. Samples not amplified with the specific RT-PCR were subjected to a nested-RT-PCR (Lentivirus PCR) that amplified a 650 pb fragment of *pol* of HIVs and SIVs (supplementary methods,

<http://links.lww.com/QAD/B242>). Prospective screening was also performed on 28,415 samples, diagnosed as HIV positive between March 2013 and June 2015 by the Centre Pasteur of Cameroun (CPC), using all the HIV-1 and HIV-2 antigens of the serological immunoassay, as previously described [13].

We then proceeded to phenotypic and genotypic characterization of the prototypic strain RBF168. The infectivity of the RBF168 strain was analysed after six serial passages in human PBMCs by calculating the TCID₅₀ (tissue culture infective dose 50%) as previously described [14], and compared to those of seven primary isolates representative of HIV genetic diversity (supplementary methods, <http://links.lww.com/QAD/B242>). To determine the residue evolution at gag-30 position during the six passages, we amplified a 493 pb fragment of *gag* using an in-house RT-PCR (supplementary methods, <http://links.lww.com/QAD/B242>). Natural genetic polymorphism of the cART targets : protease (PR), reverse transcriptase (RT), integrase (IN), and gp41, was also determined on viral RNA from three plasma samples collected before treatment initiation (supplementary methods, <http://links.lww.com/QAD/B242>), and compared to that of the second P strain U14788; mutations were subjected to genotypic resistance interpretation using the last version (v27) of the ANRS resistance algorithm (<http://www.hivfrenchresistance.org/>).

Lastly, patient RBF168 was followed up from March 2004 to March 2017; monitoring of CD4 cell count was performed by Beckman Coulter flow cytometer systems (Beckman, Brea, California) and of plasma viral load (pVL) by the RealTime HIV-1 Viral Load assay (Abbott, Des Plaines, IL) [6, 15].

RESULTS

Validation of specific tools led to selection of the candidate peptide CP684 for serological screening, and to definition of the analytical sensitivity of the group P specific RT-PCR as close to 2 Log cp/mL, with a specificity of 100% (supplementary results, <http://links.lww.com/QAD/B242>).

Using peptide CP684, 992 of the 1083 (91.6%) samples from the collections were found negative (median OD at 0.029), including all the simian samples. Seventy-three (6.7%) gave equivocal results ($0.1 \leq \text{OD} < 0.5$), with median OD at 0.146. Eighteen samples corresponding to 14 patients (1.6%) were reactive with $\text{OD} \geq 0.5$ (median at 1.189), six samples being highly reactive from 1.518 to 2.438. Group P specific RT-PCR performed on samples of these 14 patients gave negative results ; the non-specific Lentivirus RT-PCR identified HIV-1/M variants (CRF02_AG, n=4 and CRF11-like, n=1), HIV-1/O (n=6), and one dual HIV-1/M+O (data not shown) ; two samples were not amplified. Finally, no group P strain was found among the 28,415 HIV positive samples diagnosed by the CPC, which were reactive to HIV-1/M (99%), HIV-1/O or HIV-1/M+O (0.9%), HIV-2 (0.08%) infections, and HIV-1/N (1 case).

Infectivity testing of the six successive amplifications showed an extremely low median at 261 TCID₅₀/mL [149; 447], relative to the strains belonging to the other type or groups, especially HIV-1/M with a mean TCID₅₀/mL at 250,625 (n=4), while HIV-2 and HIV-1/O showed TCID₅₀/mL at 1,171 (n=1) and 22,627 (n=2), respectively. Genotypic characterisation of the gag-30 position in each of the six successive amplifications revealed no evolution with conservation of methionine.

Concerning the cART targets, the natural polymorphism of RBF168 was similar between the three sequences before treatment. Based on the ANRS algorithm used to predict genotypic resistance of HIV-1/M viruses, the highest number of mutations was detected in the PR with 11 resistance associated mutations, conferring natural (or possible) resistance to atazanavir, tipranavir and saquinavir (table 1). The presence of T69S, A98G, K103R and the atypical mutation V179E in the RT, had no genotypic impact (table 1). The IN harboured no resistance mutation; but the L44M found in the gp41 led to resistance to enfuvirtide. The genotypic profile of strain U14788 was similar to RBF168, leading to the same interpretation (table 1), except for the IN, with presence of a T97A mutation leading to genotypic resistance to elvitegravir.

Monitoring of patient RBF168 started in March 2004 at HIV diagnosis; she did not receive cART until December 2008, according to the French guidelines at that time. During this period, the mean pVL was at 4.7 Log cp/mL and the mean CD4 count at 326 mm⁻³, ranging from 260 (June 2004) to 430 (December 2005) (Figure 1a). In February 2009, cART was initiated with tenofovir+emtricitabine+lopinavir/ritonavir, but switched after 2 months to abacavir+lamivudine+lopinavir/ritonavir, due to tenofovir adverse effects. This allowed to achieve undetectability (<1.6 Log cp/mL) at M3 (Figure 1b), maintained until the last monitoring in March 2017 (Figure 1b). During cART, the mean CD4 cell count was at 648 mm⁻³, with the last point at 687 mm⁻³ in March 2017.

DISCUSSION

Only two viral sequences and scarce data on HIV-1/P infection were available until now; thus, all new data are needed to improve our knowledge of this group and to assess its potential for transmission and pathogenicity.

We screened for new infections using a sero-molecular strategy for group P specific detection, as previously done for other HIV/SIV variants [12, 13, 16]. After validation of specific tools, we tested a large number of samples from collections and prospective diagnosis. Despite a wide panel of simian samples or samples with HIV atypical reactivities (profiles similar to that of RBF168 at diagnosis), we failed to detect a new HIV-1/P strain. The same is true in older collections from central Africa and in the thousands of HIV positive samples, diagnosed currently in Cameroon. This confirms that HIV-1 group P infection is extremely rare [2, 7].

Reasons for this limited spread are likely multifactorial. In order to better estimate the emergence of this group, we performed evolutionary analysis using the two HIV-1/P sequences previously analysed [8], and three additional RBF168 sequences (supplementary methods and results, <http://links.lww.com/QAD/B242>). This analysis indicates that HIV-1/P strains share a tMRCA dating back to the 1960s. Thus, it would appear that HIV-1/P emerged more recently than strains M and O [8], and possibly around the same time period as group HIV-1/N [17]. Another factor could be specific viral properties; weak counteracting of human cell restricting factors was already demonstrated for tetherin [8]. Focusing on infectivity, our work showed that the serial isolates of RBF168 were consistently and reproducibly less infective, in particular relative to HIV-1/O (the closest phylogenetic group) and HIV-2 (the lowest pathogenic HIV known so far). When searching for residue evolution in gag-30 position, we failed to identify a switch from methionine to arginine (harboured by the second P strain, U14788), or lysine (found in other HIV-1 variants). However, this evolution probably requires further passages. This apparent lack of adaptation of RBF168 to human hosts contrasts with the high replication level *in vivo* (mean pVL at 4.7 Log cp/mL). In this context, it is crucial to screen for other viral determinants of adaptation.

The follow-up of this single patient during the first five-year ART-free period, showed she remained symptom-free, with a relative stability of the CD4 cell count, despite high viral replication; this appears similar to the situation encountered in non-pathogenic SIV infections harbouring a high level of replication in their symptom-free hosts [18]. The singular natural polymorphism of the cART targets (distinct to that of the closest phylogenetic group O), did not prevent determination of an efficient therapy, that led to virological success during an 8-year follow-up, with a significant increase of CD4 of 600 between nadir and zenith; this suggests no difficulty for therapeutic management of this infection.

In conclusion, we have provided new insights into the last described divergent group. We have shown that HIV-1/P strains are extremely rare, suggesting that this variant expanded in a limited manner after its recent emergence in the human population, and that subsequently numbers have declined. Phenotypic characteristics of the prototypic strain, associated with data on the natural course of RBF168 infection, suggest that HIV-1/P could be less adapted and less pathogenic than the other HIV-1 strains, accounting for its lack of success in the human population. These observations have led to the hypothesis that HIV-1/P infection could evolve towards or even already correspond to a dead-end infection.

ACKNOWLEDGEMENTS

EAG and JCP conceived and managed the study. EAG, FDO, ML, VL, PAN performed the experiments. DLR and FF performed computational analyses. PM and FS provided sample collections. EAG, DLR and JCP wrote the paper. All the authors were involved at all stages of manuscript development.

We thank François Cordonnier, Houria Ichou, Claire Gervais, Sylvie Leporcher, Sebastien Delannoy, Fanny Lermachain, of the Department of Infectious Diseases at Louis Mourier Hospital and the team of the Laboratory of Virology, Rouen University Hospital for their technical support. We are also grateful to Nikki Sabourin-Gibbs, Rouen University Hospital, for her help in editing the manuscript.

CONFLICT OF INTEREST AND SOURCE OF FUNDING: This work was supported by *Santé Publique France* and Rouen University Hospital. FF was supported by a BBSRC studentship to DLR.

ACCEPTED

REFERENCES

1. Mourez T, Simon F, Plantier JC. **Non-M variants of human immunodeficiency virus type 1.** *Clin Microbiol Rev* 2013; **26(3)**:448-461.
2. Rodgers MA, Vallari AS, Harris B, Yamaguchi J, Holzmayer V, Forberg K, et al. **Identification of rare HIV-1 Group N, HBV AE, and HTLV-3 strains in rural South Cameroon.** *Virology* 2017; **504**:141-151.
3. Keele BF, Van Heuverswyn F, Li Y, Bailes E, Takehisa J, Santiago ML, et al. **Chimpanzee reservoirs of pandemic and nonpandemic HIV-1.** *Science* 2006; **313(5786)**:523-526.
4. Van Heuverswyn F, Li Y, Bailes E, Neel C, Lafay B, Keele BF, et al. **Genetic diversity and phylogeographic clustering of SIVcpzPtt in wild chimpanzees in Cameroon.** *Virology* 2007; **368(1)**:155-171.
5. D'Arc M, Ayouba A, Esteban A, Learn GH, Boue V, Liegeois F, et al. **Origin of the HIV-1 group O epidemic in western lowland gorillas.** *Proc Natl Acad Sci U S A* 2015; **112(11)**:E1343-1352.
6. Plantier JC, Leoz M, Dickerson JE, De Oliveira F, Cordonnier F, Lemeé V, et al. **A new human immunodeficiency virus derived from gorillas.** *Nat Med* 2009; **15(8)**:871-872.
7. Vallari A, Holzmayer V, Harris B, Yamaguchi J, Ngansop C, Makamche F, et al. **Confirmation of putative HIV-1 group P in Cameroon.** *J Virol* 2011; **85(3)**:1403-1407.
8. Sauter D, Hue S, Petit SJ, Plantier JC, Towers GJ, Kirchhoff F, et al. **HIV-1 Group P is unable to antagonize human tetherin by Vpu, Env or Nef.** *Retrovirology* 2011; **8**:103.
9. Wain LV, Bailes E, Bibollet-Ruche F, Decker JM, Keele BF, Van Heuverswyn F, et al. **Adaptation of HIV-1 to its human host.** *Mol Biol Evol* 2007; **24(8)**:1853-1860.
10. Takehisa J, Kraus MH, Ayouba A, Bailes E, Van Heuverswyn F, Decker JM, et al. **Origin and biology of simian immunodeficiency virus in wild-living western gorillas.** *J Virol* 2009; **83(4)**:1635-1648.
11. Depatureaux A, Charpentier C, Leoz M, Unal G, Damond F, Kfutwah A, et al. **Impact of HIV-1 group O genetic diversity on genotypic resistance interpretation by algorithms designed for HIV-1 group M.** *J Acquir Immune Defic Syndr* 2011; **56(2)**:139-145.
12. Simon F, Souquiere S, Damond F, Kfutwah A, Makuwa M, Leroy E, et al. **Synthetic peptide strategy for the detection of and discrimination among highly divergent primate lentiviruses.** *AIDS Res Hum Retroviruses* 2001; **17(10)**:937-952.
13. Kfutwah A, Lemeé V, Ngono HV, De Oliveira F, Njouom R, Plantier JC. **Field evaluation of the Abbott ARCHITECT HIV Ag/Ab Combo immunoassay.** *J Clin Virol* 2013; **58 Suppl 1**:e70-75.
14. Japour AJ, Mayers DL, Johnson VA, Kuritzkes DR, Beckett LA, Arduino JM, et al. **Standardized peripheral blood mononuclear cell culture assay for determination of drug susceptibilities of clinical human immunodeficiency virus type 1 isolates. The RV-43 Study Group, the AIDS Clinical Trials Group Virology Committee Resistance Working Group.** *Antimicrob Agents Chemother* 1993; **37(5)**:1095-1101.
15. Mourez T, Delaugerre C, Vray M, Lemeé V, Simon F, Plantier JC. **Comparison of the bioMérieux NucliSENS EasyQ HIV-1 v2.0-HIV-1 RNA quantification assay versus Abbott RealTime HIV-1 and Roche Cobas TaqMan HIV-1 v2.0 on current epidemic HIV-1 variants.** *J Clin Virol* 2015; **71**:76-81.

16. De Oliveira F, Mourez T, Vessiere A, Ngoupo PA, Alessandri-Gradt E, Simon F, et al. **Multiple HIV-1/M + HIV-1/O dual infections and new HIV-1/MO inter-group recombinant forms detected in Cameroon.** *Retrovirology* 2017; **14(1)**:1.
17. Wertheim JO, Worobey M. **Dating the age of the SIV lineages that gave rise to HIV-1 and HIV-2.** *PLoS Comput Biol* 2009; **5(5)**:e1000377.
18. Klatt NR, Silvestri G, Hirsch V. **Nonpathogenic simian immunodeficiency virus infections.** *Cold Spring Harb Perspect Med* 2012; **2(1)**:a007153.

ACCEPTED

Table 1. Natural polymorphism of RBF168 and U14788 strains in the Protease, Reverse Transcriptase, Integrase and gp41.

Region	Type of mutation	Mutations at resistance positions*		
		Specific to RBF168	Shared by the two strains	Specific to U14788
PR	RAM	I15V	L10V, V11I, L33V, M36L, Q58E, D60E, I62V, H69R, A71V, L89I	
	Atypical mutation		K20C, L63K,	
	Interpretation of mutations on drug resistance	R: saquinavir	R: atazanavir	I: saquinavir
RT	RAM		T69S, A98G, K103R	
	Atypical mutation		V179E, L210Y	K101Q, E138D
	Interpretation of mutations on drug resistance		No resistance	No resistance
IN	RAM			T97A
	Interpretation of mutations on drug efficacy			R:elvitegravir
gp41	RAM		L44M	
	Interpretation of mutations on drugs resistance		R: enfuvirtide	

RAM: Resistance Associated Mutation, PR: Protease, RT: Reverse Transcriptase IN: Integrase

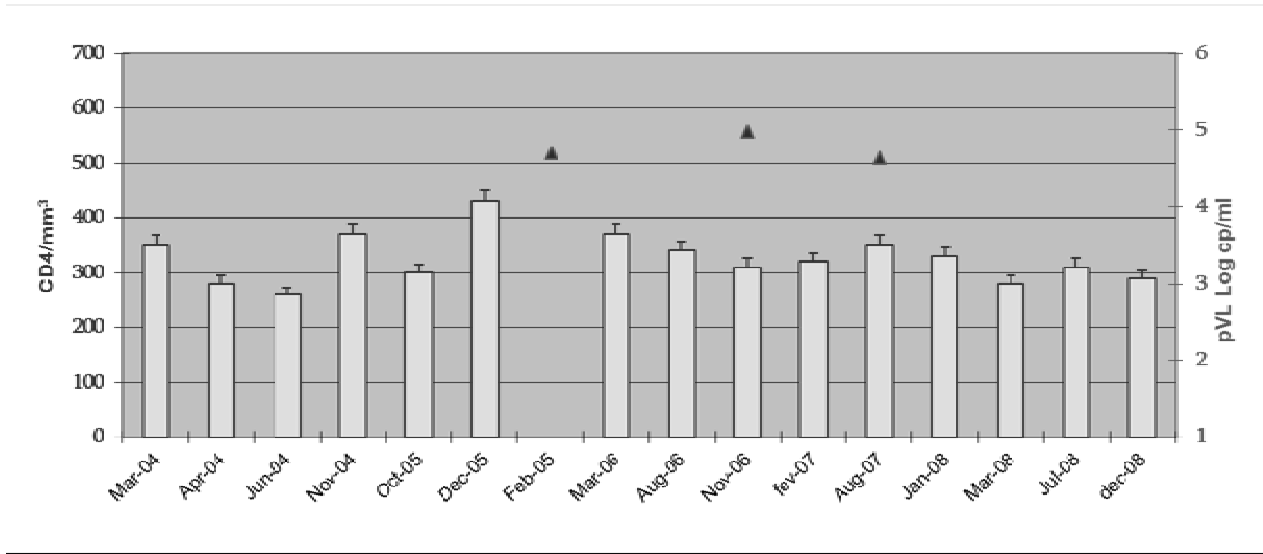
Atypical mutation: mutation not described as associated with resistance, but present at resistance position.

*Mutations found at positions associated with resistance according to the ANRS HIV-1 genotypic drug resistance interpretation algorithm 2017 v27.

I: Intermediate, R:Resistant, drugs not mentioned are interpreted as susceptible.

ACCEPTED

a)



b)

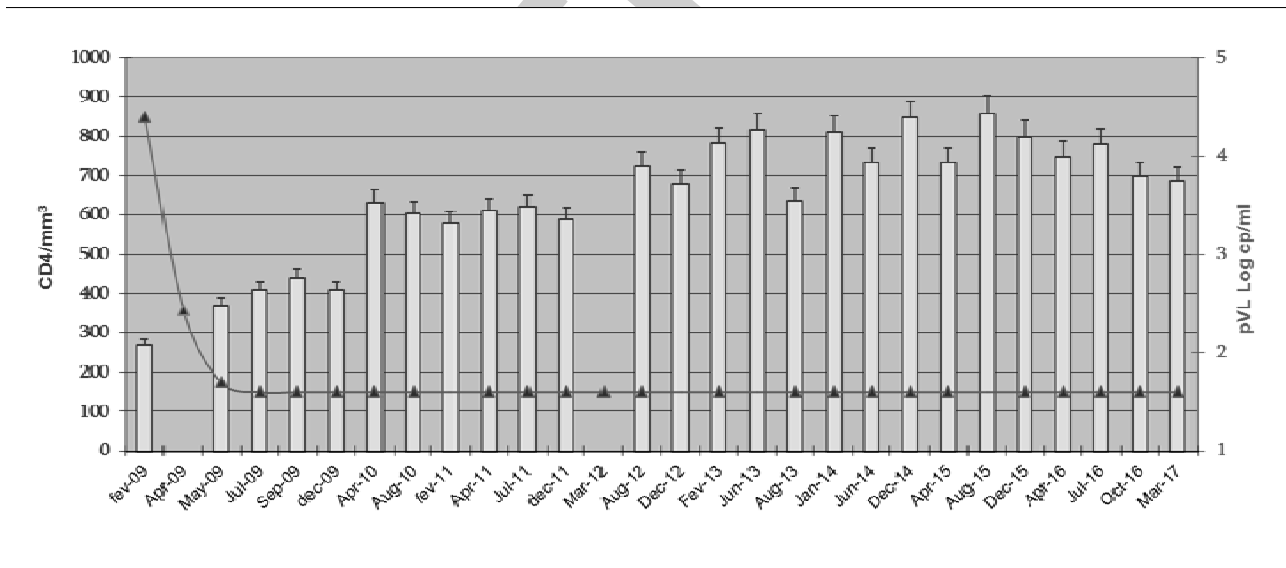


Figure 1. Biological monitoring of patient RBF168. CD4 positive cell count (bar chart) and plasmatic viral load (pVL ▲) are represented before (a) and after (b) treatment initiation. Undetectability is mentioned at 1.6 log cp/ml corresponding to the quantification threshold of the RealTime HIV-1 Viral Load assay.