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Running head: Therapeutic response in a HIV-1+ patient

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Our results indicate that the salvage treatment was successful in this patient despite his background of resistance to almost all NRTIs and NNRTIs.

As for the DRV/r plus RAL combination, Akil Jackson et al. carried out a study which analyzed DRV/r and RAL plasma and intracellular concentrations in HIV-infected individuals during co-administration. In this study, HIV-infected patients on antiretroviral therapy received RAL 400 mg twice daily for 21 days; DRV/r 800/100 mg once daily was added for 14 days and patients were randomized to continue RAL twice daily or to switch to 800 mg once daily, then they all stopped RAL intake and continued DRV/r once daily for 14 days. Drug concentrations in plasma and peripheral blood mononuclear cells were measured. No remarkable interactions between DRV/r and RAL in plasma or cells were seen. Raltegravir intracellular concentrations were assessed for the once-daily 800 mg dose for the first time. (**11**) More recently, once-daily DRV/r

has received approval in treatment-naïve patients and represents an excellent option for first-line therapy. (**12**)

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Notwithstanding the difficult virological presentation, complicated by HBV co-infection, we designed an effective treatment combination that included RAL+DRV/r+TDF with the aid of precise molecular tools. Our patient presented a sustained immunological and virological response overcoming difficulties posed by a complex CXCR4-tropic CRF11-cpx virus.

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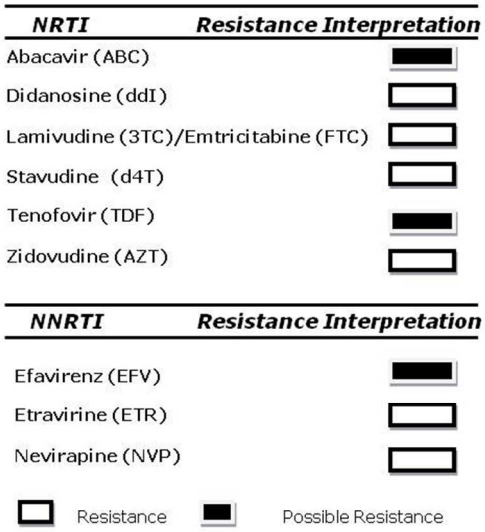

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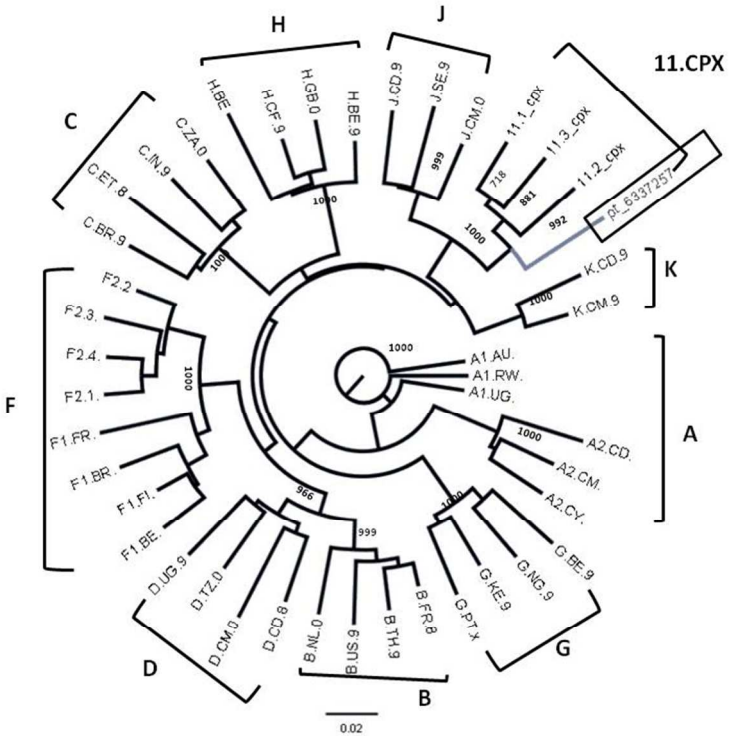

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Fig.1 Profile of Drug Resistance Mutations at Salvage Therapy Initiation.

Fig. 2 Neighbour joining tree of 2010 HIV-1/SIVcpz complete genome and the patient's sequence (pt_6337257) that are marked. Bootstrap support is shown for key nodes. Horizontal branch lengths are drawn to scale with the bar at the bottom indicating 0.02 nucleotide substitution per site.

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Dear Editor,
we submit a revised version of the manuscript entitled "FAVORABLE THERAPEUTIC RESPONSE WITH AN ANTIRETROVIRAL SALVAGE REGIMEN IN A HIV-1 POSITIVE SUBJECT INFECTED WITH A CRF11-CPX VIRUS". We have responded to the queries posed by the reviewer as described in the e-mail of October 28.

1) It is not clear how the authors concluded that the majority of virions in this patient use the CXCR4 coreceptor. Most HIV-1 isolates use the CCR5 coreceptor, and there is some correlation between V3 sequence and coreceptor use, but the sequence with accession number KF381391 has so many "R", "Y" and other ambiguity codes in it, that it is not possible for the Geno2Pheno or similar programs to make an accurate prediction of coreceptor use.

To determine the virus tropism we used a FPR significance level of 10%, according to the Recommendations from the European Consensus Group on clinical management of HIV-1 tropism testing (LPR Vandekerckhove et al.; *European guidelines on the clinical management of HIV-1 tropism testing*; The Lancet Infectious Diseases, Volume 11, Issue 5, Pages 394 - 407, May 2011), while the reviewer used a FPR cut-off of 1%: for this reason we concluded that patient harbours a CXCR4-tropic virus. Moreover, the reviewer changed the sequence with accession number KF381391: all ambiguity codes were substituted with N code (as reported in the geno2pheno report attached by the reviewers). These changes resulted in several aminoacids substitutions, that affected and worsened the geno2pheno interpretation. Ambiguity are usually present in HIV sequences (especially in the gp120 hypervariable domains), owing to the high heterogeneity and variability of viral population. In our opinion, we would not modify the referenced sequence, thus introducing aminoacids changes not present in our original sequence and indeed in the sample (i.e.: in our sequence the 11th codon is AGY, coding for Ser for both AGT and AGC; in the reviewers sequence at the same position it is reported a AGN codon, that could encode Ser for AGT and AGC and Arg for AGA and AGG).

2) The drug resistance profile reported for this patient does not match the results I get using the Stanford drug resistance database
(http://sierra2.stanford.edu/sierra/servlet/JSierra?action=sequenceInput):

KF381391 result:
NRTI Resistance Mutations: M41LM, A62AV, T69N, K70KR, V75GISV, M184V, T215FIST
NNRTI Resistance Mutations: Y181I
Other Mutations: K49KR, K122E, D123N, S162CS, K173T, Q174K, D177E, T200EQ, I202V, Q207E, R211K, E224D, T240R, P243A, V245Q
Nucleoside RTI

lamivudine (3TC) High-level resistance
abacavir (ABC) High-level resistance
zidovudine (AZT) High-level resistance
stavudine (D4T) High-level resistance
didanosine (DDI) High-level resistance
emtricitabine (FTC) High-level resistance
tenofovir (TDF) Intermediate resistance

Non-Nucleoside RTI
efavirenz (EFV) Intermediate resistance
etravirine (ETR) High-level resistance
nevirapine (NVP) High-level resistance
rilpivirine (RPV) High-level resistance

Our drug resistance profile reported for this patient was carried out in May 2013, with Stanford version 6.2.0 that has been active from 29.05.2012 up to 05.06.2013. After this version, there was the version 6.3.0 up to 19.09.2013; and then until today the version 6.3.1 (that I think you have used). This explains the slightly different profiles that we got.

3) Please carefully check for typos in the paper. For example, please change "...41-year-old men, was evaluated from 2007 to 2010 in Bangui, Central African Repubblic, where he performed..." to "...41-year-old man, was evaluated from 2007 to 2010 in Bangui, Central African Republic, where he was tested with..."
We agree with the reviewer.

4) It is very highly unusual for a HIV-seronegative man (the paper says he repeatedly tested negative, with just one positive test: ELISA only, not western blot?? in 2007?) to harbor an X4 drug resistant virus. Was the 2007 ELISA very strongly positive? What was the reason for this man being repeatedly tested? What is his risk for infection?

The transmission of resistant variants is well documented: for this reason all guidelines suggest to test patients before starting therapy to identify drug resistance transmission. On CXCR4 variants transmission there are not confirmed data yet, but, in contrast with previous studies, recent reports suggest that CXCR4 viruses are likely to be transmitted as well (reviewed in: C. Hedskog et al.; *Transmission of the X4 Phenotype of HIV-1: Is There Evidence Against the ''Random Transmission'' Hypothesis?*; JID 2012, 205:163-165).

In 2007, our patient was tested only for HIV-1 Ab Elisa in Bangui, Central African Republic, whereby we have only the qualitative data (positive / negative), that is positive according to the Abbott Elisa test. We do not have a clear explanation for the repetition of tests, we can only say that both tests resulted negative in 2009 and 2010. His risk factors for HIV-1 infection were blood transfusions and unprotected heterosexual intercourses.

5) In the paragraph pointed out in comment 3 above, the paper says the patient hod only one positive test, in 2007, never another. But on the next page the paper says that the patient has a positive ELISA and western blot in 2012. Combining the statements into one paragraph explaining that the patient had negative results in Bangui but positive tests in Milan might reduce confusion. We agree with the reviewer.

6) The authors should use the "Subtype Reference Alignment" with nonrecombinants plus CRF11_cpx sequences. The paper states that the complete genome alignment was used, but there are hundreds of complete genomes in the LANL HIV genome alignment, and a very few have been carefully chosen for "references".
http://www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html
The phylogenetic tree is acceptable as it is, this paper is not primarily about the phylogeny, so just clarifying that the subtype reference alignment was used, should be sufficient. We agree with the reviewer.

Best wishes,
Pamela Tau and Stefano Rusconi