17th Conference on Retroviruses and Opportunistic Infections. 2010 February 16-19, San Francisco CA USA.

Poster 281

No evolution of HIV-1 Total DNA and 2-LTR Circles After 48 Weeks of Raltegravir-Containing Therapy in Patients With Controlled Viremia: a Sub-study of Randomized EASIER-ANRS 138 Trial

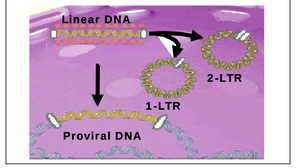
C Delaugerre[1], I Charreau[2], J Braun[2], ML Néré[1], N de Castro[3], P Yeni[4], J Ghosn[5], F Simon[1], JP Aboulker[2], JM Molina[3] and the ANRS 138 study group.

1-Virology Dpt, Saint-Louis Hospital-APHP, Paris, 2-INSERM SC 10, Villejuif, 3-Infectious Diseases Dpt, Saint-Louis Hospital-APHP, Paris, 4-Infectious Diseases Dpt, Bichat-Claude Bernard Hospital-APHP, Paris, 5-Internal Medicine and Infectious Diseases Dpt, Kremlin Bicêtre Hôpital-APHP, Kremlin Bicêtre, FRANCE

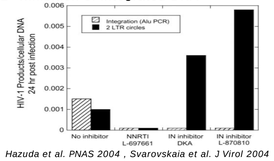
constance.delaugerre@sls.aphp.fr

HIV DNA Forms

HIV-1 Retrotranscription leads to : linear DNA + circular DNA (1- or 2-LTR) + integrated DNA
= **HIV-1 Total DNA**



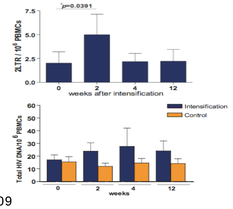
Integration inhibition by integrase inhibitors (*in vitro*)
1- Increase of 2-LTR circles
2- Inhibition of integrated DNA



Hazuda et al. PNAS 2004 , Svarovskaia et al. J Virol 2004

Integrase inhibitors effect : *in vivo*

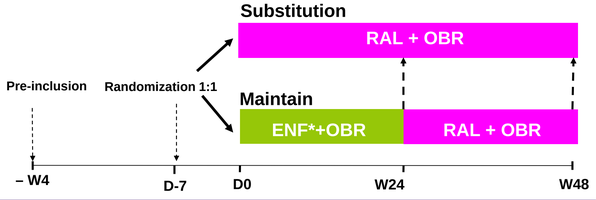
- Intensification with raltegravir (RAL) of patients controlled with stable HAART regimen
  - ➢ Transient and significant increase in episomal HIV-1 DNA at W2 in patients with RAL intensification compared to baseline
  - ➢ HIV total (and proviral DNA) remain stable during intensification compared to control group (along week 12)



Buzon et al. CROI 2009

EASIER Study Design

a non-inferiority randomized multicentric study

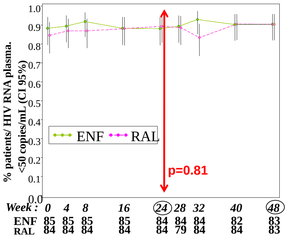


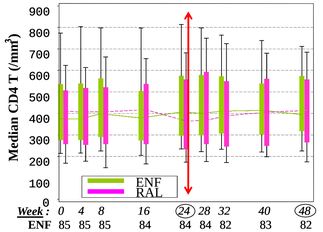
Inclusion criteria:
- ✓ Highly treatment-experienced HIV-1 infected pts
- ✓ With a 3 months unchanged optimized background regimen (OBR) + ENF*
- ✓ Plasma HIV-1 RNA levels <400 cp/mL for at least 3 months

*ENF=enfuvirtide

de Castro et al. CID 2009

Virological and immunological W48 response (N=169)





de Castro et al. IAS 2009

Objective

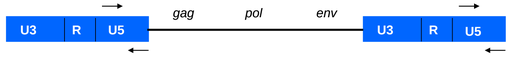
Our objective was to analyze the effect of RAL on the evolution of HIV-1 total DNA and 2-LTR forms in patients included in the ANRS 138 EASIER study

Patients and Methods

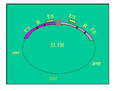
- **Patients**
  - The 30 first patients were selected in both arm (ENF and RAL)
- **Methods**
  - Viral DNA was extracted from whole blood (MagnaPure, Roche) at D0 and at week 24, and also at week 48 for patients in RAL arm
  - Number of cells was determined by the Albumine gene quantification, and the number of PBMC was estimated according to whole blood count
  - Quantification were performed in triplicates

Real-time PCR-based quantification

- HIV-1 total DNA was determined with primers that anneal in the LTR region (Avettand-Fènoël 2009)



- HIV-1 2LTR were measured with primers that span the 2LTR circle junction (Sharkey 2000, Gueudin 2008)



Patients baseline characteristics

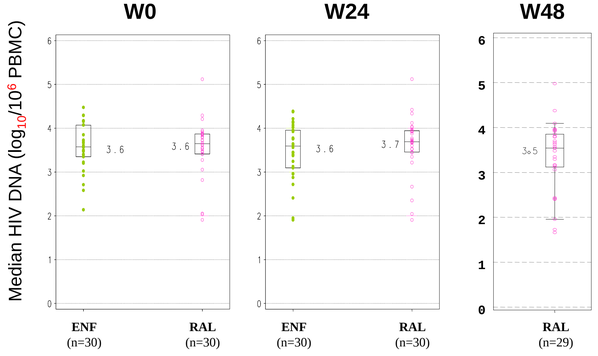
	Maintain ENF n=30	Substitution RAL n=30	Total n=60
Male, no. (%)	25 (83)	27 (90)	52 (87)
CDC Stade C, no. (%)	18 (60)	18 (60)	36 (60)
Nadir CD4 /mm³, median [IQR]	64 [11; 118]	25 [10; 104]	37 [10; 118]
Inclusion CD4 /mm³, median [IQR]	375 [271; 535]	425 [269; 552]	400 [270; 543]
HIV-1 RNA inclusion <50 c/mL, no. (%)	26 (87)	26 (87)	52 (87)
Duration ARV (years), median [IQR]	12.6 [11.1; 14.7]	14.6 [12.1; 15.5]	14.0 [11.7; 15.2]
Duration ENF (years), median [IQR]	2.2 [1.5 ; 3.2]	2.6 [1.9 ; 3.4]	2.5 [1.7 ; 3.3]
Number of drugs OBT (ENF included), median [IQR]	4 [4; 5]	4 [3; 5]	4 [3; 5]
≥ 1 PI/r	30 (100)	29 (97)	59 (98)

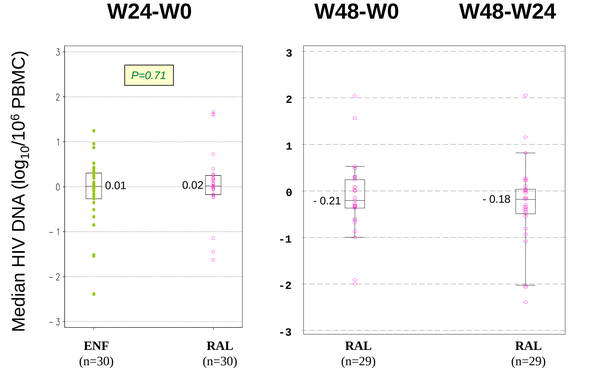
Baseline characteristics for the 60 patients are similar to the overall trial population (4)

Virological and immunological outcome (n=60)

	Maintain ENF	Substitution RAL	Total
W24	n=30	n=30	
CD4 /mm³, median [IQR]	416 [282; 563]	422 [234; 582]	416 [282; 570]
HIV-1 RNA <50 c/mL no. (%)	28 (93)	25 (83)	53 (88)
W48	n=30	n=29	
CD4 /mm³, median [IQR]	384 [306; 573]	446 [288; 587]	403 [288; 587]
HIV-1 RNA <50 c/mL no. (%)	25 (83)	27 (93)	52 (88)

HIV-1 Total DNA evolution





References

1. **Avettand-Fènoël V.,** et al., *J Med Virol*. 2009 Feb;81(2):217-23
2. **Burgard M**., et al., AIDS 2009 vol 23(16):2165-71.
3. **Buzon MJ**., et al., CROI 2009 (423a)
4. **De Castro N**., et al., CID 2009 Oct 49(8):1259-67 and IAS 2009 (MOPEB066)
5. **Gueudin M**., et al., *AIDS*. 2008 Nov 30;22(18):2543-5
6. **Hazuda DJ**., et al., *PNAS* 2004 vol 3;101(31):11233-8.
7. **Kostrikis LG**., et al., J Virol. 2002 Oct;76(20):10099-108
8. **Sharkey M**., et al.,. Nat Med. 2000 Jan;6(1):76-81.
9. **Svarovskaia ES**., et al., *J Virol*. 2004; Apr;78(7):3210-22
10. **Viard JP.,** et al., AIDS. 2004 Jan 2;18(1):45-9.

HIV-1 2LTR circle quantification

2LTR detectable* at W0

patients	arm	W0 copies/10⁶ PBMC	W24 copies/10⁶ PBMC
#4	ENF	210	<20
#7	ENF	22	<20
#17	RAL	36	<20
#3	RAL	125	<20
#4	RAL	130	<20
#2	RAL	53	<20

*Threshold >20 copies/10⁶ PBMC

2LTR detectable* at W24

patients	arm	W0 copies/10⁶ PBMC	W24 copies/10⁶ PBMC
#1	ENF	<20	63
#8	ENF	<20	39
#10	RAL	<20	20

➢ For all these patients, plasma HIV-1 RNA suppression below 50 copies/ml was sustained between W0 and W24

➢ No 2LTR circles were detected at W48 in the 29 patients under RAL-containing regimen

Discussion

- Median HIV-1 total DNA at D0 (3.6 log/10⁶ PBMC) was high in these heavily pretreated patients compared to values in patients at an earlier stage of HIV infection (2.7 $\log_{10}$/10⁶ PBMC, J Virol Kostrikis 2002). This higher level may be related to the low nadir of CD4 count (37 cells) (Burgard AIDS 2009).
- In this randomized trial, we found no difference in HIV-1 total DNA evolution after 24 weeks under raltegravir compared to enfuvirtide in patients with a sustained plasma HIV-1 RNA below 400 copies/ml.
- No significant individual HIV-1 DNA viral load difference was observed after 48 weeks of RAL suggesting a stable HIV-1 DNA amount in successful treated patients as previously described with standard HAART (Viard AIDS 2004).
- HIV-1 2LTR DNA does not increase at 24 or 48 weeks on raltegravir. This result may be related to the sample timing, the 2-LTR increase being described early after the raltegravir initiation *(Buzon et al. CROI 2009)*.
- However, HIV-1 2LTR DNA detection was observed in 9 patients (6 at W0 and 3 at W24) despite controlled viremia below 50 copies/mL suggesting an ongoing replication.
- HIV-1 2LTR DNA circles quantification could not be proposed as a sensitive surrogate marker of antiviral activity of raltegravir in controlled patients except in the early steps of the inhibition of integration (ie. at week 2).

Conclusion

In patients virologically controlled with potent regimens, including integrase inhibitor, the evolution of the viral reservoir illustrated by the HIV-1 DNA level is stable and non-dynamic within the 48-week short-term of follow up.

Aknowledgements

The Patients

The EASIER ANRS 138 team and

Investigators

BASTIDES Frédéric	Tours
BECK-WIRTH Geneviève	Mulhouse
BOUE François	Clamart
CHENNEBAULT Jean-Marie	Angers
COTTE Laurent	Lyon
DE TRUCHIS Pierre	Garches
DEBAB Yasmine	Rouen
DUPON Michel	Bordeaux
ELHARRAR Brigitte	Créteil
GERARD Laurence	Paris
GHOSN Jade	Bicêtre
GIL Helder	Besançon
GILQUIN Jacques	Paris
HOEN Bruno	Besançon
JEANBLANC François	Lyon
KATLAMA Christine	Paris
LAFEUILLADE Alain	Toulon
LASCOUX-COMBE Caroline	Paris
LEVY Yves	Créteil

Investigators

MARCHOU Bruno	Toulouse
MAY Thierry	Nancy
MEYNARD Jean-Luc	Paris
MOLINA Jean-Michel	Paris
MORLAT Philippe	Bordeaux
PIROTH Lionel	Dijon
POIZOT-MARTIN Isabelle	Marseille
RAFFI François	Nantes
RAGNAUD Jean-Marie	Bordeaux
REYNES Jacques	Montpellier
SALMON-CERRON Dominique	Paris
SIMON Anne	Paris
SLAMA Laurence	Paris
VERDON Renaud	Caen
VIARD Jean-Paul	Paris
WEISS Laurence	Paris
YAZDANPANAH Yazdan	Tourcoing
YENI Patrick	Paris

Merck Sharp & Dohme-Chibret Laboratories, Paris, France, graciously provided raltegravir for this trial