PC-9
#300

21st CROI
Boston
March 3-6, 2014

Effect of Raltegravir Intensification in the Cytokine Profile of Treated HIV+ Individuals

Marta Massanella[1], Dan Ouchi[2], Josep M Llibre[3], Silvia Marfil[2], Maria C Puertas[2], Jordi Puig[3], Eugènia Negredo[3,4], Douglas D Richman[1], Javier Martínez-Picado[2,4,5], Bonaventura Clotet[2,3,4], Julià Blanco[2,4] and the Discor-ral and IntegRal collaborative groups.

[1]University of California San Diego, La Jolla, CA, US, [2]Research AIDS Institute - IrsiCaixa-, Institut de Recerca en Ciències de la Salut Germans Trias i Pujol (IGTP), Badalona, Spain. [3]Lluita contra la SIDA foundation, Hospital Universitari Germans Trias i Pujol, Badalona, Spain. [4]Universitat de Vic, Spain, [5]ICREA, Spain

IrsiCaixa
Institut de Recerca de la Sida

Marta Massanella
e-mail: mmassanellaluna@ucsd.edu
Julià Blanco,
e-mail: jblanco@irsicaixa.es

BACKGROUND

► Antiretroviral therapy (ART) rapidly suppresses viral replication and increases in most of the cases CD4 T-cell counts, but only partly reduces T-cell activation, cell death and soluble inflammatory markers.[1,2]

► Increased levels of inflammatory markers in ART-treated individuals have been associated with several clinical outcomes, such as death, cardiovascular risk, AIDS defining events and non-AIDS defining events.[3]

► Although the reasons for this incomplete effect of cART in immune restoration are still elusive, factors could include an incomplete CD4 T-cell reconstitution and HIV persistence (HIV antigens coming from long-lived reservoirs or from low level HIV replication in sanctuary tissues).[2,4]

► Long term (48 weeks) intensification of cART with Raltegravir showed evidence for an effective blockade of residual viral replicaiton and reduced CD8 T-cell activation in the Discor-ral[5,6] and IntegRal studies[7-9].

OBJECTIVE

To evaluate the effect of Raltegravir intensification in several soluble plasma markers in the Discor-ral and IntegRal studies

METHODS

► IntegRal[7-9] and Discor-ral[5,6] studies: inclusion criteria were >18 years, stable ART composed of 2 NRTIs + etiher 1PI or 1 NNRTI , HIV VL < 50 copies/mL at baseline and during at least the previous year for the IntegRal or two years in the Discor-Ral study (4 determinations). **CD4 T-cell counts:** no restriction was defined for the IntegRal Study, while <350 cells/µl during the previous two years for the Discor-Ral study. In both studies, subjects were randomly assigned 2:1 to add Raltegravir (400 mg twice daily) or continue their cART regimen.

► Samples: Plasma samples were obtained from EDTA treated blood, aliquoted and stored at -80 ºC at different time points (Figure 1). Samples from the IntegRal study cover the full intensification period (48 weeks)[8] and an additional 12-week period after Raltegravir discontinuation[9]. Samples from the Discor-Ral study include longer intensification periods (up to 60 weeks) but lack additional samples after Raltegravir discontinuation.

► Quantification of soluble markers: Frozen plasma were assayed using a Bio-Plex Pro Human Cytokine panel (dilution ¼, BioRad),and inlcuded: MIP-1β, IP-10, TRAIL, TNF-α, IFN-γ, IFN-α, G-CSF, GM-CSF, MCP-1, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 IL-10, IL-12, IL-13, IL-15 and IL-17. For the determination of soluble VCAM-1 (sVCAM-1) and soluble ICAM-1 (sICAM-1) plasma samples were diluted 1/200 and tested using a BioPlex pro human cytokine 2-plex also from BioRad. Plasma levels of D-dimer were analyzed using the D-dimer HS 500 quantitative detection kit . Plasma levels of soluble CD14 (sCD14) were analyzed using a commercial ELISA kit.

► Statistical Analysis: We used the Mann-Whitney U test to compare medians between different arms or different studies and the signed-rank test (paired test) to compare longitudinal differences within groups (baseline-week 48 and week 48-week 60).The relationship between soluble plasma markers and different co-variables was examined using the nonparametric Spearman test. Regression coefficients and p-values for each cytokine are reported from multivariate linear regression model: cytokine = intercept + HCV status + 2LTR detection + age + PI/NNRTI based cART + nadir + CD4 counts + gender. Specifically, the final multivariate model was derived using a bootstrapped AIC based stepwise selection method. Moreover, the study of longitudinal changes within groups and the mean change comparison between arms (slope coefficient), from baseline to week 48, were evaluated using linear mixed models: cytokine = intercept + group + week + group*week. A statistically significant interaction term (group*week) identifies significantly different slopes among treatment groups. All analyses were done using the R package (v 3.0.2).

RESULTS

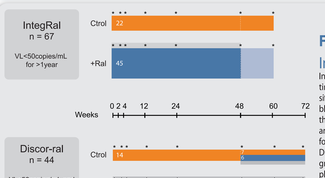


Figure 1. Schematic representation of IntegRal and Discor-Ral studies.
In both studies, enrolled individuals were randomized 1:2 to continue with previous cART (Ctrl, control groups in orange) or intensify their therapy with Raltegravir (+Ral, intensified groups in blue). The extent of Raltegravir intensification was 48 weeks for the IntegRal study and 72 weeks for the Discor-Ral study (grey areas).The follow up of the IntegRal study was 60 weeks, allowing for the analysis of the effect of Raltegravir discontinuation. In the Discor-Ral study six patients from the control arm started Raltegravir for 24 weeks (blue area). In all groups, asterisks denote sampling times.

Table 1. Patient characteristics

	IntegRal			Discor-Ral			
	Control n=22	Intensified n=45	*p*-value between groups[a]	Control n=14	Intensified n=30	*p*-value between groups[a]	***p*-value between Studies[b]**
Age, years, Median [IQR]	45 [37-50]	44 [41-49]	0.569	48 [42-51]	45 [44-53]	0.221	**0.007**
Females, n (%)	6 (27.3)	6 (9.0)	*0.187*	3 (13.3)	4 (14.3)	*0.932*	*1*
Hepatitis C co-infection, n (%)	5 (22.7)	13 (28.1)	*0.81*	7 (50.0)	6 (20.0)	***0.005***	*0.83*
ART at Baseline PI-based regimen, n (%)	8 (36.4)	14 (31.1)	*0.878*	8 (57.1)	18 (60.0)	*0.878*	***0.01***
Time from HIV-1 infection diagnosis years, Median [IQR]	10.4 [3.9-14.7]	11.4 [7.5-17]	*0.795*	14,6 [4.8-19.9]	14,3 [7.3-20.9]	*0.678*	***0.02****
Time with suppressive (VL<50 copies/mL) ART years, Median [IQR]	4.9 [2.7-7.9]	6.6 [2.5-7.3]	*0.311*	5.6 [3.5-9.7]	5.8 [3.5-8.5]	*0.868*	*0.07*
CD4 T cell at baseline, (cell/µl),Median [IQR]	503 [371-600]	530 [434-678]	*0.333*	242 [188-292]	253 [208-301]	*0.668*	***<0.0001***
Nadir CD4 T-cell count, (cell/µl) Median [IQR]	84 [41-254]	251 [85-405]	*0.06*	67 [14-108]	90 [24-117]	*0.435*	***<0.0001***

PI, protease inhibitors; ART, antiretroviral treatment; VL, plasma viral load (HIV-1 RNA copies /ml);
[a] *p*-value between groups: Mann-Whitney U test, [b] *p*-value between studies: : Mann-Whitney U test

Baseline characteristics of immunoconcordants and immunodiscordants

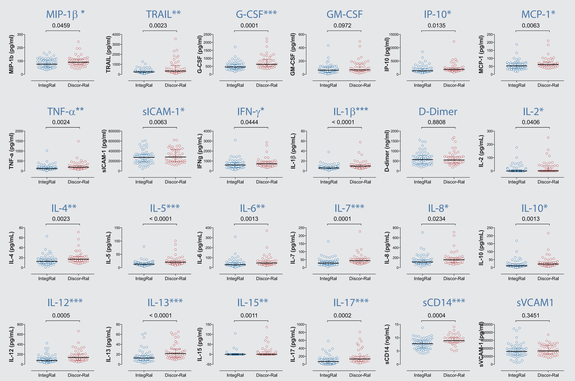


Figure 2. Baseline plasma levels of soluble inflammatory markers.
The levels of the indicated soluble markers were assessed prior to Raltegravir intensification in patients recruited in the IntegRal (n = 67, blue symbols) or the Dicor-Ral (n = 44, red symbols) studies. Control and intensified individuals were grouped for this baseline analysis.
Asterisks next to the soluble marker denote significant *p*-values: * 0.05 < *p*-value < 0.005, ** 0.005 < *p*-value < 0.0005, *** *p*-value < 0.0005

Baseline levels of soluble plasma markers

	Univariate Model						Multivariate Model					
	HCV	2-LTR	PI	Age	Nadir	Male	CD4	HCV	2-LTR	PI	Age	Nadir	Male	CD4
MIP-1β	0.4016	0.1513	0.0098 Δ	0.6137	0.7164	0.3934	0.0788 ∇	--	0.0460 ∇	0.0128 Δ	--	--	--	--
IP-10	0.0000 Δ	0.2303	0.0222 Δ	0.1690	0.0098 ∇	0.1591	0.0000 ∇	0.0001 Δ	0.1370	0.1264	--	--	--	0.0311 ∇
TRAIL	0.7541	0.2514	0.0447 Δ	0.5208	0.3057	0.0238 ∇	0.0033 ∇	--	0.0536 ∇	0.0248 Δ	--	--	--	0.0389 ∇
TNF-α	0.9343	0.7240	0.0103 Δ	0.7321	0.1598	0.0381 ∇	0.0964 ∇	--	0.1401	0.0474 Δ	--	--	--	--
IFN-γ	0.9842	0.8915	0.0135 Δ	0.1529	0.3864	0.0523 ∇	0.3884	--	--	0.1241	0.0433 ∇	0.0488 ∇	--	--
G-CSF	0.8303	0.8504	0.0003 Δ	0.1716	0.0906 ∇	0.0348 ∇	0.0274 ∇	--	--	0.0364 Δ	0.1067	0.0145 ∇	--	--
GM-CSF	0.7466	0.7462	0.1471	0.1542	0.8502	0.1400	0.2504	--	--	--	0.0686 ∇	--	--	0.0625 ∇
MCP-1	0.8743	0.0448 ∇	0.0667 Δ	0.9838	0.7633	0.5321	0.3443	--	0.0271 ∇	0.0770 Δ	--	--	--	--
IL-1β	0.5009	0.3147	0.0617 Δ	0.7870	0.0224 ∇	0.0355 ∇	0.0580 ∇	--	0.1185	0.0309 Δ	--	--	--	0.0559 ∇
IL-4	0.8769	0.7060	0.0047 Δ	0.2613	0.3098	0.0281 ∇	0.1782	--	0.1271	0.0753 Δ	0.0783 ∇	--	--	0.0606 ∇
IL-5	0.7119	0.8630	0.0019 Δ	0.5655	0.0371 ∇	0.0915 ∇	0.0091 ∇	--	--	0.0886 Δ	0.1632	--	--	0.0186 ∇
IL-6	0.6826	0.4846	0.0084 Δ	0.6661	0.2215	0.0623 ∇	0.0364 ∇	--	--	--	0.1074	--	--	0.0823 ∇
IL-7	0.9029	0.7090	0.0041 Δ	0.5879	0.0549 ∇	0.1044	0.0071 ∇	--	0.1612	0.0912 Δ	0.1063	--	--	0.0152 ∇
IL-8	0.8458	0.9137	0.0105 Δ	0.4047	0.8987	0.4474 ∇	0.3776	--	0.1299	0.0732 Δ	0.0955 ∇	--	--	--
IL-10	0.8164	0.8789	0.0001 Δ	0.4217	0.0891 ∇	0.1180	0.0081 ∇	--	--	--	0.0720 ∇	--	--	0.0517 ∇
IL-12	0.8925	0.7300	0.0097 Δ	0.5236	0.1026	0.0318 ∇	0.0122 ∇	--	--	--	--	--	--	0.0071 ∇
IL-13	0.9055	0.6882	0.0037 Δ	0.3451	0.0233 ∇	0.0590 ∇	0.0016 ∇	--	--	0.0880 Δ	--	--	0.1202	0.0348 ∇
IL-17	0.7191	0.4331	0.0312 Δ	0.3579	0.0934 ∇	0.0333 ∇	0.0270 ∇	--	--	--	--	0.0194 ∇	0.1065	--
sICAM-1	0.0000 Δ	0.0279 ∇	0.9684	0.6373	0.7401	0.1207	0.0772 ∇	0.0004 Δ	0.0279 ∇	--	--	--	--	--
sVCAM-1	0.0000 Δ	0.0251 ∇	0.4512	0.1260	0.1179	0.4575	0.0084 ∇	0.0003 Δ	0.0447 ∇	--	--	--	--	0.0518 ∇
sCD14	0.3429	0.5479	0.3788	0.0000 Δ	0.0038 ∇	0.1671	0.0094 ∇	--	0.0198 Δ	--	0.0004 Δ	0.0272 ∇	--	--
D-dimer	0.8466	0.3177	0.8514	0.0757 Δ	0.8113	0.4947	0.9536	--	0.1127	--	--	--	--	--

Figure 3. Analysis of baseline levels of soluble plasma marker.
Heat map of the association of baseline levels of the different soluble plasma markers analyzed with the following parameters: HCV co-infection (HCV column), presence of 2-LTR during the study period (2-LTR column), the presence of PIs on the cART regimen (PI column, PI=1, NNRTI=0), age, Nadir CD4 T-cell counts (Nadir column), gender (Gender column, male=1, female=0) and CD4 T-cell counts at study baseline (CD4 columns). Left part shows results from a univariate analysis (spearman test), while right side data correspond to a multivariate analysis carried out using the AIC-based stepwise procedure. In all cases, color codes are as follow: no color means p-value>0.1, yellow 0.1<p-value<0.05; orange 0.05<p-value<0.01 and red p-value<0.01. Upward arrowheads (Δ) indicate positive correlations while downward (∇) arrowheads indicate negative correlations.

Effect of raltegravir intensification.

	Comparison baseline - Week 48						Linear Mixed Model		
	IntegRal		Discor-Ral		Both		Both		
	Ctrol	+Ral	Ctrol	+Ral	Ctrol	+Ral	Ctrol Slope	+Ral Slope	Ctrol vs +Ral
MIP-1b	0.7605	0.6811	0.1937	0.1783	0.2425	0.5791	0.19	0.98	0.28
IP-10	0.0147 Δ	0.3001	0.0580 ∇	0.0258 ∇	0.0022 ∇	0.0015 ∇	0.08 ∇	0.01 ∇	0.81
TRAIL	0.3942	0.0163 ∇	0.0758 ∇	0.4461	0.4866	0.0366 ∇	0.18	0.62	0.17
TNF-a	0.4859	0.0582 ∇	0.0676 ∇	0.1213	0.3846	0.7370	0.11	0.36	0.42
IFN-g	0.4331	0.2176	0.0785 ∇	0.0011 ∇	0.0530 ∇	0.0005 ∇	0.00 ∇	0.00 ∇	0.96
G-CSF	0.1387	0.0040 ∇	0.0001 ∇	0.0008 ∇	0.0942 ∇	0.3359	0.06 ∇	0.01 ∇	0.97
GM-CSF	0.4488	0.7613	0.1189	0.0937 ∇	0.0762 ∇	0.2526	0.01 ∇	0.01 ∇	0.45
MCP-1	0.6632	0.1921	0.2166	0.5763	0.5068	0.5485	0.16	0.29	0.57
IL-1b	0.9826	0.6169	0.1937	0.0329 ∇	0.3126	0.2821	0.02 ∇	0.05 ∇	0.42
IL-4	0.3380	0.3170	0.0906 ∇	0.0011 ∇	0.5189	0.0655 ∇	0.13	0.00 ∇	0.70
IL-5	0.9306	0.9573	0.1937	0.0208 ∇	0.2579	0.0774 ∇	0.01 ∇	0.00 ∇	0.89
IL-6	0.2763	0.0233 ∇	0.1353	0.0797 ∇	0.6469	0.7143	0.17	0.39	0.52
IL-7	0.4080	0.4212	0.1353	0.0116 ∇	0.4716	0.2889	0.03 ∇	0.03 ∇	0.60
IL-8	0.3604	0.4389	0.0245 ∇	0.0007 ∇	0.2823	0.0730 ∇	0.03 ∇	0.02 ∇	0.64
IL-10	0.7049	0.2659	0.0494 ∇	0.0842 ∇	0.1925	0.8526	0.02 ∇	0.08 ∇	0.33
IL-12	0.1770	0.0799 ∇	0.0017 ∇	0.0089 ∇	0.1752	0.5385	0.26	0.17	0.89
IL-13	0.2959	0.8582	0.1189	0.0091 ∇	0.4489	0.0549 ∇	0.94	0.14	0.37
IL-17	0.8469	1.0000	0.0625 ∇	0.1257	0.0421 ∇	0.3003	0.11	0.19	0.58
sICAM-1	0.2397	0.6169	0.0009 Δ	0.0000 Δ	0.0012 Δ	0.0003 Δ	0.01 Δ	0.00 Δ	0.15
sVCAM-1	1.0000	0.9288	0.0040 ∇	0.0077 ∇	0.1141	0.0755 ∇	0.22	0.61	0.49
sCD14	0.4445	0.0445 Δ	0.5625	0.5421	0.3072	0.2301	0.39	0.70	0.63
D-dimer	0.8617	0.0379 ∇	0.0855 ∇	0.2228	0.8224	0.0156 ∇	0.96	0.13	0.36

Figure 4. Longitudinal analysis of soluble plasma markers
Left part shows the heat map of the *p*-values obtained by comparing the levels of plasma soluble markers at baseline and week 48 in the IntegRal and Discor-Ral studies or in a global analysis of both studies. *P*-values are given for the control (Ctrol) and intensified (+ Ral) groups as indicated. Right part shows the results from a linear mixed model analysis showing *p*-values for slope (change of every marker per week) in the Ctrol and +Ral groups and the interaction slope/group that indicates the difference among groups. Color codes are as follow: no color means p-value>0.1, yellow 0.1<p-value<0.05; orange 0.05<p-value<0.01 and red p-value<0.01. Upward arrowheads (Δ) indicate positive correlations while downward arrowheads (∇) indicate negative correlations.

Effect of raltegravir intensification in levels of D-Dimer

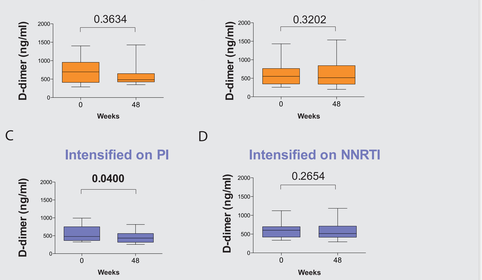


Figure 5. Longitudinal changes in plasma level of D-dimer according to cART regimen.
Individuals from the IntegRal and Discor-Ral studies were classified according to their cART regimen, PI- or NNRTI-based. The level of D-dimer in plasma at baseline and after 48 weeks of study was compared. Figure shows changes for Control individuals on PI-based regimens (A, n=15), control individuals on NNRTI-based regimens (B, n=17), intensified individuals on PI-based regimens (C, n=27) and intensified individuals on NNRTI based regimens (D, n=33). Comparisons were performed using the signed-rank test (paired test) and p-values are shown for each group

CONCLUSIONS

► Our analysis of two separate studies (IntegRal and Discor-Ral) points to a poor association between plasma inflammatory markers and the detection of residual viral replication (assessed by 2-LTR circles) in chronic treated HIV infection

► IL-7, IL-12, TRAIL or IP-10 were independently associated with the number of CD4 T cells. However, this analysis also revealed a poor association between 2-LTR detection and baseline levels of inflammation.

► Our baseline analysis identified the strong association of HCV co-infection with three soluble markers (IP-10, sICAM-1 and sVCAM-1), and a striking association of several cytokines, TRAIL and TNF-α among other, with the cART regimen (PI- or NNRTI-based). In all cases, this latter association revealed higher levels of inflammation in individuals treated with PI-based regimens

► we failed to identify a general decrease in inflammatory markers associated with Raltegravir intensification. However, Raltegravir induced a significant reduction of D-dimer that was exclusively observed, once more, in PI-treated individuals

Our data suggest that soluble inflammatory markers show a complex relationship with several factors (age, co-infections, therapy and immune recovery) that may difficult the use of these markers in the identification of individuals with residual viral replication under cART

REFERENCES

1 Lederman MM *et al*, J Infect Dis. 2011, Oct;204(8):1217-26
2 Massanella M *et al*, Expert Rev Clin Immunol. 2013, Nov;9(11):1135-49
3 Deeks SG. Annu Rev Med. 2011, Feb 18;62141-55
4 Massanella M et al, Curr HIV/AIDS Rep. 2013, Mar;10(1):33-41
5 Massanella M, Negredo E *et al*, AIDS. 2012, Sep 26;262285-2293.
6 Negredo E, Massanella M *et al*, J Antimicrob Chemother. 2013, May 14;68(10):2358-62
7 Buzon MJ, Massanella M *et al*, Nat Med. 2010, Apr;16(4):460-5.
8 Llibre JM, Buzon MJ, Massanella M, *et al*, Antivir Ther. 2012;17(2):355-64
9 Massanella M *et al*, J Acquir Immune Defic Syndr. 2013, Feb 7;63(2):152-60.

ACKNOWLEDGEMENTS

We thank Rafaela Ayen, Eulàlia Grau, Elisabeth Garcia, for technical assistance and sample processing. We also thank Adriana Capdevila from Col·legi Sant Miquel dels Sants (Vic, Spain) for preliminary statistical analysis. We finally thank patients and physicians for participating this study.