Soluble Markers Predict Immune Activation in Individuals Treated for 6 Years with Lopinavir/ritonavir (LPV/r)

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Background

Highly active combined antiretroviral therapy (HAART) is associated with significant immune reconstitution and reduction in mortality in HIV-1 infected patients. Few studies have assessed the effect of HAART on long-term immune reconstitution of 5 or more years of therapy.[1–8]

We have previously reported on a cohort of subjects using a lopinavir/ritonavir (LPV/r)-based HAART regimen followed for 6 years.9 Our data showed that after 6 years, 62 of 63 subjects remaining on study had plasma HIV-1 RNA levels <50 copies/mL. Mean increase in CD4+ T-cell count was 528 cells/mm3 ($p<0.001$) and 81% subjects had CD4+ T-cell counts >500 cells/mm3 compared to 21% at baseline (Figure 1). Mean CD4/CD8 ratio increased from 0.38 at baseline to 0.96 at year 6 ($p<0.001$). Statistically significant increases from baseline for total T-cells, B-cells and NK cells were also observed. At year 6, the mean CD4+ T-cell and CD8+ T-cell activation levels were markedly reduced compared to historical controls (23.1% vs. 4.1% and 39.3% vs. 7.7%, respectively). See Figure 2.

Figure 1.

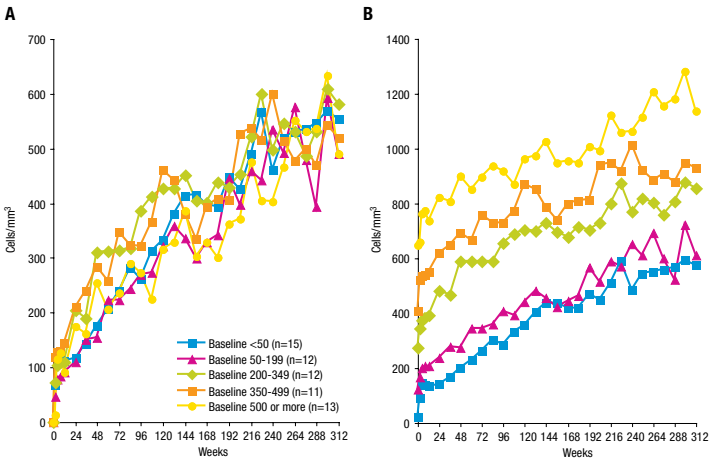
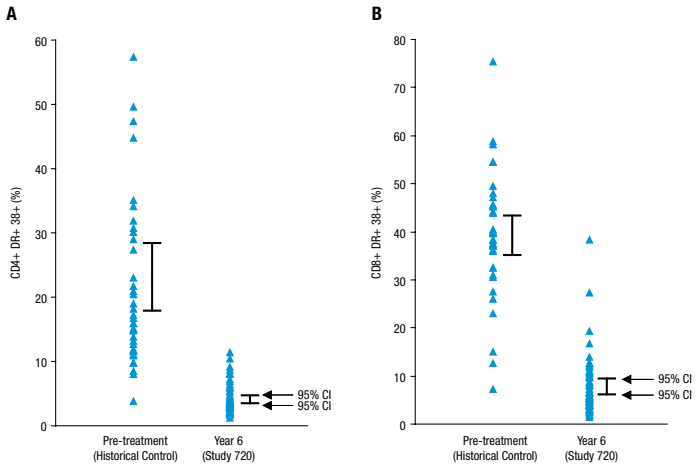


Figure 2.



Soluble markers of immune activation have been shown to be prognostic independent of CD4+ T-cell count and viral load. Specifically, serum neopterin and IgA have been shown to be predictive of HIV disease progression.[10,11]

In addition, serum TNF has been shown to be a marker of immune activation,[12] Soluble TNF-related apoptosis-inducing ligand (TRAIL) is a serum marker for apoptosis,[13] and interferon inducible protein 10 (IP-10) is a marker of interferon induced responses.[14]

Monocyte chemoattractant protein 1 (MCP-1) exerts regulatory effects on adaptive immune responses and impairment of this may play a role in the immune dysregulation typical of AIDS.[15]

The impact of HAART on soluble immune activation markers has not been assessed in subjects receiving therapy for long periods of time.

Objective

To evaluate soluble immune activation markers in 63 ARV-naïve subjects receiving LPV/r+ 2 NRTIs for 6 years.

To identify whether there are any correlations between soluble immune activation marker levels and CD4+ T-cell count or CD4/CD8 activation at year 6 of HAART.

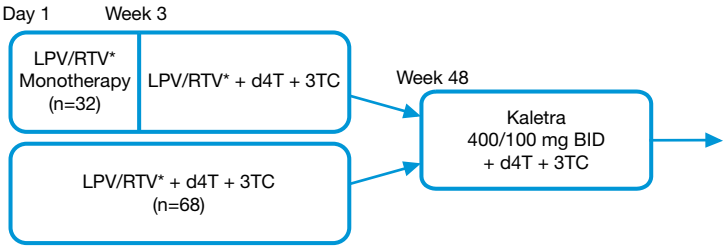
Methods

Study 720 was the initial Phase 2 clinical trial of LPV/r, and provides the longest continuous duration of observation of subjects treated with LPV/r of any study performed to date. Antiretroviral-naïve subjects entered the study and were treated with LPV/r + 2 NRTIs.

Soluble immune activation markers were assessed at baseline, 3 years, and 6 years. Interferon inducible protein-10 (IP10), MCP-1, neopterin, soluble tumor necrosis factor receptor 2 (sTNFR2) and apoptosis marker, TRAIL, were evaluated by ELISA (Biosource, Diaclone, IBL). Serum IgA, IgG and IgM levels were evaluated by immunoturbidimetry (Abbott ARCHITECT® 8200 chemical analyzer).

The relationship between baseline or year 6 values of soluble markers were assessed vs. year 6 CD4+ T-cell counts and CD4/CD8 activation marker levels were assessed using linear regression.

Figure 3. Study 720 Design and Baseline Characteristics



* LPV/RTV dosed at either 200/100 mg BID, 400/100 mg BID or 400/200 mg BID.

Results

Change in Soluble Immune Activation Markers Through 6 Years

- IgA levels were elevated at baseline and did not change significantly through year 6.
- IgG ($p<0.001$) and IgM ($p=0.0034$ at year 3, $p=0.0029$ at year 6) values decreased significantly from baseline to year 3 and year 6.
- IP10 levels significantly decreased ($p<0.0001$ at year 3, $p=0.0012$ at year 6) from baseline to year 3 and year 6. IFN a did not change significantly through year 6.
- Neopterin ($p<0.0001$) and soluble TNFR2 ($p=0.0001$ at year 3 and $p=0.0012$ at year 6) decreased significantly from baseline to year 3 and year 6. MCP-1 decreased significantly through year 3 ($p=0.001$) and returned to pre-therapy levels by year 6.
- TRAIL ($p=0.0217$ at year 3 and $p=0.0285$ at year 6) decreased significantly from baseline to year 3 and year 6.
- Table 1 shows changes in the soluble immune markers through year 6.

Table 1. Mean Values for Soluble Immune Activation Markers Over Time

	Reference Range	Baseline	Year 3	Year 6
	Immunoglobulin Production			
IgA (mg/dL)	59 – 292	303 ± 165	278 ± 143	280 ± 148
IgG (mg/dL)	596 – 1584	1486 ± 445	1152* ± 299	1149* ± 337
IgM (mg/dL)	35 – 213	124 ± 72	90* ± 78	91* ± 80
	Innate Immunity			
IFN a (pg/mL)	0 – 266	23.2 ± 71	27.7 ± 58	14.9 ± 43
IP10 (pg/mL)	42 – 269	364 ± 196	247* ± 158	266* ± 146
	Inflammation			
MCP1 (pg/mL)	43.3 – 93.6	145 ± 98	92* ± 74	122 ± 63
Sol TNFR2 (ng/mL)	1.91 – 8.51	14.0 ± 11.4	8.2* ± 3.7	9.1* ± 4.0
Neopterin (nmol/L)	<10 normal	21.0 ± 19.9	9.3* ± 7.4	9.2* ± 7.8
	Apoptosis			
TRAIL (pg/mL)	430 – 1440	1458 ± 933	1228* ± 733	1271* ± 788

* Statistically significant mean change from baseline ($p<0.05$).

Correlations of Baseline Soluble Immune Activation Markers with Year 6 CD4/CD8 Values

- Baseline values of certain soluble markers were significantly associated with absolute CD4+ T-cell counts or CD4 or CD8 activation at year 6. These are described in Table 2.

– Absolute year 6 CD4+ T-cell count was significantly correlated with baseline IgA, neopterin and MCP-1. Baseline IgA was also significantly correlated with year 6 CD4 activation levels.

– The association between baseline soluble TNFR2 level, absolute year 6 CD4+ T-cell count and activation level were driven by data from a single subject and when this subject was removed from the analysis, no correlation was noted.

- When change in CD4+ T-cell count was used instead of absolute year 6 CD4+ T-cell count, no significant associations were noted with any of the soluble immune markers.

Table 2. Correlation (R Values) Between Baseline Soluble Immune Activation Markers and Year 6 CD4/CD8 Values

Marker	Year 6 CD4+ T-cell Count	Year 6 CD4 Activation	Year 6 CD8 Activation
IgA	–0.37*	0.30*	0.04
IgG	0.04	0.15	0.01
IgM	0.05	–0.07	–0.01
IFNa	0.14	–0.13	–0.15
IP10	0.002	0.19	0.13
MCP1	–0.31*	0.05	–0.07
Sol TNFR2	–0.30*	0.44*	0.20
Neopterin	–0.27*	0.22	0.17
TRAIL	–0.04	–0.11	–0.10

* Statistically significant (p<0.05) correlation between baseline soluble marker and Year 6 CD4/CD8 value.

Correlations of Year 6 Soluble Immune Activation Markers with Year 6 CD4/CD8 Values

- We evaluated the association between year 6 values of the soluble immune activation markers and year 6 CD4+ T-cell count or T-cell activation markers. See Table 3.
- Statistically significant correlations were observed between year 6 IgA and year 6 CD4+ T-cell count, and between soluble TNFR2 and year 6 CD4 activation level.
- In addition, year 6 neopterin was positively correlated with both CD4 and CD8 activation at year 6. See Figure 4.
- Mean neopterin level was lower (8.1 nmol/L vs. 17.0 nmol/L) in subjects with low CD4 or CD8 (<8% or 19%, respectively) activation compared to those with higher CD4/CD8 (>8% and 19%, respectively) activation (p=0.004). See Figure 5.

Figure 4. Correlatiaons of Year 6 CD4/CD8 Activation with Year 6 Neopterin

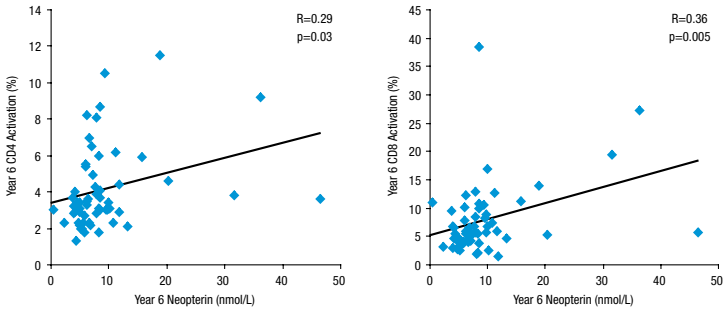


Figure 5. Higher Year 6 Neopterin in Subjects with Higher Year 6 Activation Levels

Table 3. Correlation (R Values) Between Year 6 Values of Soluble Immune Activation Markers and Year 6 CD4/CD8 Values

Marker	Year 6 CD4+ T-cell Count	Year 6 CD4 Activation	Year 6 CD8 Activation
IgA	–0.34*	0.16	–0.02
IgG	–0.06	0.05	–0.03
IgM	0.09	0.06	0.05
IFNa	–0.19	–0.06	–0.13
IP10	–0.19	0.16	0.10
MCP1	–0.14	–0.05	0.02
Sol TNFR2	–0.22	0.26*	0.11
Neopterin	–0.16	0.29*	0.36*
TRAIL	0.17	0.04	0.05

* Statistically significant (p<0.05) correlation between year 6 soluble marker level and year 6 CD4/CD8 value.

Discussion

- In this population of subjects with successful LPV/r-based virologic treatment for 6 years, most of the soluble immune markers decreased with antiretoviral therapy through year 3 and year 6. Most notable were neopterin and IP10, which decreased to within normal limits.
- IgA levels did not change significantly during therapy in this cohort of patients who were responding well to antiretroviral therapy (62/63 subjects had plasma HIV-1 viral loads <50 copies/mL with a mean increase of CD4+ T-cell counts of 528 cells/mm3). The clinical significance of this is not clear; however, prior studies have shown that elevated IgA may be indicative of specific immune dysfunction.[16]
- This finding may suggest that although potent antiretroviral therapy effectively suppresses HIV replication and produces robust immune restoration, there may be certain aspects of HIV infection or immune dysfunction which contribute to lack of change in IgA levels over time.
- Although correlations were noted with baseline immune markers and year 6 CD4 or CD8 values, these correlations were modest and somewhat variable across markers examined. Thus, no definitive conclusions regarding the pre-treatment predictive value of soluble markers of immune activation and HAART-associated immune reconstitution can be drawn from these data.
- This finding supports conclusions from other studies which have noted that neopterin may be a useful marker to evaluate disease progression and prognosis.[10, 11]

Conclusions

- In this study of patients successfully responding to an LPV/r-based regimen, soluble immune markers decreased with increasing CD4+ T-cell count increase.
- These data provide further evidence of the importance of neopterin as a soluble immune marker.
- Further study is required to determine how this information may be used clinically, in addition to information provided by CD4+ T-cell count.

References

1. Smith K et al. *AIDS* 2004;18:2953–56.
2. Kaufmann G et al. *CID* 2005;41:361–72.
3. Le Moing V et al. 12th CROI, Boston, MA, USA, 2005, #609.
4. Esteve A et al. 12th CROI, Boston, MA, USA, 2005, #611.
5. Smith KY et al. 3rd IAS, Rio de Janiero, Brazil, 2005, #WEPe16.7B06.
6. Keruly, J, Moore, R. 13th CROI, Denver, CO, USA, 2006, #529.
7. Gras L et al. 13th CROI, Denver, CO, USA, 2006, #530.
8. Gulick RM et al. *AIDS* 2003;17:2345–2349.
9. Landay A et al. *CID* 2007;44:749–754.
10. Mildvan et al. *CID* 2005;40:853–858.
11. Fahey et al. NEJM 1990;322:166–172.
12. Martinson JA et al. *Immunology* 2007;120:526–535.
13. Aukrust P et al. *J Infect Dis* 1994;169:420–424.
14. Herbeuval JP et al. *Natl Acad Sci* 2005;102(39):13974–13979.
15. Fantuzzi L et al. *J Leukoc Biol* 2003;74:719-725.
16. French et al. *HIV Med* 2007;8:148–55.

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