This is the peer reviewd version of the followng article:

Dynamics of adaptive and innate immunity inpatients treated during primary human immunodeficiency virus infection: Results from Maraviroc in HIV Acute Infection (MAIN) randomized clinical trial / Ripa, M.; Pogliaghi, M.; Chiappetta, S.; Galli, L.; Pensieroso, S.; Cavarelli, M.; Scarlatti, G.; DE BIASI, Sara; Cossarizza, Andrea; De Battista, D.; Malnati, M.; Lazzarin, A.; Nozza, S.; Tambussi, G.. - In: CLINICAL MICROBIOLOGY AND INFECTION. - ISSN 1198-743X. - 21:9(2015), pp. 1-4. [10.1016/j.cmi.2015.05.007]

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

01/05/2024 02:44

# Accepted Manuscript

Dynamics of adaptive and innate immunity in patients treated during Primary HIV Infection: results from MAIN randomised clinical trial

Marco Ripa, Manuela Pogliaghi, Stefania Chiappetta, Laura Galli, Simone Pensieroso, Mariangela Cavarelli, Gabriella Scarlatti, Sara de Biasi, Andrea Cossarizza, Davide de Battista, Mauro Malnati, Adriano Lazzarin, Silvia Nozza, Giuseppe Tambussi, M.D



- PII: S1198-743X(15)00444-9
- DOI: 10.1016/j.cmi.2015.05.007
- Reference: CMI 261
- To appear in: Clinical Microbiology and Infection
- Received Date: 11 November 2014
- Revised Date: 23 January 2015
- Accepted Date: 2 May 2015

Please cite this article as: Ripa M, Pogliaghi M, Chiappetta S, Galli L, Pensieroso S, Cavarelli M, Scarlatti G, de Biasi S, Cossarizza A, de Battista D, Malnati M, Lazzarin A, Nozza S, Tambussi G, Dynamics of adaptive and innate immunity in patients treated during Primary HIV Infection: results from MAIN randomised clinical trial, *Clinical Microbiology and Infection* (2015), doi: 10.1016/j.cmi.2015.05.007.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

## 1 **RESEARCH NOTE**

2

# **3 FULL TITLE:**

- 4 Dynamics of adaptive and innate immunity in patients treated during Primary
- 5 HIV Infection: results from MAIN randomised clinical trial
- 6

# 7 AUTHORS:

- 8 Marco RIPA(1,2,3)\*, Manuela POGLIAGHI(1,2,3)\*, Stefania
- 9 CHIAPPETTA(1,2,3), Laura GALLI(3), Simone PENSIEROSO(4),
- 10 Mariangela CAVARELLI(4), Gabriella SCARLATTI(4), Sara DE BIASI(5),
- 11 Andrea COSSARIZZA(5), Davide DE BATTISTA(6), Mauro MALNATI(6),
- 12 Adriano LAZZARIN(1,3), Silvia NOZZA(2,3), Giuseppe TAMBUSSI(2,3)
- 13
- 14 \*These authors contributed equally to this work.
- 15

### 16 **AFFILIATIONS**:

- 17 1) Università Vita-Salute San Raffaele, Department of Infectious and Tropical
- 18 Diseases. Milan, Italy
- 19 2) IRCCS Ospedale San Raffaele, Vaccine and Immunotherapy Research
- 20 Center, Milan, Italy
- 21 3) IRCCS Ospedale San Raffaele, Department of Infectious and Tropical
- 22 Diseases, Milan, Italy

- 23 4) IRCCS Ospedale San Raffaele, Unit of Viral Evolution and Transmission,
- 24 Milan, Italy
- 25 5) Università di Modena e Reggio Emilia, Department of Surgery, Medicine,
- 26 Dentistry and Morphological Sciences, Modena, Italy
- 27 6) IRCCS Ospedale San Raffaele, Unit of Human Virology, Milan, Italy

28

#### 29 **RUNNING TITLE:**

- 30 Adaptive and innate immunity in primary HIV infection
- 31

### 32 CORRESPONDING AUTHOR:

- 33 Giuseppe Tambussi, M.D.
- 34 Vaccine and Immunotherapy Research Center
- 35 Department of Infectious and Tropical Diseases
- 36 IRRCS Ospedale San Raffaele
- 37 Via Stamira d'Ancona, 20
- 38 20127 Milano Italy
- 39 phone number: +39 0226433722
- 40 fax number: +39 0226427030
- 41 e-mail address: tambussi.giuseppe@hsr.it

42

#### 43 AUTHORSHIP/CONTRIBUTION

- 44 MR and MP equally contributed to the work and wrote the manuscript. GT
- 45 designed the clinical trial. SN, MP, SC, MR followed the patients. LG

- 46 supervised statistical analysis and helped to write the manuscript. SP, MC and
- 47 GS performed B-cells and DC investigations. SDB and AC performed T-cells
- 48 staining. DDB and MM performed NK-cells staining.

## 50 ACKNOWLEDGEMENTS

We thank all the patients enrolled in the MAIN Study. A special thank to CRIV
Staff: Liviana Della Torre, Andrea Galli, Maria Rita Parisi, and to Monica
Tolazzi for technical assistance.

Results partially presented during 7th Conference on HIV Pathogenesis,
Treatment and Prevention (IAS, 2013), Keystone Symposium: "Immune
Activation in HIV Infection: Basic Mechanisms and Clinical Implications"
(2013) and 20th Conference on Retroviruses and Opportunistic Infections
(CROI, 2013).

### 69 WORD COUNTS:

- 70 Abstract: 94
- 71 Text: 989
- 72

### 73 ABSTRACT

We evaluated the dynamics of innate and adaptive immunity in patients treated
with combined antiretroviral therapy (cART) during primary HIV infection
(PHI), enrolled in a prospective randomised trial (MAIN, EUDRACT 2008007004-29).
After 48 weeks of cART, we documented a reduction in activated B-cells and
CD8<sup>+</sup> T-cells. Moreover, natural killer (NK) and dendritic cells (DC)
frequencies were measured, finding a decrease in CD16<sup>+</sup>CD56<sup>dim</sup> with a

- 81 reciprocal rise in CD56<sup>high</sup> NK and an increase in myeloid and plasmacytoid
- 82 DC.

83 In conclusion, 48 weeks of cART during PHI showed significant benefits on84 both innate and adaptive immunity.

85

# 86 **KEYWORDS**:

- 87 Primary HIV-1 infection; B cells; NK cells; dendritic cells; immune activation
- 88
- 89
- 90
- 91

#### 92 **TEXT**

During Primary HIV Infection (PHI) the huge amount of viral replication
harms the immune system by a direct viral cythopatic effect, but mostly
through massive T-cell activation [1,2], responsible for severe CD4<sup>+</sup> T-cell
depletion; combined antiretroviral therapy (cART) can only partially restore
this damage [3].

98 Few authors reported the dynamics of adaptive and innate immunity during 99 PHI, the effect of early initiation of cART and their impact on CD4<sup>+</sup> T-cell 100 recovery. It has been shown that not only T- [3,4] but even B- [5-8], natural 101 killer (NK) [9,10] and dendritic cells (DC) [11] compartments are deranged 102 since PHI and that cART can improve, although not completely, these 103 impairments.

Aim of this study was to evaluate T, B, NK and DC subsets during PHI and toassess the effect of cART on the adaptive and innate immune system.

106

We analyzed 19 patients treated during PHI enrolled in a monocenter,
randomised, open-label, proof-of-concept clinical trial (MAIN study,
EUDRACT number: 2008-007004-29). Patients started a protease-inhibitorbased regimen and were randomized to maraviroc as associated drug. Blood
was collected at baseline (BL), and at week (W) 48 and processed to preserve
frozen Peripheral Blood Mononuclear Cells (PBMCs).

Staining and polychromatic flow-cytometry were performed on cryopreserved
PBMCs at BL and W48. Gating strategies are described in Figure 1. CD8<sup>+</sup> and

115	CD4 <sup>+</sup> T-cells were defined as naive (NV), central memory (CM), effector
116	memory (EM), and terminally differentiated (TD) on the basis of CCR7 and
117	CD45RA expression; activated cells were defined by HLA-DR expression.
118	Treg were defined as CD4 <sup>+</sup> CD8 <sup>-</sup> CD25 <sup>bright</sup> FoxP3 <sup>+</sup> CD127 <sup>-</sup> . B-cells were
119	divided in transitional (TR), naive (NV), resting memory (RM), activated
120	memory (AM) and tissue-like memory (TLM) on the basis of CD27 and CD21
121	expression. Plasma cells (PC) were defined as CD27 <sup>+</sup> CD38 <sup>+</sup> . NK cells were
122	divided in CD56 <sup>high</sup> and CD56 <sup>dim</sup> CD16 <sup>+</sup> subsets. Among DC, CD11c <sup>+</sup> myeloid
123	(mDC) and CD123 <sup>+</sup> plasmacytoid (pDC) DCs were identified.

124

An intention-to-treat analysis was performed. Results are described as median (interquartile range) or frequency (%). Wilcoxon signed-rank test and Spearman rank correlation coefficient were applied. All tests were two-sided and a P-value<0.05 was considered as significant. Statistical analysis was performed by SPSS 20 (IBM).

130

Patients BL characteristics are shown in table 1. Median HIV-RNA load was
5.8log<sub>10</sub>cp/mL (5.2/6.4), and CD4:CD8 ratio was very low [0.2 (0.15/0.42)],
mainly because of massive CD8<sup>+</sup> T-cell activation.

134 After 48W of cART,  $CD4^+$  T-cell significantly increased [349cell/µL 135 (241/398), p<0.001], while HIV-RNA decreased [-5.8log<sub>10</sub>cp/ml (-6.4/-5.2), 136 p<0.001], reaching <50cp/ml in 18/19 patients.

#### ACCEPTED MANUSCRIPT

137	At W48, among $CD4^+$ T-cells we observed a decrease of NV and CM [NV: -
138	18.1% (-29.9/-8.0), CM: -28.9% (-41.5/-21.6); p<0.001] and an increase in TD
139	[62.5% (40.3/70.1), p<0.001]; among $CD8^+$ T-cells, we documented an
140	increase in EM [20.8% (-10.6/32.7), p=0.030] and TD [9.7% (3.5/20.4),
141	p=0.005].
142	Cell activation was measured in both $CD4^+$ and $CD8^+$ T-cells. While
143	$CD4^{+}HLA-DR^{+}$ T-cells did not significantly change (p=0.053), we found a
144	significant reduction in activated CD8 <sup>+</sup> T-cell [-45.2% (-55.4/-30.1), p<0.001].
145	At baseline, both $CD4^+HLA-DR^+$ and $CD8^+HLA-DR^+$ T-cells were inversely

147 respectively). At W48 a greater reduction in  $CD4^+HLA-DR^+$  T-cells was 148 associated with a higher gain of  $CD4^+$  T-cells (r=-0.460, p=0.048).

correlated with CD4:CD8 ratio (r=-0.505, p=0.027 and r=-0.728, p<0.001,

- 149 The T-reg population frequency did not significantly change after 48W of150 treatment (p=0.091).
- 151 The analysis of the B-cell populations revealed a 48-week decrease in
- 152 activated subsets, TLM, AM and PC [TLM: -7.1% (-10.5/-3.0), p<0.001; AM:
- 153 -5.2% (-15.8/-3.3), p<0.001; PC: -8.7% (-13.0/-2.2), p<0.001], and an increase
- 154 in RM [9.9% (7.5/17.4), p<0.001].

- 155 At baseline, CD4:CD8 ratio inversely correlated with AM and TLM [r=-0.571,
- 156 p=0.011 and r=-0.715, p=0.001, respectively], while higher HIV-RNA was
- 157 associated with higher PC frequencies (r=0.519, p=0.023). Notably, after 48W
- 158 a greater reduction of HIV-RNA was associated to a more significant decrease
- 159 of PC (r=0.647, p=0.003).

Among NK cells, at 48W we observed a decrease in CD16<sup>+</sup>CD56<sup>dim</sup> [-9.3% (-160 18.4/2.9), p=0.011] and an increase in CD56<sup>high</sup> cells [0.4% (-0.2-1.0), 161 162 p=0.033]. Finally, both pDC and mDC percentages increased at W48 [pDC: 0.1% (0.1-163 164 0.2), p=0.001; mDC: 0.2% (0.1-0.3), p=0.003]. 165 166 Aims of this study were to assess alterations of innate and adaptive immunity 167 during PHI and to evaluate cART impact on different cellular subsets. 168 Antiretroviral therapy was able to decrease CD8<sup>+</sup> T-cell activation in patients with PHI, while no effect was documented on CD4<sup>+</sup>HLA-DR<sup>+</sup> T-cells. A 169 170 greater decrease of CD4<sup>+</sup>HLA-DR<sup>+</sup> was correlated to a more significant 171 increase in CD4<sup>+</sup> T-cells, while no relationships were found between decrease 172 in CD8<sup>+</sup>HLA-DR<sup>+</sup> T-cells and CD4<sup>+</sup> T-cell gain. Interestingly, we observed a 173 decrease in NV and CM CD4+ T-cells, coupled with an increase in TD CD4+ 174 and CD8+ T-cells, in contrast to what is described in chronic infected patients

175 [12].

Few reports described B-cells kinetics in PHI [13-15]. Notably, at the onset of infection we observed increased frequencies of activated B-cells. Dysregulated B-cell subsets, such as TLM, AM and PC, decreased significantly at W48, suggesting a possible role of cART in preserving humoral immunity. Moreover, we documented a significant increase in RM B-cells, responsible for an efficient secondary immune response. We also studied some parameters to assess the innate immunity in patients treated during PHI: a reciprocal variation of CD16<sup>+</sup>CD56<sup>dim</sup> and CD56<sup>high</sup> NK was observed, while among DC we documented an increase in both mDCs and pDCs, usually reduced during CHI [16].

186

We showed that during PHI a lower CD4:CD8 ratio was significantly associated not only to a more significant CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation, but also to higher levels of B-cell dysregulated subsets, as measured by AM and TLM. This finding underlines the importance of CD4:CD8 ratio as a surrogate marker of immune function during HIV infection.

192 Despite the small sample size, due to the well-known underestimated 193 incidence of patients diagnosed during PHI, our study showed that during PHI 194 both the adaptive and the innate immune system cell subsets were skewed 195 toward an activated phenotype. Immune activation was significantly 196 dampened by cART, in particular for CD8<sup>+</sup> T-cells, B-cells and NK cells. 197 Moreover, our findings suggested that CD4:CD8 ratio could be a useful 198 parameter to indirectly assess baseline immune activation during primary HIV 199 infection.

200

201

202

203

#### 205 **REFERENCES**

- 206 1. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 Infection.
- 207 N Engl J Med 2011; 364(20):1943–54.
- 208 2. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M.
- 209 Massive infection and loss of memory CD4+ T cells in multiple tissues during
- 210 acute SIV infection. Nature **2005**; 434(7037):1093–7.
- 211 3. Streeck H, Jessen H, Alter G, et al.. Immunological and virological impact
- 212 of highly active antiretroviral therapy initiated during acute HIV-1 infection. J
- 213 Infect Dis **2006**; 194(6):734–9.
- 4. Tilling R, Kinloch S, Goh LE, et al. Parallel decline of CD8+/CD38++ T
- 215 cells and viraemia in response to quadruple highly active antiretroviral therapy
- 216 in primary HIV infection. AIDS 2002; 16(4):589–96.
- 5. Moir S, Fauci AS. B cells in HIV infection and disease. Nat Rev Immunol
- 218 **2009**; 9(4):235–45.
- 219 6. Moir S, Fauci AS. Insights into B cells and HIV-specific B-cell responses in
- 220 HIV-infected individuals. Immunol Rev **2013**; 254(1):207–24.
- 221 7. Amu S, Ruffin N, Rethi B, Chiodi F. Impairment of B-cell functions during
- 222 HIV-1 infection. AIDS **2013**; 27(15):2323–34.
- 8. Moir S, Ho J, Malaspina A, Wang W, et al. Evidence for HIV-associated B
- 224 cell exhaustion in a dysfunctional memory B cell compartment in HIV-
- 225 infected viremic individuals. J Exp Med **2008**; 205(8):1797–805.
- 226 9. Alter G, Teigen N, Davis BT, et al. Sequential deregulation of NK cell
- subset distribution and function starting in acute HIV-1 infection. Blood **2005**;

#### 228 106(10):3366-9

- 229 10. Mavilio D, Lombardo G, Benjamin J, et al. Characterization of CD56-
- 230 /CD16+ natural killer (NK) cells: a highly dysfunctional NK subset expanded
- 231 in HIV-infected viremic individuals. Proc Natl Acad Sci U S A 2005;
- 232 102(8):2886–91.
- 233 11. Huang J, Yang Y, Al-Mozaini M, et al. Dendritic cell dysfunction during
- 234 primary HIV-1 infection. J Infect Dis **2011**; 204(10):1557–62.
- 235 12. Robbins GK, Spritzler JG, Chan ES, et al. Incomplete reconstitution of T
- 236 cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials
- 237 Group protocol 384. Clin Infect Dis. **2009;** 1;48(3):350-61.
- 238 13. Titanji K, Chiodi F, Bellocco R, et al. Primary HIV-1 infection sets the
- stage for important B lymphocyte dysfunctions. AIDS **2005**; 19(17):1947–55.
- 14. Moir S, Buckner CM, Ho J, et al. B cells in early and chronic HIV
  infection: evidence for preservation of immune function associated with early
  initiation of antiretroviral therapy. Blood **2010**; 116(25):5571–9.
- 243 15. Pensieroso S, Galli L, Nozza S, et al. B-cell subset alterations and
- correlated factors in HIV-1 infection. AIDS **2013** ; 27(8):1209–17.
- 16. Manches O, Frleta D, Bhardwaj N. Dendritic cells in progression and
  pathology of HIV infection. Trends Immunol 2013; pii: S14714906(13)00156-7
- 248

249

# 251 FIGURE CAPTIONS:

### **252** Figure 1 – Gating strategies

- 253 FSC: forward scatter; SSC: side scatter.
- A) T-cells. NV: naïve; CM: central memory; EM: effector memory. TD:
- 255 terminally differentiated.
- 256 B) Treg.
- 257 C) B-cells. TR: transitional; NV: naïve; RM: resting memory; AM: activated
- 258 memory; TLM: tissue-like memory; PC: plasma cells.
- D) NK cells.
- 260 E) Dendritic cells. pDC: plasmacytoid dendritic cells; mDC: myeloid dendritic
- cells.

Characteristics (N=19)			
Age, years	39 (33/41)		
Sex			
М	18 (9	95%)	
F	1 (5	5%)	
Risk factor			
MSM	12 (63%)		
Heterosexuals	7 (37%)		
Fiebig			
III	7 (37%)		
IV	1 (5%)		
V	11 (58%)		
	W0	W48	
$CD4^+$ T-cells, cells/ $\mu L$	382 (269/470)	705 (614/854)	
CD4 <sup>+</sup> T-cells %	12.7 (10.1/22.1)	35.2 (32.2/39.3)	
$CD8^+$ T-cells, cells/ $\mu L$	1539 (884/2697)	733 (587/1263)	
CD8 <sup>+</sup> T-cells %	70.7 (53.0/72.9)	36.7 (32.7/45.1)	
CD4:CD8 ratio	0.20 (0.15/0.42)	1.01 (0.72/1.13)	
HIV-RNA, log <sub>10</sub> cp/ml	5.8 (5.2/6.4)	0.0 (0.0/1.6)	



## ACCEPTED MANUSCRIPT



CD127

FOXP3





Chillip Mark