

Advanced Course in Clinical Parasitology (ACCP)

Barcelona, September 7th 2011

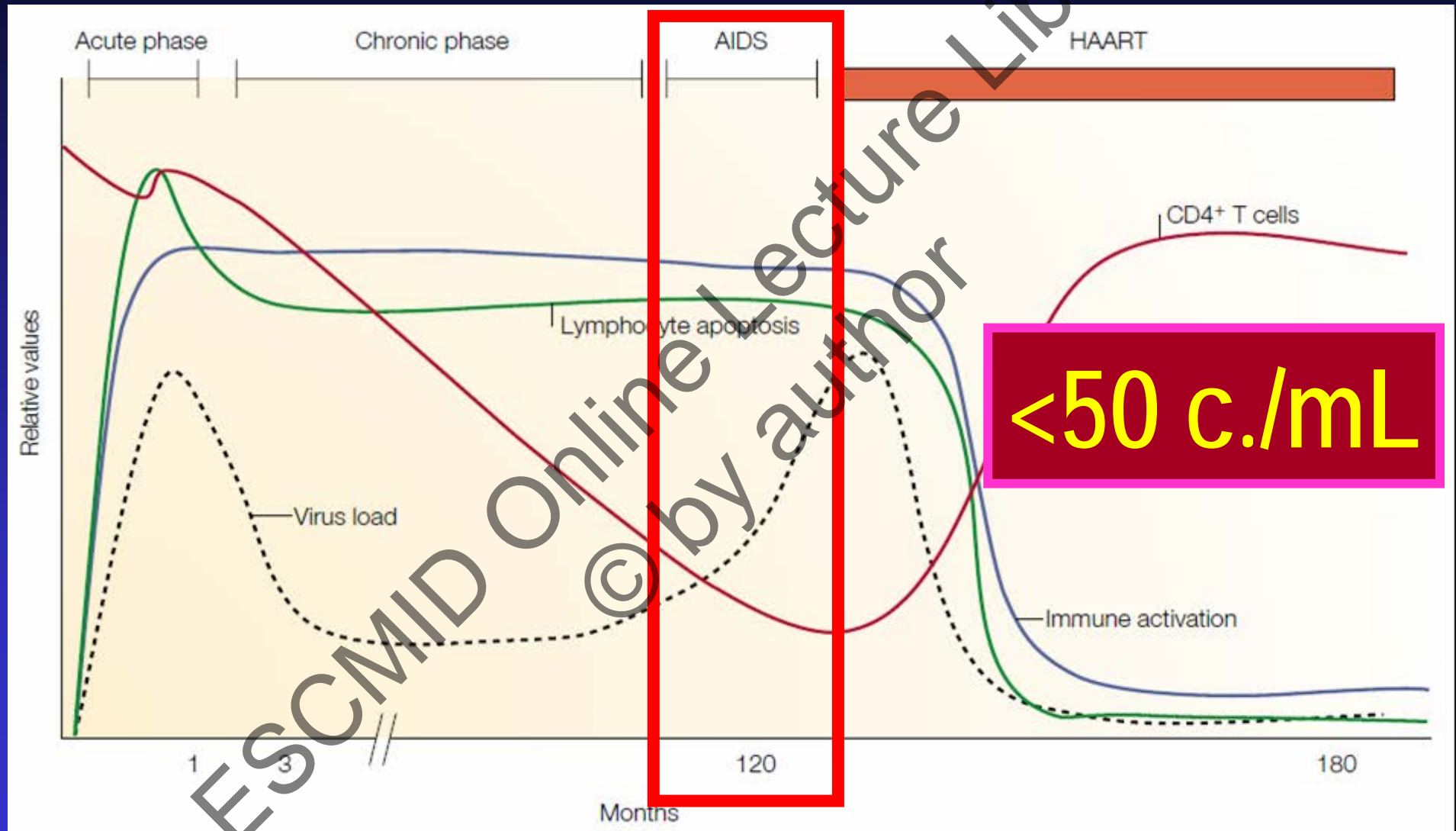
**Restoration of T Cell Responses to T. gondii
after Successful HAART in Patients with AIDS
with Previous Toxoplasmic Encephalitis**

Dr. José M. Miró

Infectious Diseases Service
Hospital Clinic – IDIBAPS
University of Barcelona
Barcelona (Spain)

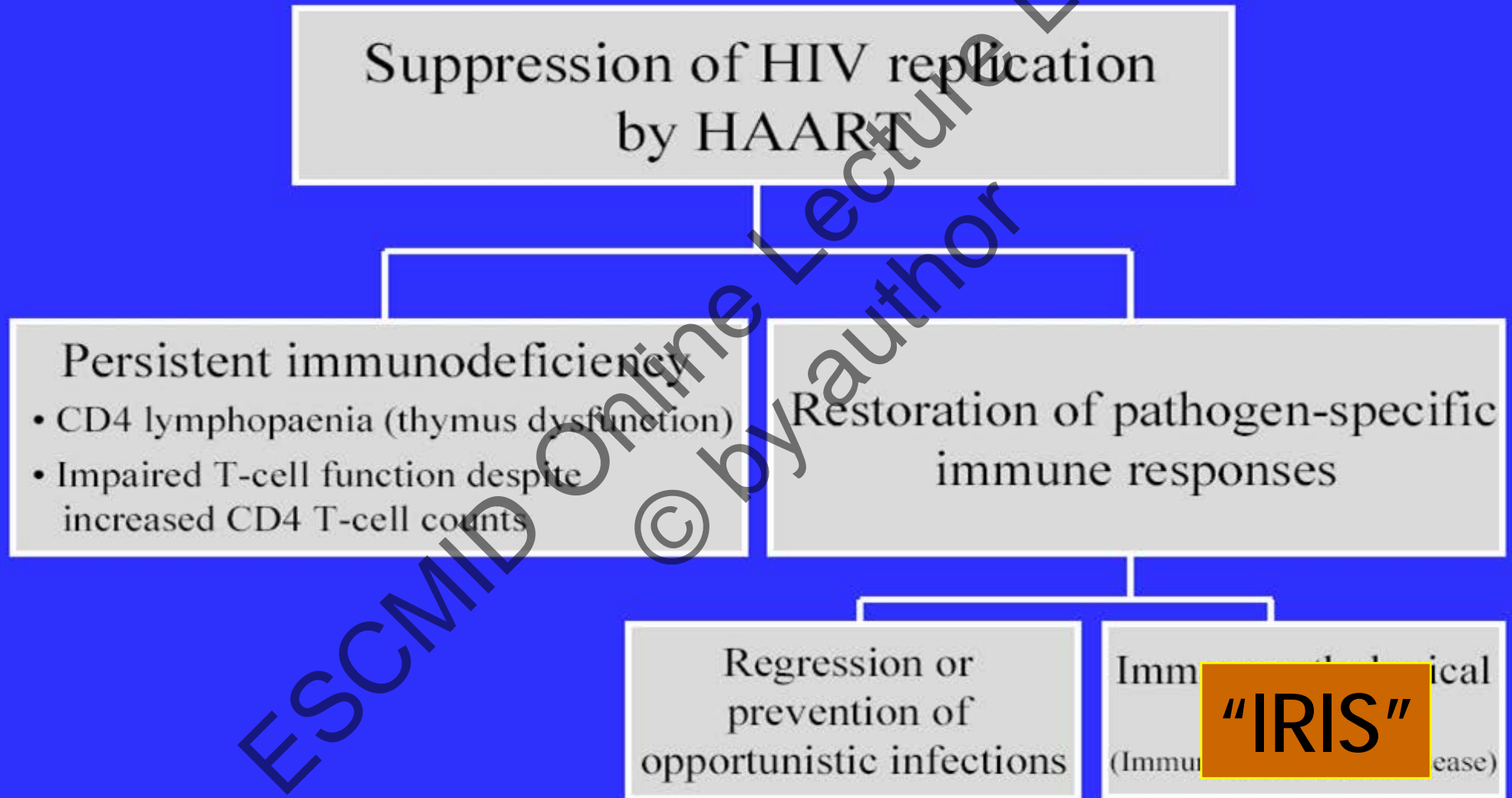
E-mail address: jmmiro@ub.edu

Natural history of HIV infection



Immune Restoration Induced by HAART

Hirsch M et al. Clin Infect Dis. 2004.



Immune Reconstitution Induced by cART

Immunological Changes During the 1st Year

• 0-3 mo.

↑ Memory CD4+/CD8+ T-cells
Peripheral redistribution from LT
No T-cell functional recovery (OI risk).

• 3-9 mo.

Plasma VL BDL; ↓ ↓ immune activation;
↑ CD4+ & ↓ CD8+. Functional recovery started:
Proliferative response to recall antigens

• > 9-12 mo.

↑ Naïve CD4+/CD8+ T-cells (Thymus);
↑ Immunological *repertoire*; ↑ Th1/Th2 response
T-cell function almost fully recovered (-VIH)

Immune Reconstitution Induced by cART

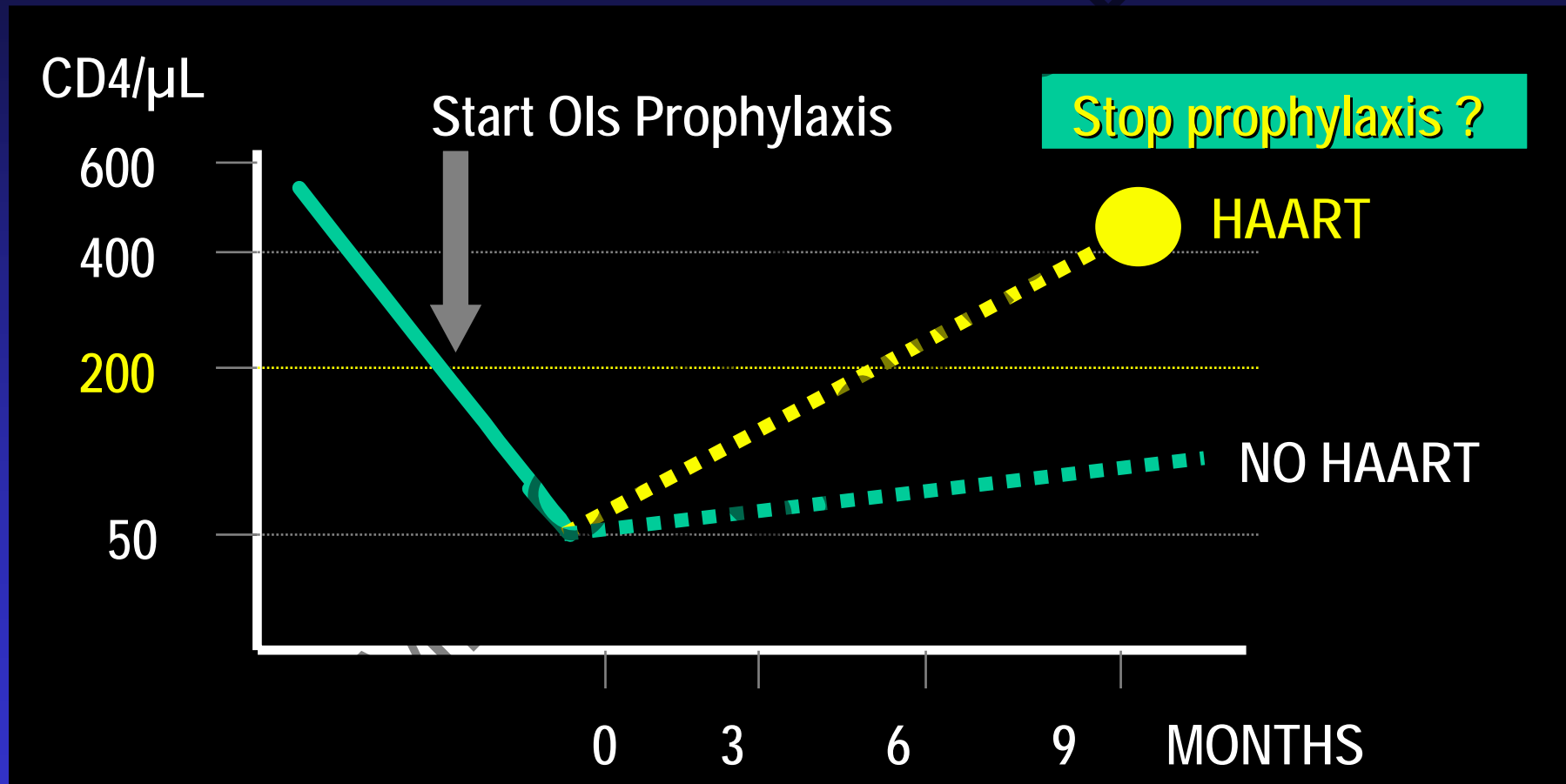
Proliferative Response to CMV and TB

Autran B. Lancet. 1998.

Proliferative Response to CMV & TB Antigens

<u>Month</u>	<u>CMV</u>	<u>TBC</u>
0	20%	0%
1	40%	25%
3	40%	40%
6	50%	60%

Is it Possible to Discontinue TE Prophylaxis in Patients with Immunological Reconstitution with HAART? = YES



Stopping prophylaxis can simplify treatment, reduce toxicity and drug interactions, bacterial resistance, lower cost of care and potentially facilitate adherence to HAART.

Guidelines for Preventing OI's among HIV-Infected Persons - 2009
USPH Service and IDSA Recommendations

DISCONTINUATION PROPHYLAXIS CRITERIA

PCP

- PP/SP D/C if CD4 >200 cells/ μ L \geq 3 mo (AI/BII).
- Restarting if CD4 < 200 cells/ μ L.

TE

- PP D/C if CD4 >200 cells/ μ L \geq 3 mo. (AI).
- **SP D/C if CD4 >200 cells/ μ L \geq 6 mo. (BII).**
- Restarting if CD4 < 200 cells/ μ L.

MAC

- D/C PP if CD4 >100 cells/ μ L \geq 3 mo. (AI).
- **SP D/C if CD4 >100 cells/ μ L \geq 6 mo. (CIII).**
- Restarting if CD4 < 50 cells/ μ L.

PCP = *P. carinii* pneumonia; TE = Toxoplasmic encephalitis; MAC = Disseminated *M. avium* infection.

GESIDA, 2008 & MMWR, 2009; Miro JM et al, CID, 2008.

Restoration of T Cell Responses to T. gondii after Successful HAART in Patients with AIDS with Previous Toxoplasmic Encephalitis

- Transversal (cross-sectional) study
- Longitudinal study

Restoration of T Cell Responses to *Toxoplasma gondii* after Successful Combined Antiretroviral Therapy in Patients with AIDS with Previous Toxoplasmic Encephalitis

Marylène Lejeune,^{1,a} José M. Miró,² Elisa De Lazzari,² Felipe García,² Xavier Claramonte,² Esteban Martínez,² Esteban Ribera,⁴ Julio Arrizabalaga,⁴ José R. Arribas,⁵ Pere Domingo,⁶ Elena Ferrer,⁷ Montserrat Plana,¹ María-Eugenia Valls,⁸ Daniel Podzamczak,⁷ Tomás Pumarola,⁸ Alain Jacquet,^{9,b} Josep Mallolas,² José M. Gatell,² Teresa Gallart,¹ and the Spanish *Toxoplasma gondii* Study Group

¹Immunology Service, Hospital Clinic-Institut d'Investigacions Biomèdiques Augustí Pi i Suñer, and ²Infectious Diseases Service, Hospital Clinic-Institut d'Investigacions Biomèdiques Augustí Pi i Suñer, University of Barcelona, Barcelona, Spain; ³Infectious Diseases Department, Hospital Universitari Vall d'Hebron, Barcelona; ⁴Infectious Diseases Unit, Hospital Donostia, San Sebastian; ⁵Internal Medicine Department, VIH Unit, Hospital La Paz, Madrid; ⁶Infectious Diseases Unit, Hospital Santa Creu i Sant Pau, Barcelona; ⁷Infectious Diseases Service, Hospital Universitari de Bellvitge, Barcelona; ⁸Microbiology Service, Hospital Clinic-Institut d'Investigacions Biomèdiques Augustí Pi i Suñer, University of Barcelona, Barcelona, Spain; and ⁹Service de Génétique Appliquée, Université Libre de Bruxelles, Brussels, Belgium; and The members of the Spanish *T. gondii* Study group are listed in Appendix 1.

OBJECTIVE

To assess if the safety of withdrawing *Toxoplasma* encephalitis (TE) secondary prophylaxis (SP) after immunological reconstitution with HAART can be supported by demonstrating *in vitro* specific T-cell responses against *Toxoplasma gondii* (Tg).

PATIENTS

Design: Prospective cross-sectional study.

Group A: 17 HIV-negative healthy individuals with chronic Tg infection (*HC group*)

Group B: 20 AIDS patients with acute TE (*TE group*)

Group C: 12 AIDS patients with previous TE treated with HAART during at least one year without immunological reconstitution ($CD4^+ < 200$ cells/mL) (*SP<200 group*)

Group D: 27 AIDS patients who discontinued TE SP after immunological reconstitution with HAART ($CD4^+ > 200$ cells/mL and plasma HIV-1 RNA viral load $< 5,000$ copies/mL for $> 3-6$ months) (*SP>200 group*)

METHODS

↳ Lymphoproliferative response

Coculture of PBMC with the soluble antigen extract of Tg (SATg) and different mitogens for 7 days.

↳ Cytokine measurements

IFN- γ production in the supernatant of PBMC culture after 72 hrs of culture stimulated with medium alone or with the soluble antigen extract of Tg (SATg).

↳ Flow cytometry analysis.

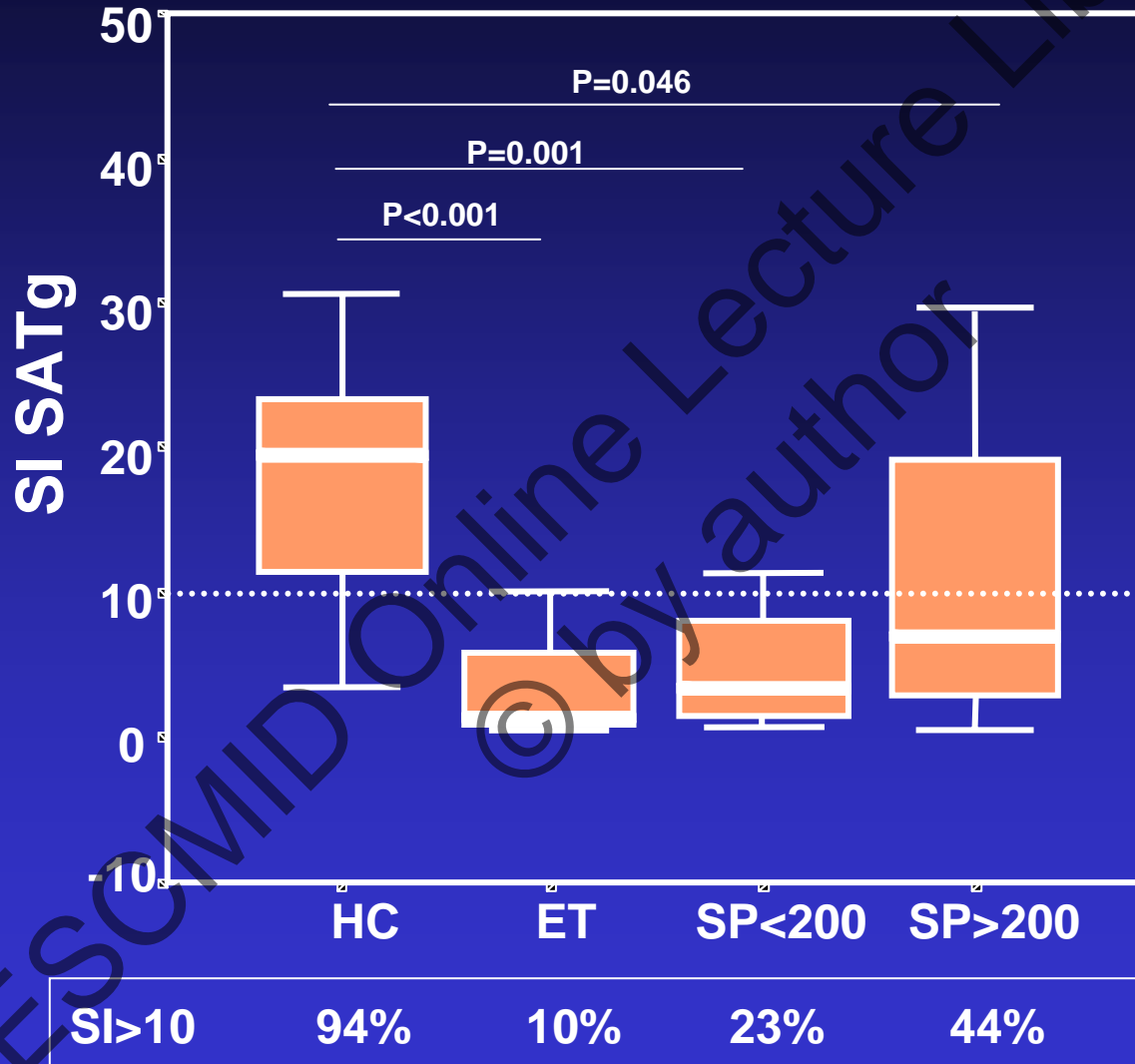
Determination of the subpopulations of CD3⁺, CD4⁺ and CD8⁺ cells by three-color flow cytometry.

Patient characteristics

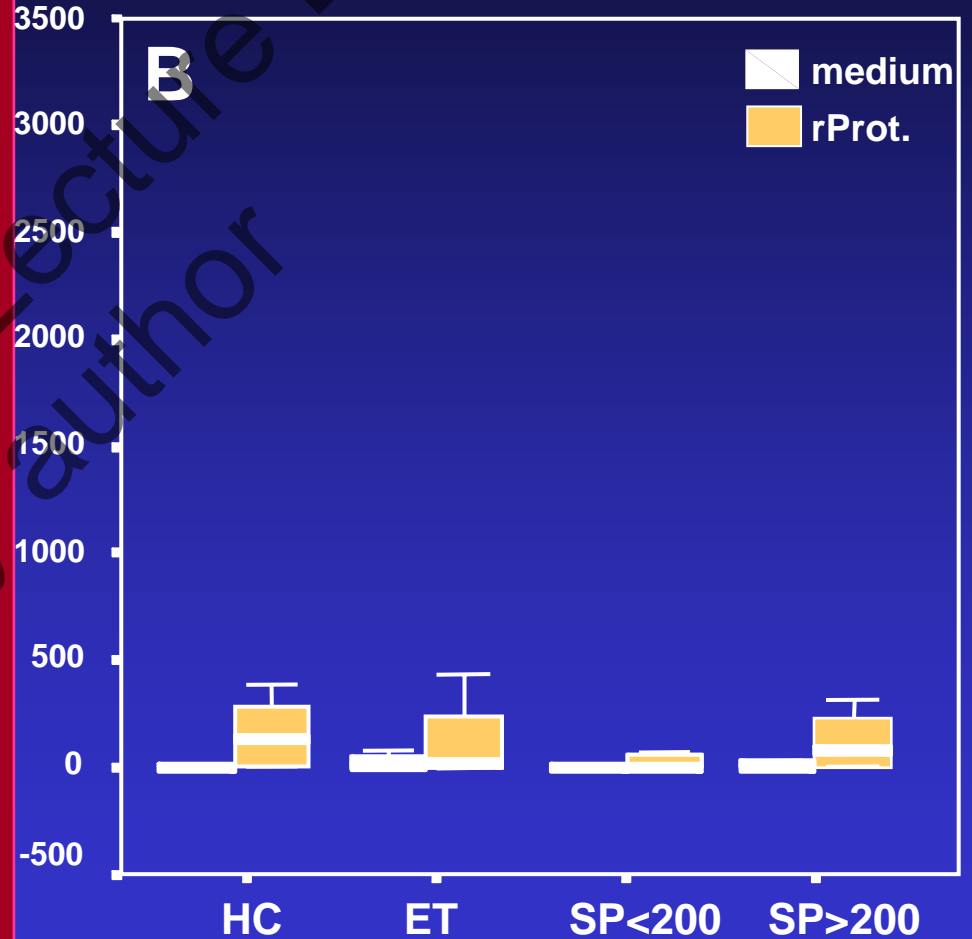
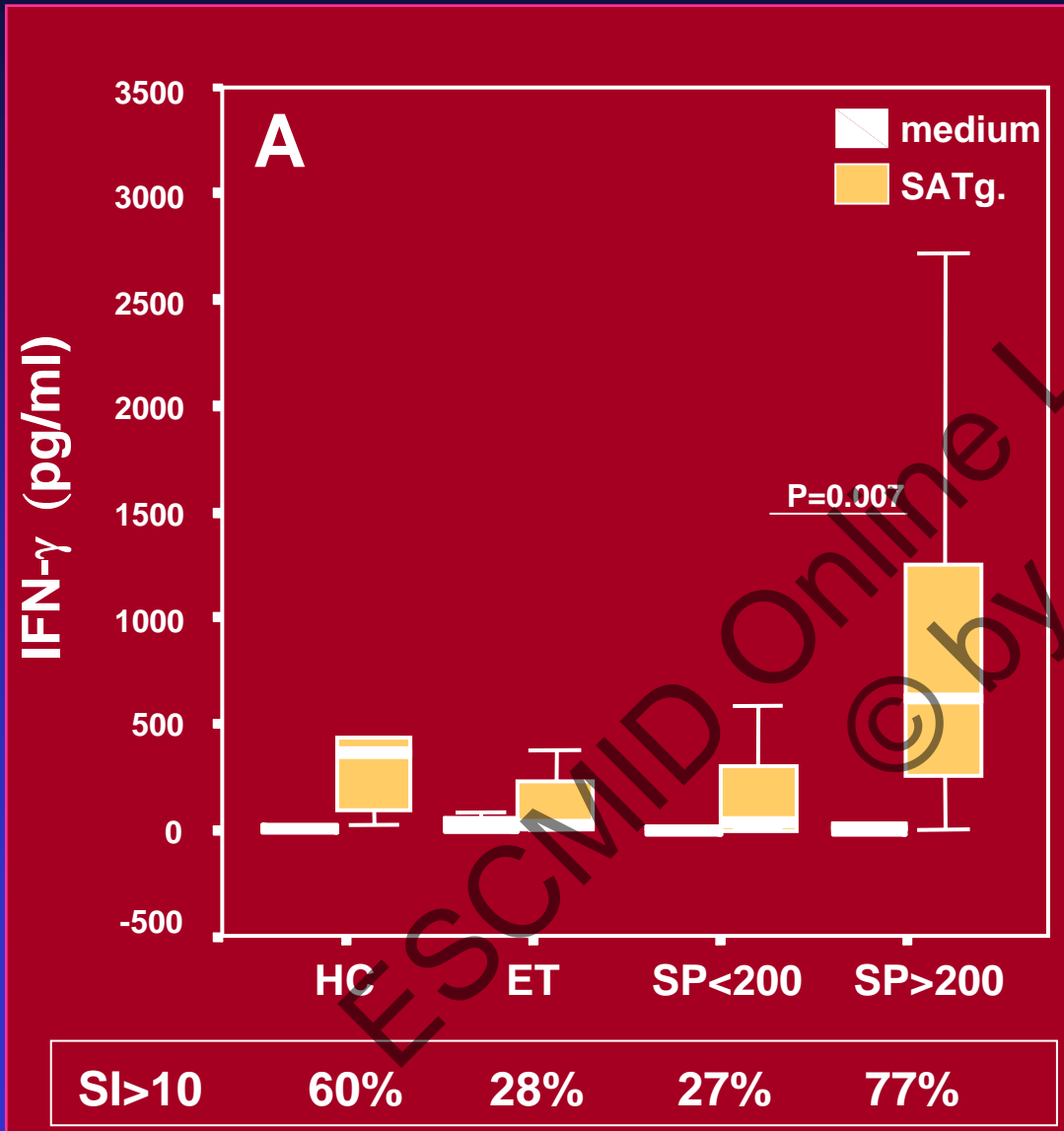
	HC group (n=17)	ET group (n=20)	SP<200 group (n=12)	SP>200 group (n=27)
Age [mean years (range)]	35 (25-50)	40 (25-63)	49 (31-70)	40 (32-65)
Sex (male/female)	9/8	9/11	2/10	5/25
HIV-1 risk category (%)				
Injected drug use	NA	50%	22%	52%
Homosexual intercourse	NA	10%	44%	24%
Heterosexual intercourse	NA	40%	34%	24%
CD4 cell count [median cells/ μ L (IQR)]	852 (793-1352)	33 (1-122)	114 (61-177)	365 (259-435)
Plasma HIV RNA				
Median [copies/mL (IQR)]	NA	70 (8270-8270)	200 (200-9945)	200 (200-200)
Median [log ₁₀ copies/mL (IQR)]	NA	3.9 (3.9-3.9)	0 (0-3.97)	0 (0-0)
Anti-toxoplasma antibody titer [median UI/mL (IQR)]	81 (42-134)	256 (122-300)	125 (55-230)	276 (115-300)

IQR: interquartile range; NA: not applicable

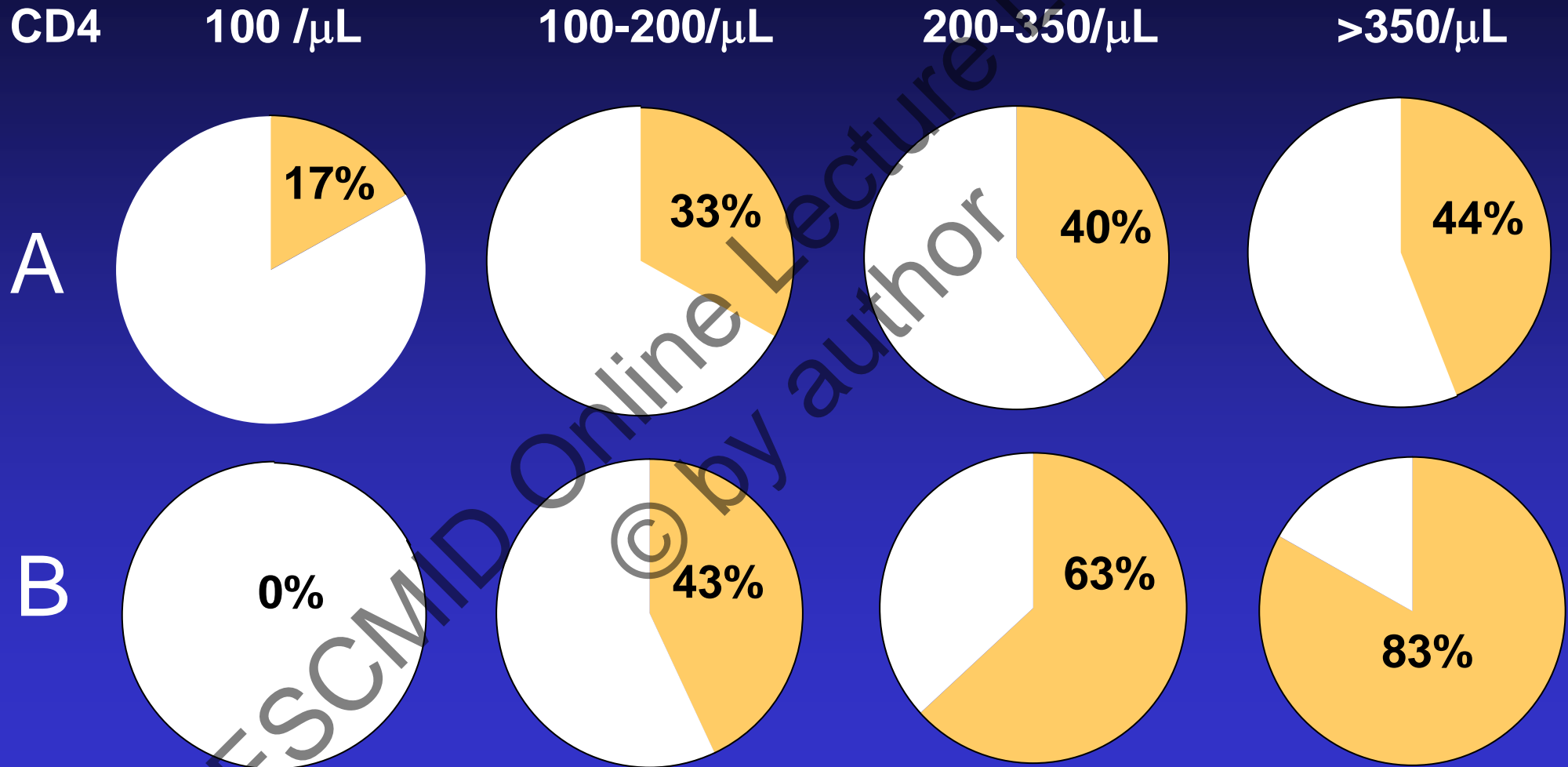
LPR (SI) to SATg



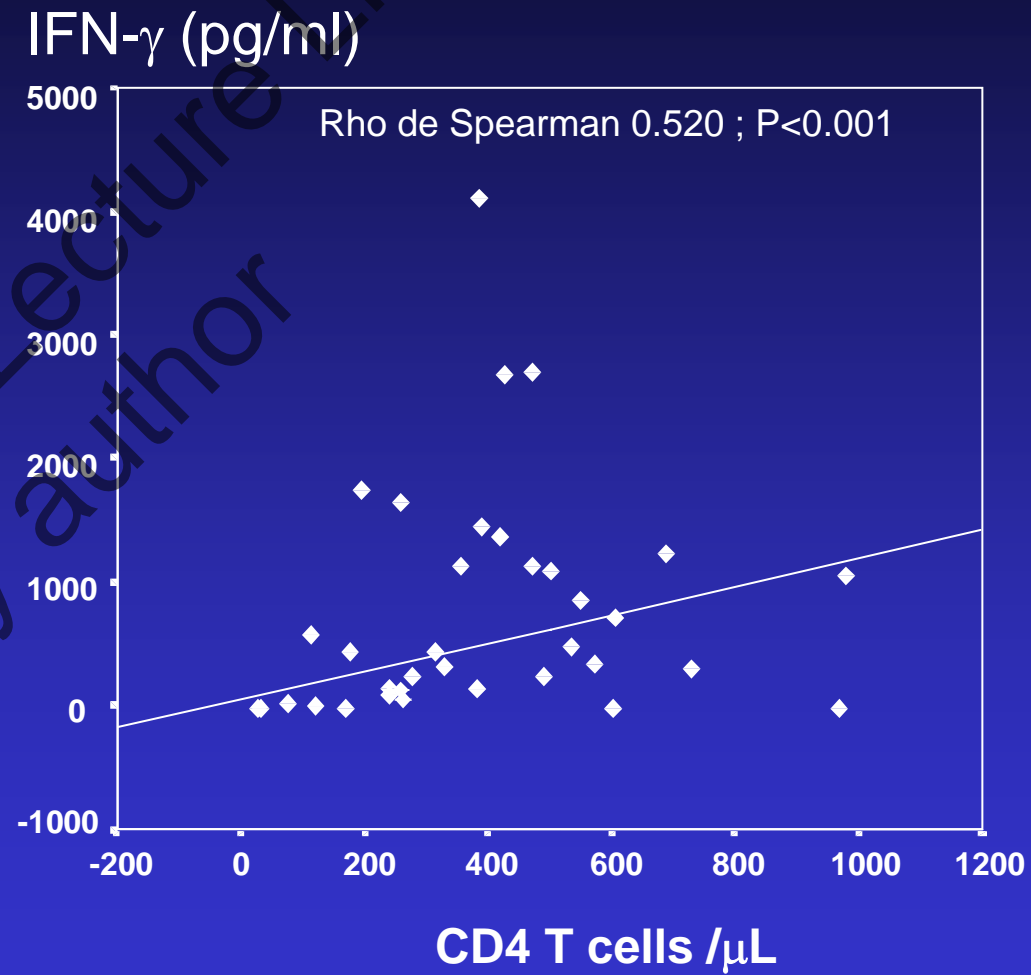
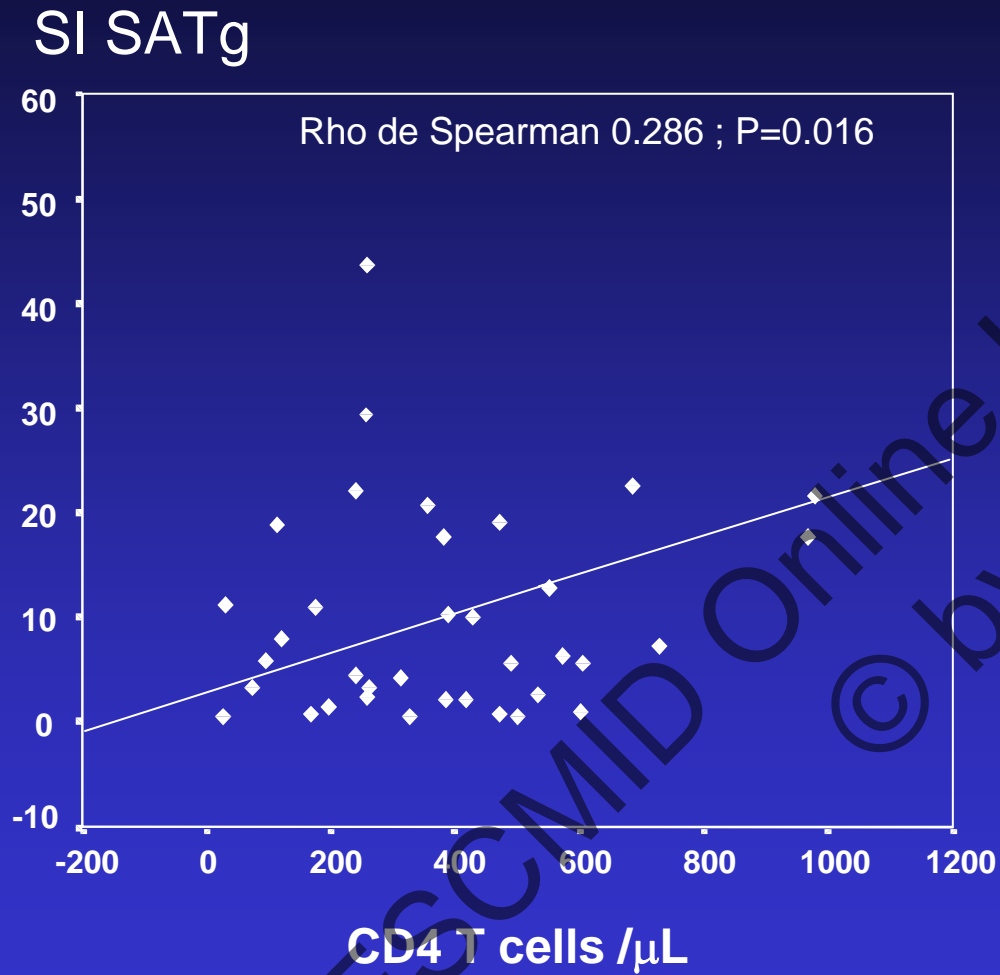
IFN- γ production at 72 hours in response to SATg (Fig. A) and to recombinant *T. gondii* proteins (Fig. B)



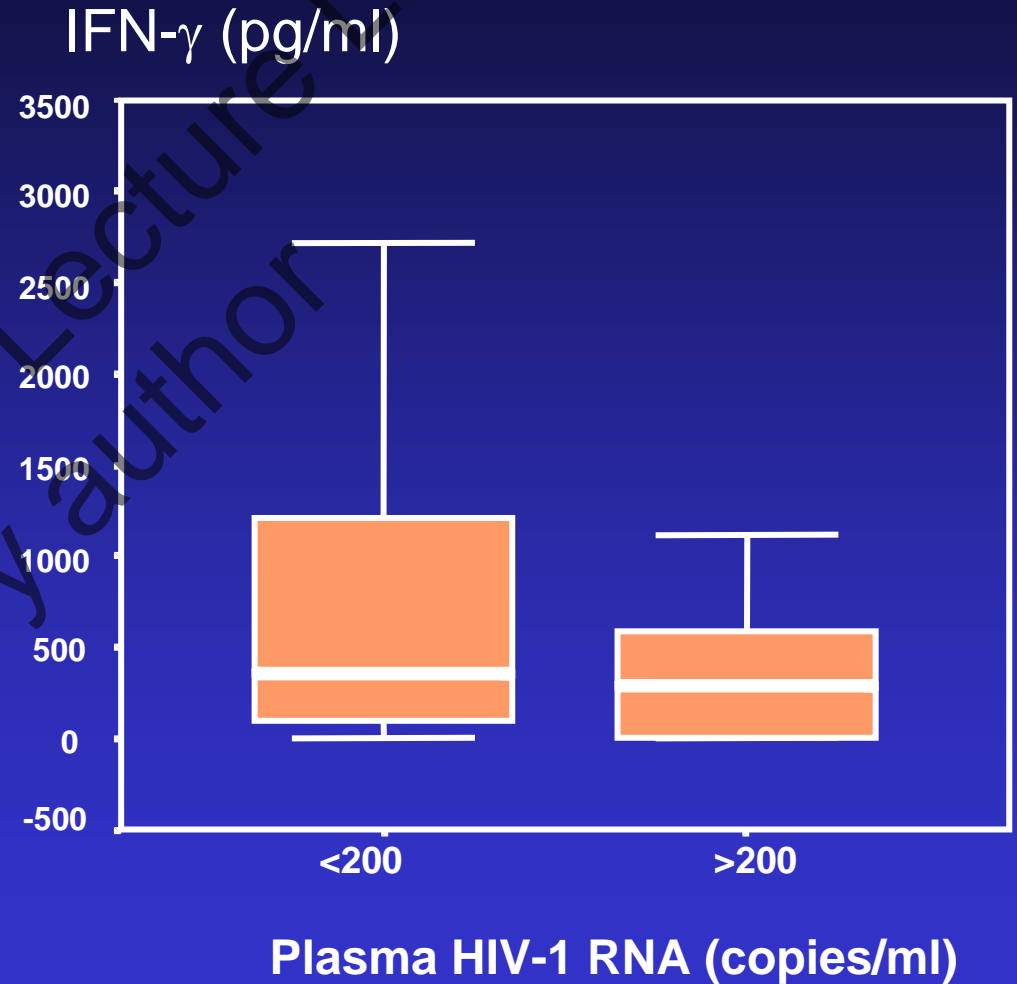
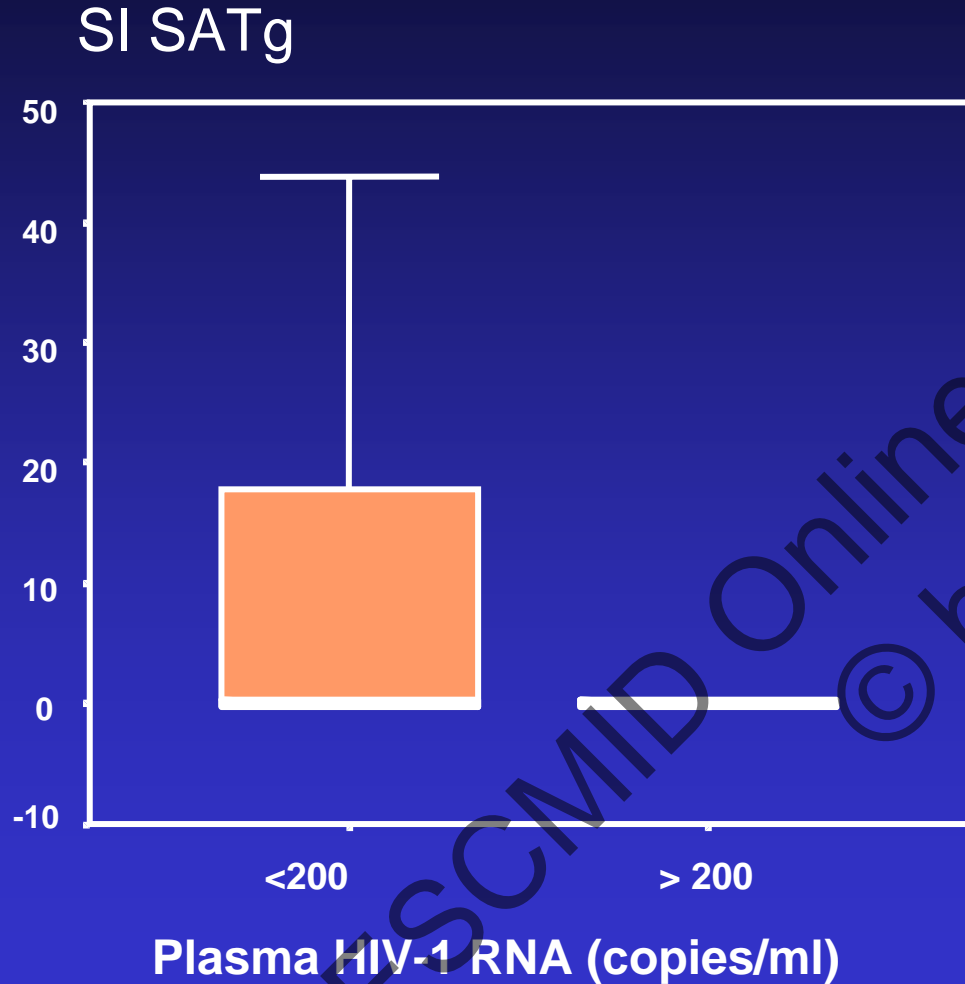
Relationship between CD4 count and the percentage of positive LPR (A) and IFN- γ production (B) to SATg.



Relationship between CD4 count and the LPR (SI) and the production of IFN- γ to SATg



Relationship between plasma viral load, and LPR (SI) and IFN- γ production to SATg



CONCLUSIONS

- T-cell responses to SATg after a TE episode were restored in most AIDS patients who discontinued TE SP after HAART-induced immunological reconstitution.
- This was especially true in terms of INF-g production, a cytokine essential for the control of *T. gondii*.
- These results support the safety of withdrawing TE SP after immunological reconstitution with HAART. These assays may be useful to establish the appropriate time for the TE SP withdrawal.

***Reconstitution of Toxoplasma gondii (Tg)-
Specific CD+ T-Cell Responses in AIDS
Patients with Acute Toxoplasmic Encephalitis
(TE) after Starting Potent Antiretroviral
Therapy (HAART): a Prospective Multicenter
Longitudinal Study.***

Miro JM*, Lejeune M*, Claramonte X, Martínez E, Ribera E¹,
Arrizabalaga J², Arribas JR³, Domingo P⁴, Podzamczar D⁵,
Crespo M¹, Sued O, García F, Plana M, Valls ME, Pumarola T,
Jacquet A⁶, Gallart T, Gatell JM.

IDIBAPS-Hosp. Clínic. University of Barcelona, Barcelona.

¹H. Vall d'Hebron, Barcelona; ²H. Ntra. Sra. de Aranzazu,
San Sebastian; ³H. La Paz, Madrid; ⁴H. Sant Pau, Barcelona;
⁵H. Bellvitge, Barcelona (Spain); ⁶Univ. Libre de Bruxelles (Belgium).

Miro JM et al. Manuscript in preparation.

OBJECTIVE

To assess the evolution of *Toxoplasma gondii*-specific T-cell immune responses in AIDS patients with acute TE after immunological reconstitution with HAART.

CONCLUSIONS

- ✍ During the acute TE episode in AIDS patients, the proliferative T-cell responses and IFN- γ production to SATg production were absent.
- ✍ T-cell responses to SATg were restored 12-18 months after the acute TE episode in most AIDS patients.
- ✍ This was also true in terms of INF- γ production, an essential cytokine for the control of *T. gondii*.
- ✍ The restoration of a positive LPR to SATg was positively correlated with the increase of CD4 T cell counts $> 200/\mu\text{L}$ and negatively correlated with the plasma HIV viral load.

CONCLUSIONS

- ✍ These results demonstrate the restoration of Tg-specific CD4 T cell responses in most AIDS patients with acute TE who reached CD4+ T cell counts above 200 cells/microL after 12-18 months of the acute TE episode.
- ✍ These data can help to better determine the appropriate timing to stop TE secondary prophylaxis in AIDS patients receiving HAART.

ACKNOWLEDGEMENTS

- This study was supported by **FIPSE** (Spanish Ministry of Health, Abbott Laboratories, Boehringer Ingelheim, Bristol Myers Squibb, GlaxoSmithKline, Merck Sharp and Dohme, **grant number 99/2010**) and the **Fundación Máximo Soriano Jiménez, Barcelona (Spain)**.
- Other members of the study: E. de Lazzari (H. Clinic); M. Crespo, V. Falco (H. Vall d'Hebron), MA Sambeat (H. Sant Pau) B. Gimeno, JA Iribarren (H. Ntra. Sra. de Aranzazu, Donosti), J. Gonzalez, JM Peña (H. La Paz, Madrid); M. Haumont (Univ. Libre de Bruxelles, Belgium).