Patogenesi: news dai congressi

Dott.ssa Stefania Piconi

I Divisione Malattie Infettive

AO L. Sacco, Milano

- **Immunoattivazione: cause e conseguenze (funzione del SI, replicazione virale e cliniche)**
- **Il recupero immunologico, significato clinico**
- **Terapia antiretrovirale precoce, quali vantaggi**
- **Infezione acuta**
- **PTC**
- **GUT e CMV**
- **Latenza virale**

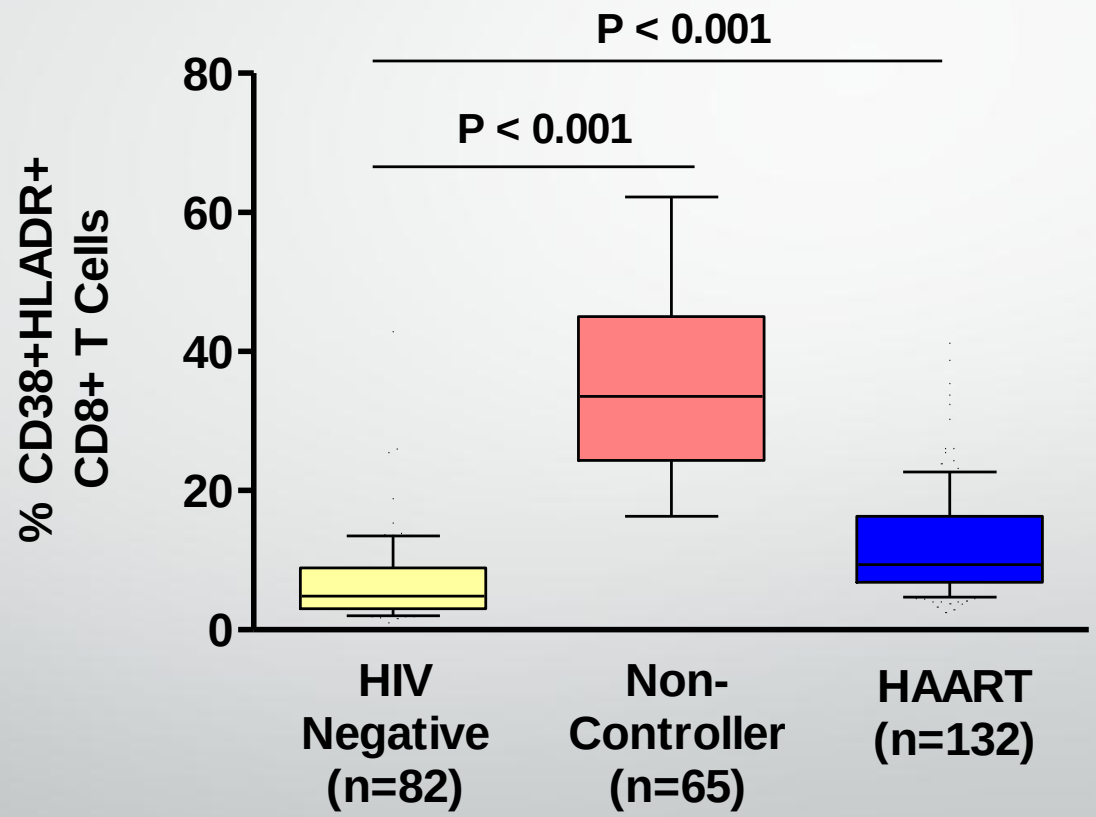
Immunoattivazione: cause e conseguenze

Immunoattivazione: cause

- HIV induce attivazione del sistema immune innato (Bhardway, Greene)
  - Il VL da solo è un debole marcatore di progressione di malattia (Rodriguez JAMA 2006)
  - L'immunoattivazione predice la progressione di malattia indipendentemente dal VL (Giorgi, Deeks)
  - Elite controllers hanno un aumento di CD38+ Tcells (Hunt)
  - Quando il VL è soppresso con la ART, persiste immunoattivazione che predice progressione
- Aumentata carica antigenica (batterica e virus erpetici) (Deeks)
- Aumentato metabolismo del triptofano (IDO) (McCune, Hunt)
- Immunologico e strutturale danno del gut, aumentata permeabilità mucosale, traslocazione di prodotti microbici in circolo (Brenchley, Douek)

Questi stimoli, attraverso attivazione di sensori cellulari dell'immunità innata, inducono produzione di citochine infiammatorie quali IL1b, IL-6 e IFN I

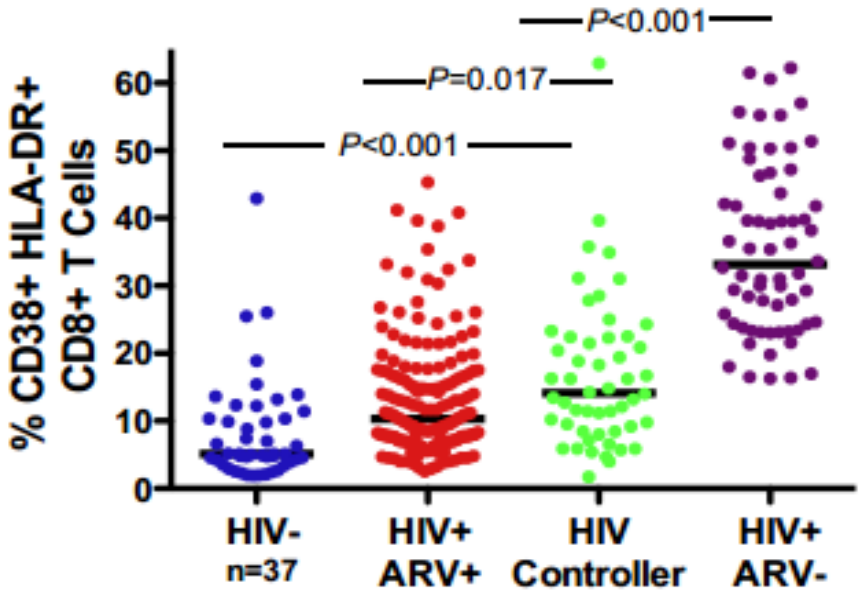
L’immunoattivazione persiste nonostante la ART virologicamente efficace



Hunt PW, et al. JID 2003,187:1534-43

HIV Controllers and Immune Activation

- Controllers also have:
  - Microbial Translocation (Douek 2006; Hunt, *JID* 2008)
  - Monocyte activation (Pereyra, *AIDS* 2012)
  - Atherosclerosis (Hsue, *AIDS* 2009; Pereyra, *AIDS* 2012)
  - Lymphoid fibrosis (Sanchez, CROI 2013)



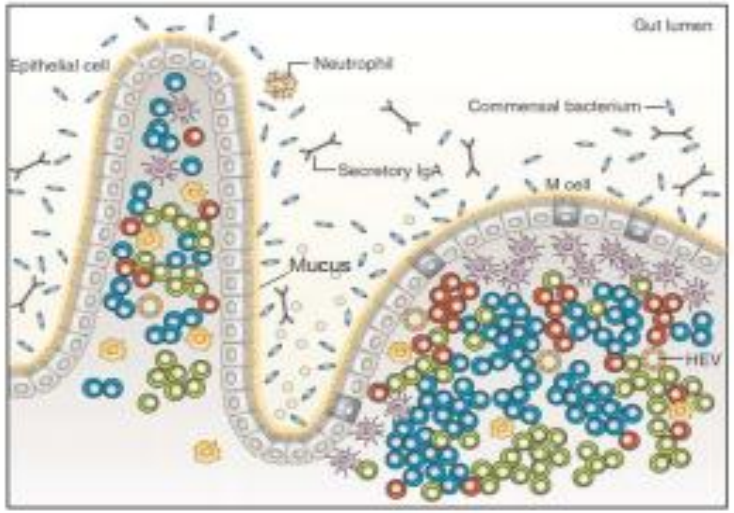
Hunt, *JID* 2008; Hunt, *PLoS One* 2011.

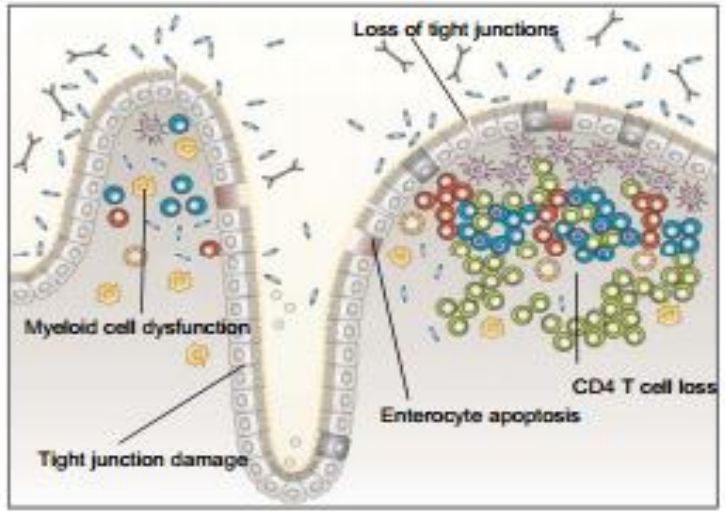
Even HIV controllers have elevated immune activation

ART reduces measures of virus and immune activation

(Hatano, *PLoS Path* 2013)

Microbial Translocation is a "Multi-Hit" Affair





Healthy Gut

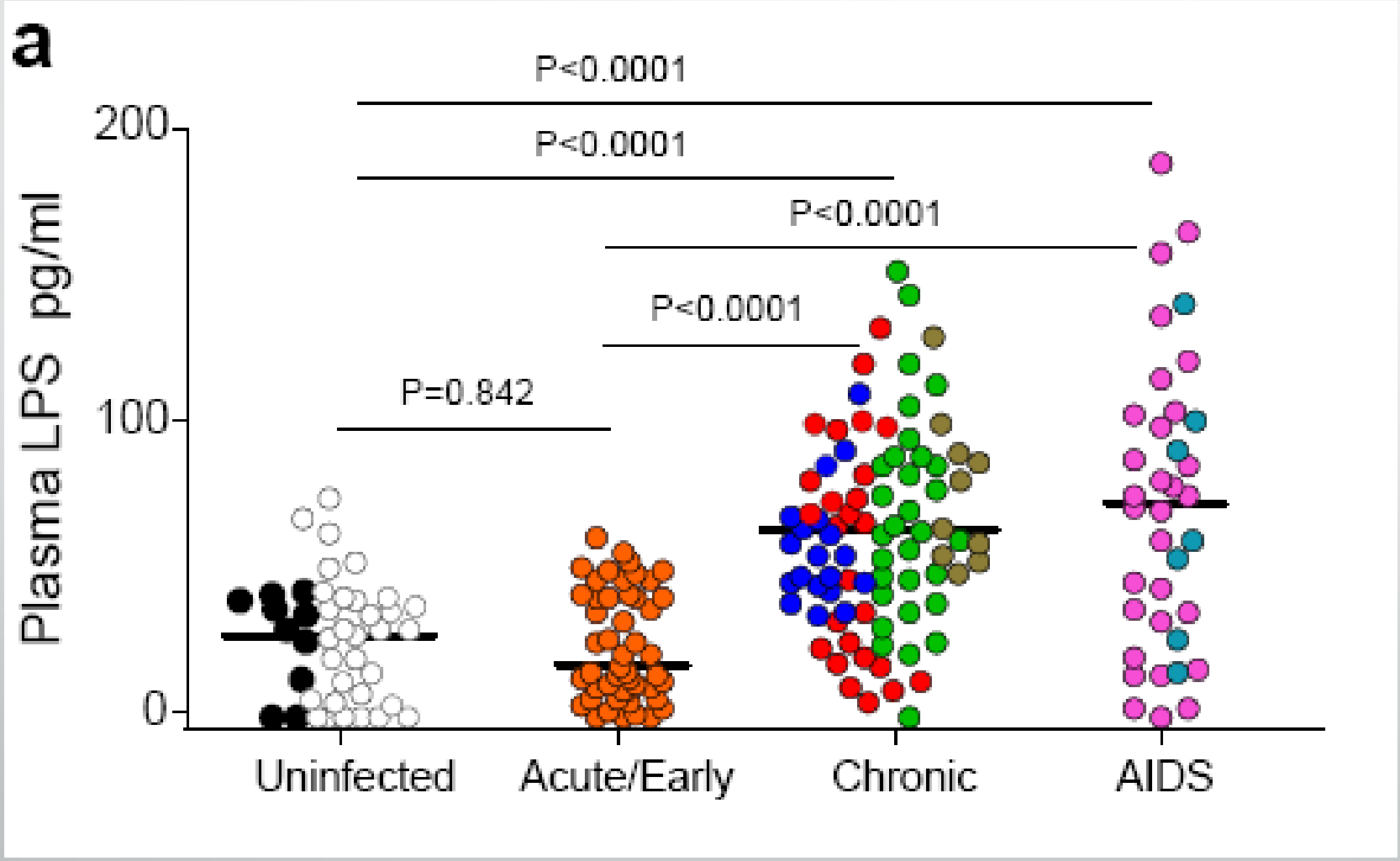
- Tight epithelial junctions, mucus
- Anti-microbial peptides
- Secreted antibodies
- Phagocytes, neutrophils, T cells
- Cross-talk maintains the barrier

HIV-Infected Gut

- Massive loss of CD4 T cells
- Preferential loss of Th17 cells
- Myeloid and B cell dysfunction
- Epithelial apoptosis
- Tight junction disruption
- Translocation of microbial products

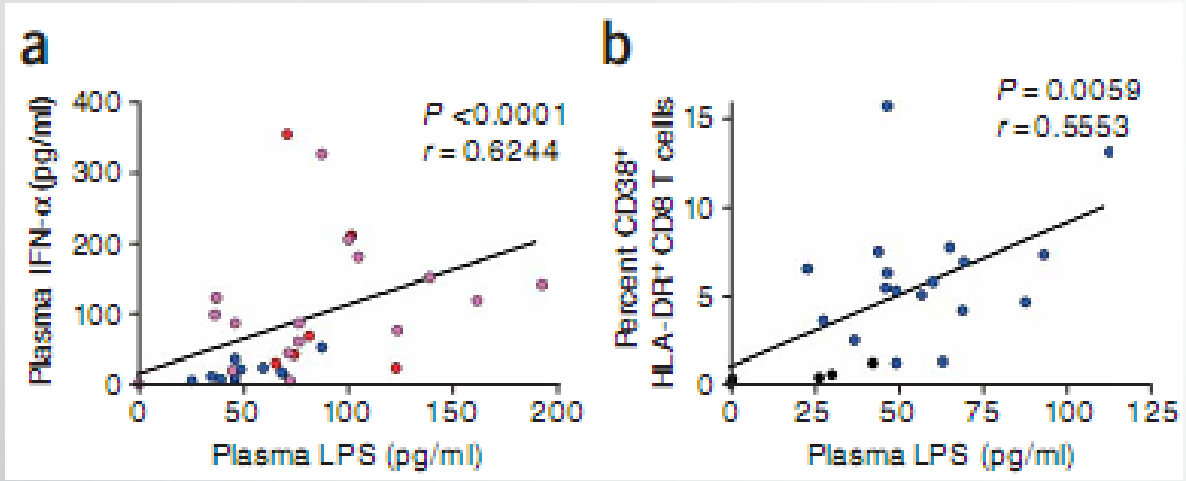
Veazey, Science 1998; Guadalupe, J Virol 2003; Brenchley, J Exp Med 2004; Mehandru, J Exp Med 2004; Brenchley Nat Med 2006; Li, JID 2008; Epple, Gut 2009

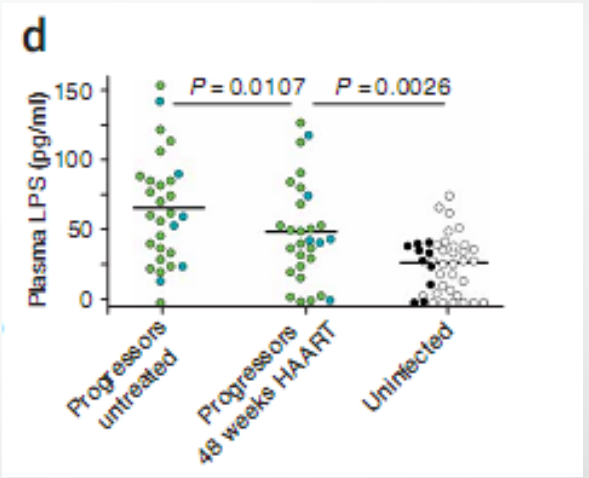
Traslocazione microbica e infezione da HIV



Brenchley et al, *Nat Med* 06

Traslocazione microbica e immunoattivazione

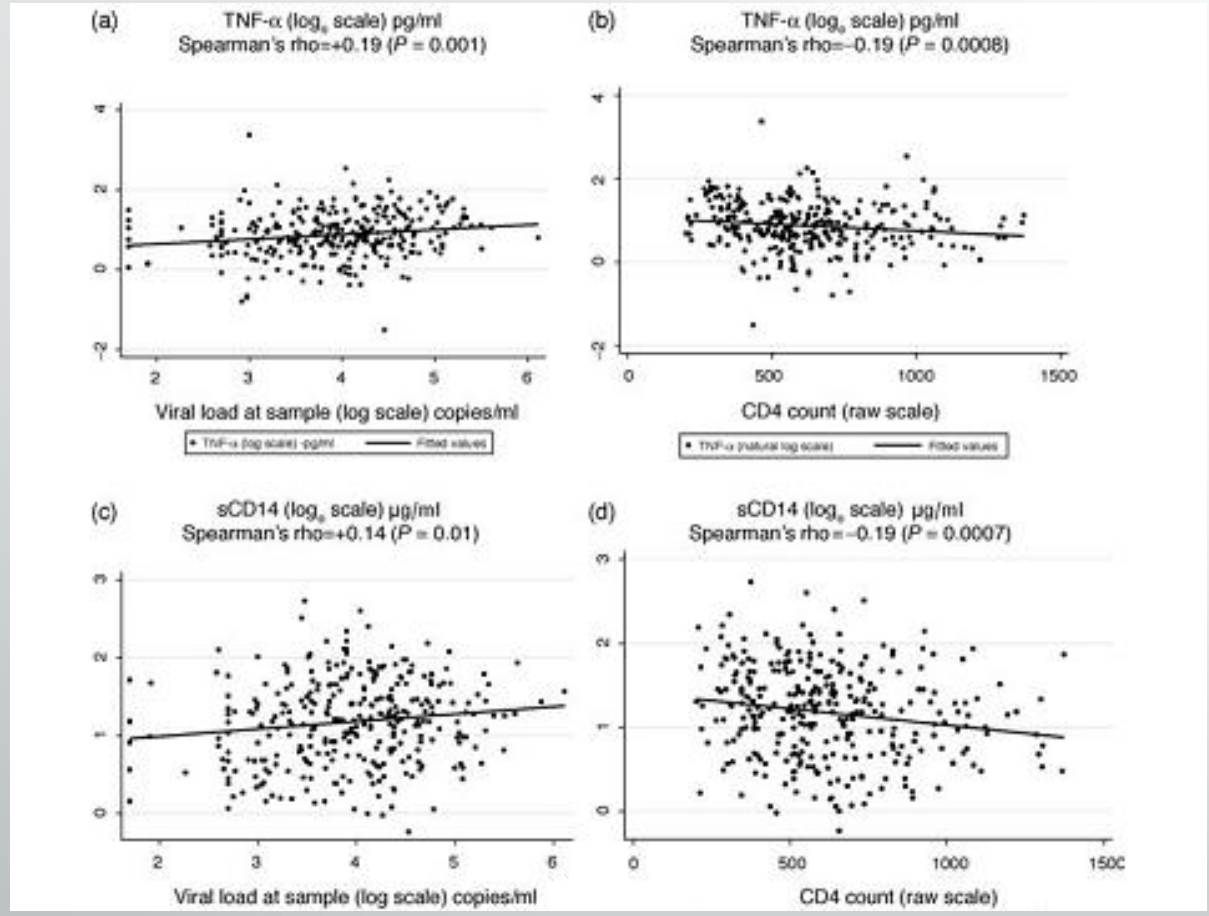


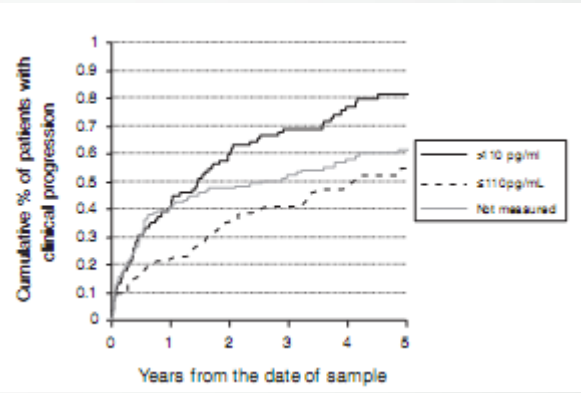


I prodotti della traslocazione microbica contribuiscono allo stato di immunoattivazione sistemica e si associano alla progressione dell'infezione

Brenchley et al, Nat Med 06

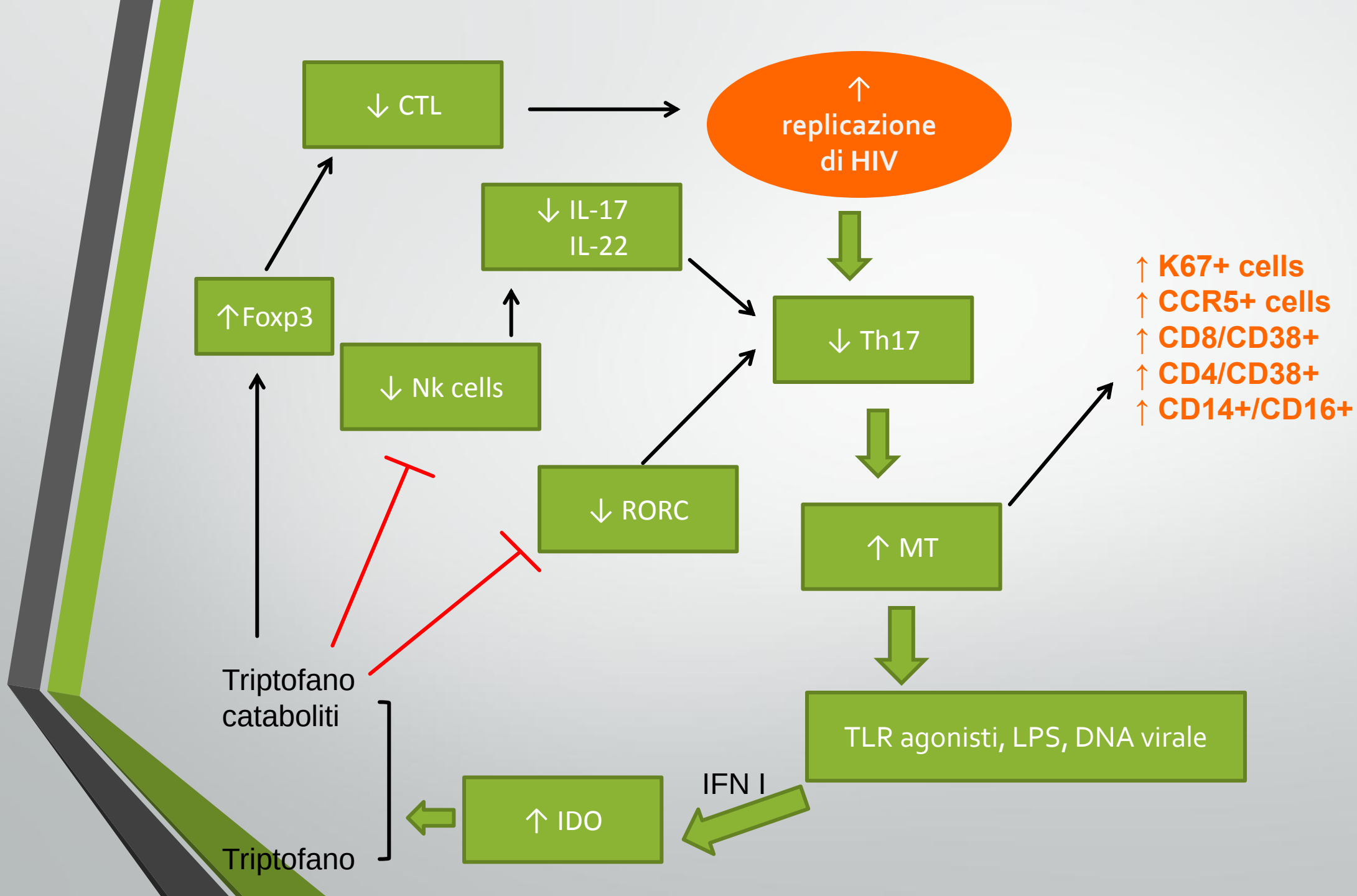
Traslocazione microbica e outcome clinico



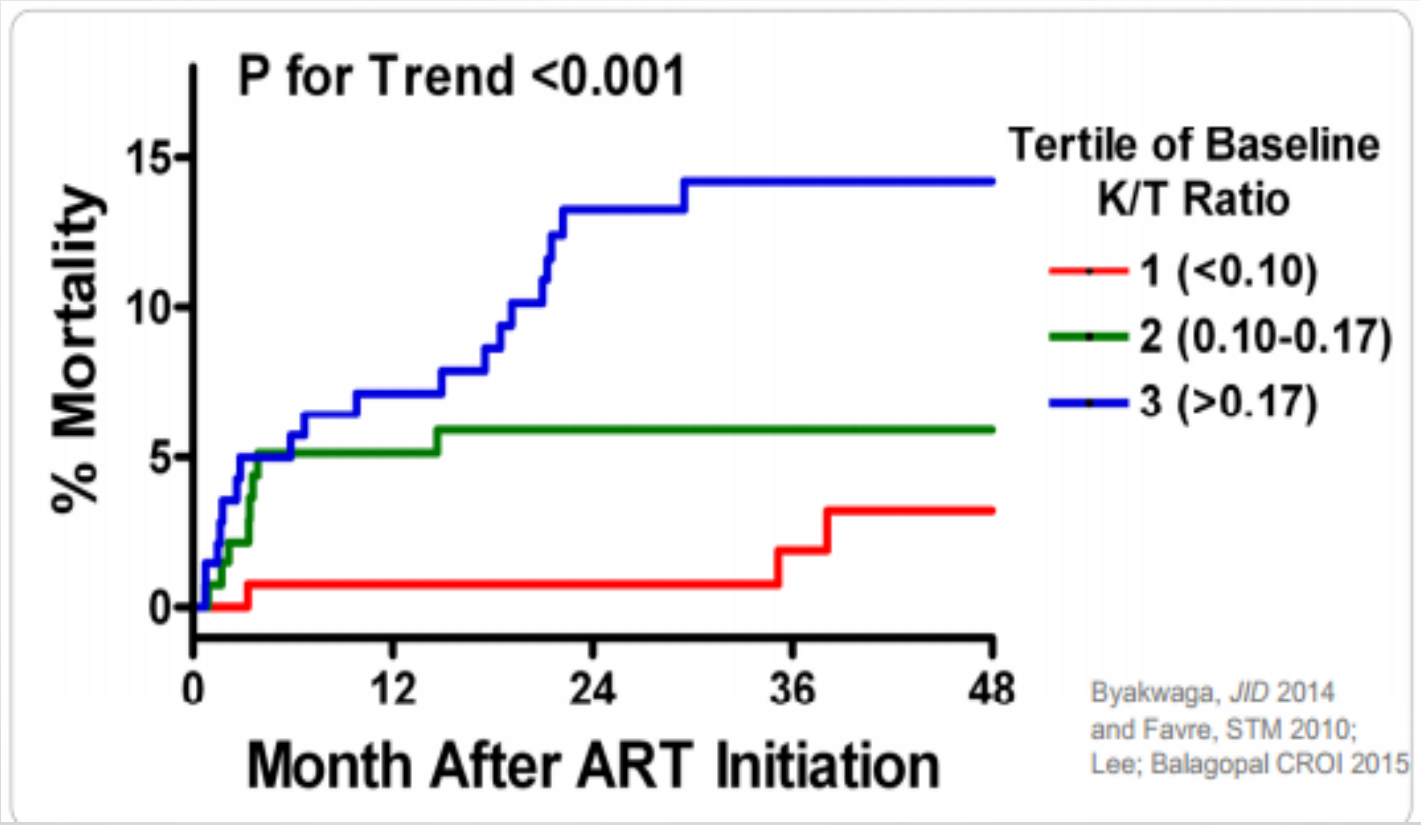


La traslocazione microbica correla con la progressione della malattia e l'immunoattivazione

Marchetti G AIDS, 2011 25:1385-94


↓ CTL
↑ replicazione di HIV
↓ IL-17 IL-22
↑Foxp3
↓ Nk cells
↓ Th17
↑ K67+ cells
↑ CCR5+ cells
↑ CD8/CD38+
↑ CD4/CD38+
↑ CD14+/CD16+
↓ RORC
↑ MT
Triptofano cataboliti
TLR agonisti, LPS, DNA virale
IFN I
↑ IDO
Triptofano

IDO pathway e patogenesi di HIV

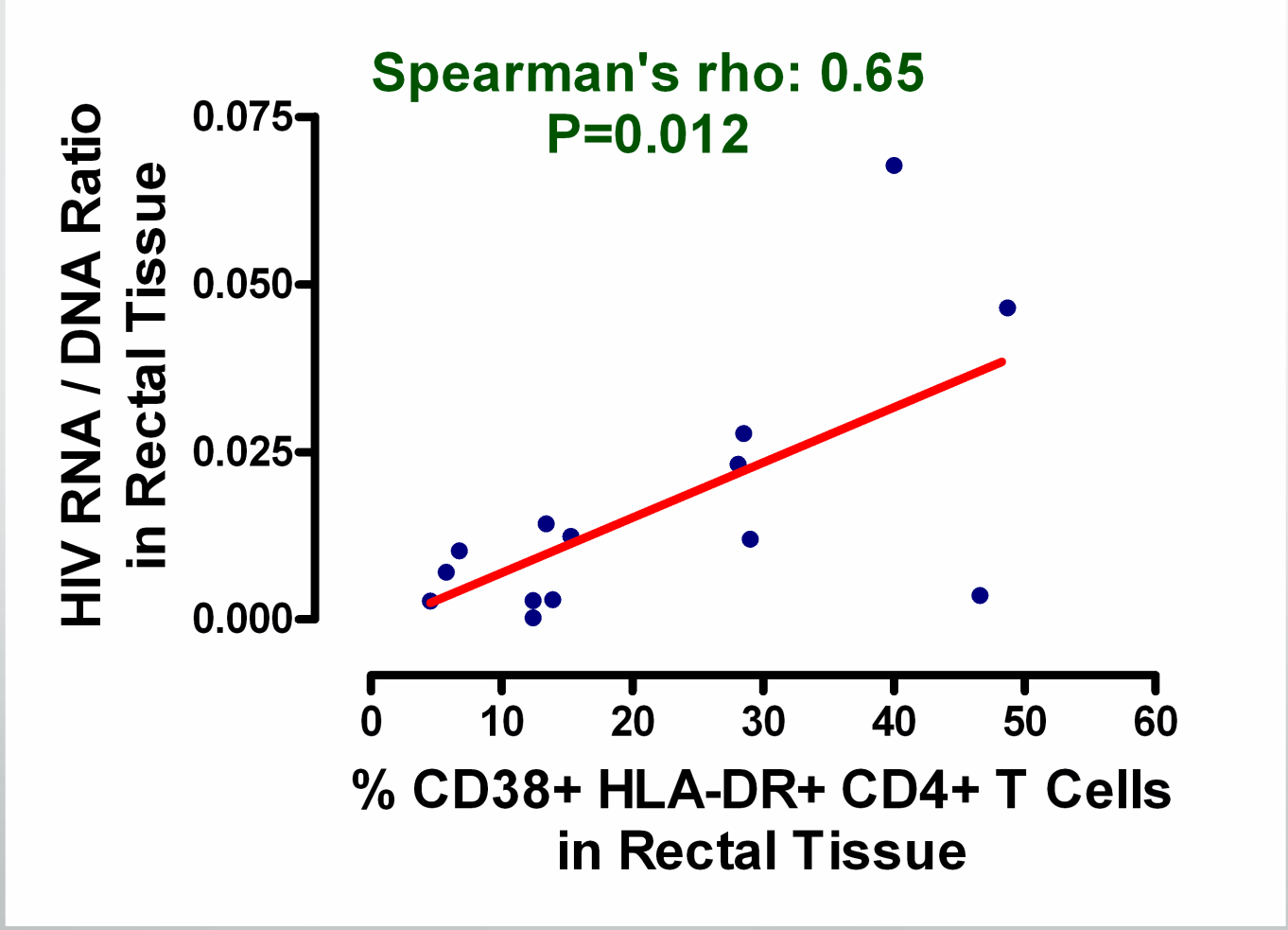


L'elevata attivazione di IDO (rapporto K/T) predice l'aumentata mortalità in una coorte di HIV ugandesi in ART (corretto per BMI, CD4 e CD8+38+)

Immunoattivazione: conseguenze sulla funzione del SI e sulla replicazione virale

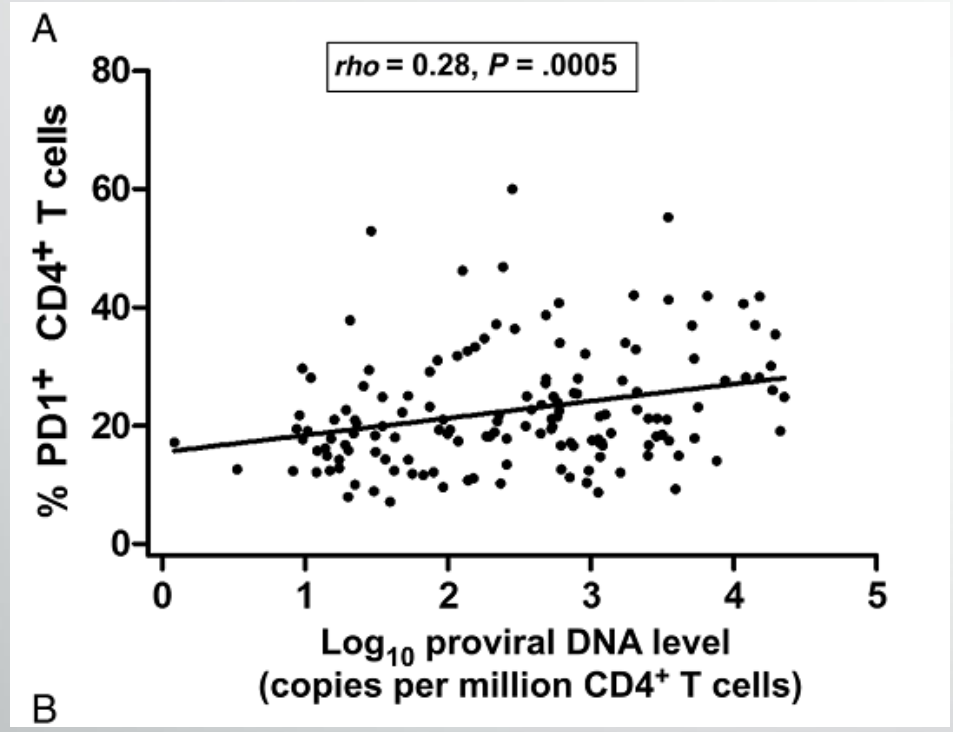
- Si associa alla persistenza virale (Hunt)
- Altera l'immunoricostituzione se persiste nonostante la ART (Kelley CF CID, 2009 Piconi S AIDS 2010)
- Distrugge le sedi anatomiche del SI (fibrosi) (Estes JD JID,207)
- Altera la risposta immune HIV-specifica cellulare e umorale (Appay V J Exp. Med 2000)
- Favorisce l'esaurimento del SI (Yi JS Immunology 2010) ed un'eccessiva apoptosi non antigene-specifica (Vanderfort TH Future Med 2010)

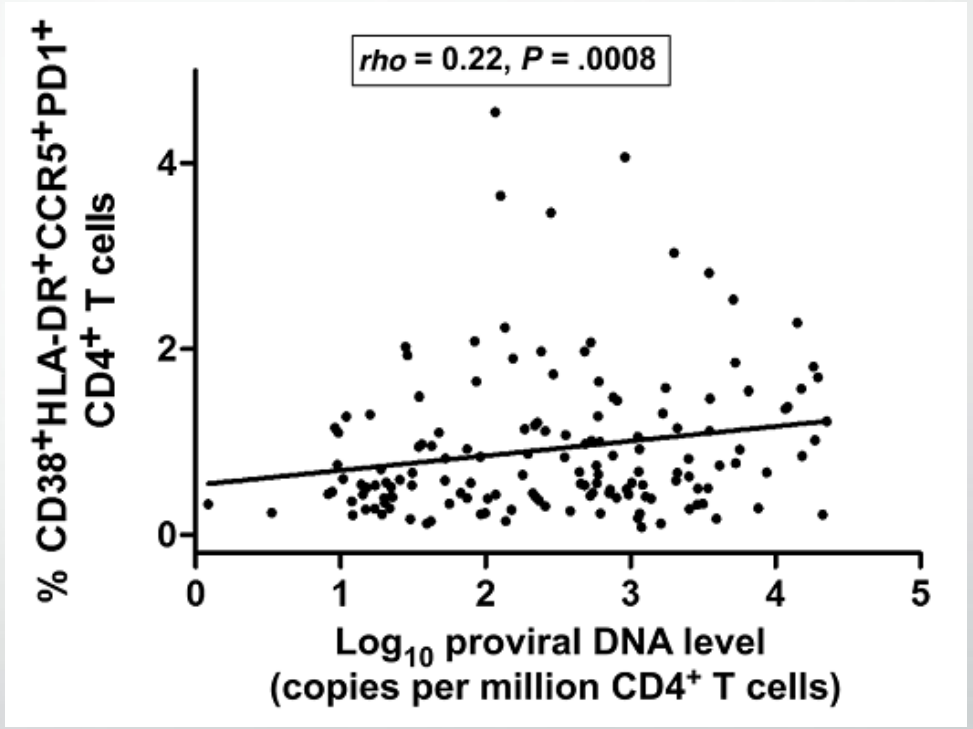
Associazione tra persistenza virale e immuno-attivazione tissutale



Hunt, Yukl e Wong

Cell-Based Measures of Viral Persistence Are Associated With Immune Activation and Programmed Cell Death Protein 1 (PD-1)–Expressing $CD4^+$ T cells

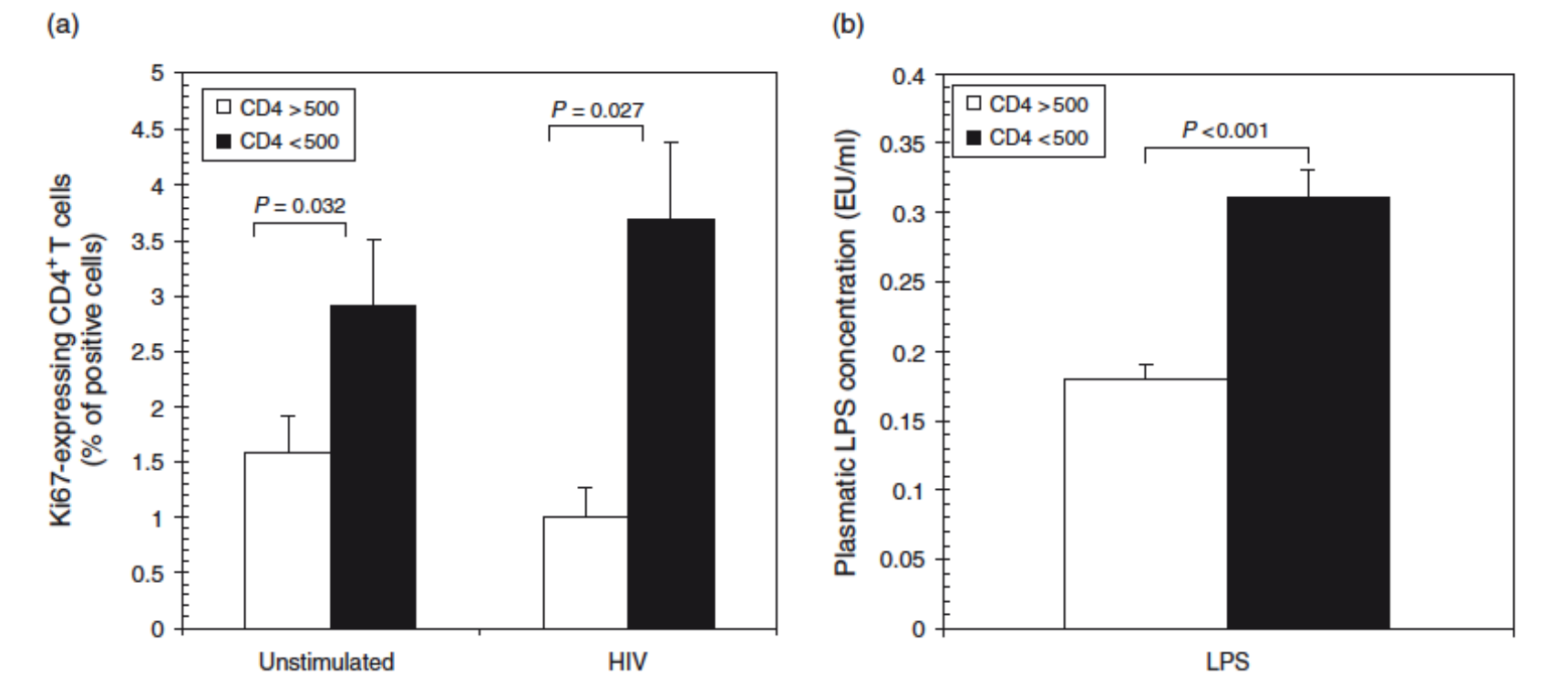




Associazione positiva tra HIV-DNA e PD-1^+ TCD4+ e CD38-$DR^+CCR5^+PD1^+$

Hatano H. JID 2013;208:50-6

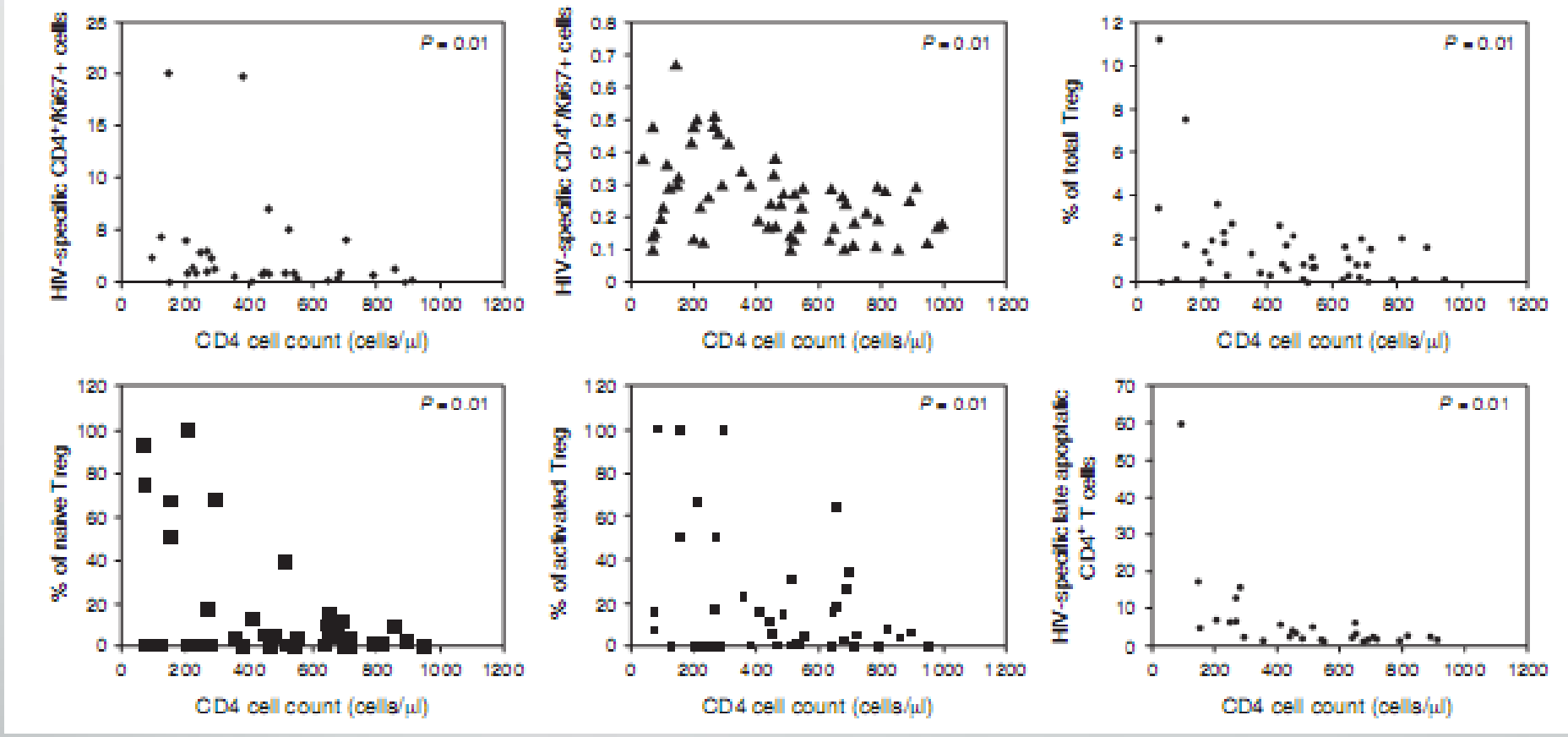
Immune activation, apoptosis, and Treg activity are associated with persistently reduced $CD4^+$ T-cell counts during antiretroviral therapy



Il mancato recupero dei CD4 si associa ad aumentata immuno-attivazione, CD4+/Ki67+, MT e %Treg

Piconi S. AIDS 2010:24:1991-2000

Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4$^+$ T-cell counts during antiretroviral therapy



Nei pazienti con CD4<500: correlazione inversa tra i CD4/LPS,T attivati, T reg e T apoptotiche

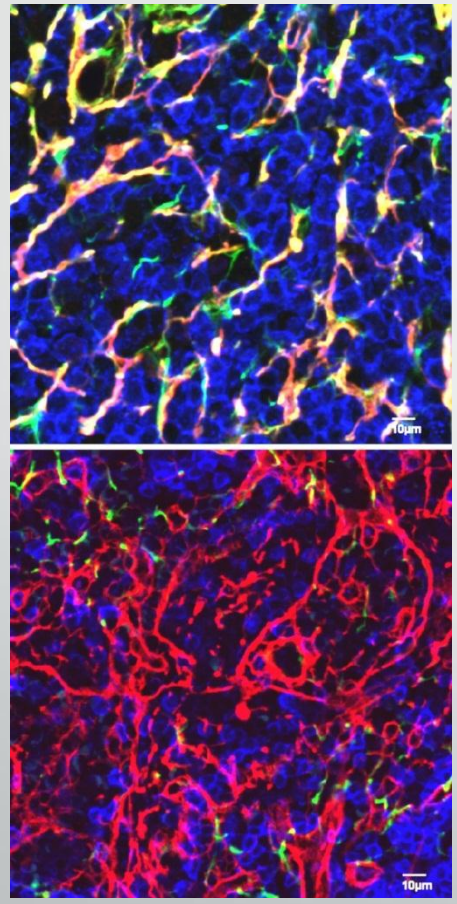
Piconi S. AIDS 2010:24:1991-2000

Lymphoid Tissue Damage in HIV-1 Infection Depletes Naïve T Cells and Limits T Cell Reconstitution after Antiretroviral Therapy

Ming Zeng[1], Peter J. Southern[1], Cavan S. Reilly[2], Greg J. Beilman[3], Jeffrey G. Chipman[3], Timothy W. Schacker[4], Ashley T. Haase[1]*

1Department of Microbiology, Medical School, University of Minnesota, Minneapolis, Minnesota, United States of America, 2 Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota, United States of America, 3Department of Surgery, Medical School, University of Minnesota, Minneapolis, Minnesota, United States of America, 4Department of Medicine, Medical School, University of Minnesota, Minneapolis, Minnesota, United States of America

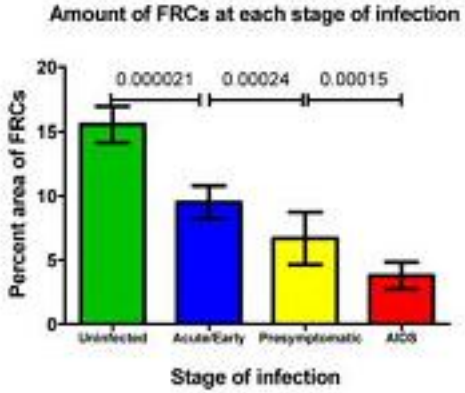
PLoS Pathogens2012;8(1):e1002437

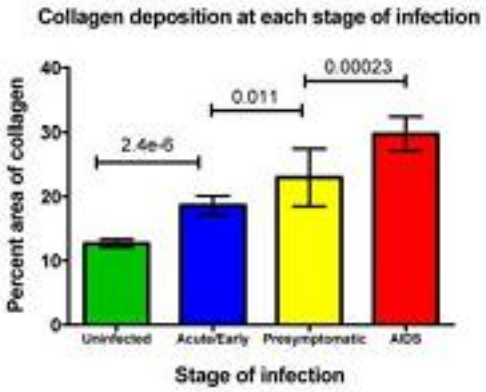


CD3+

Collagen 1 +

Desmin +





Infiammazione cronica → risposta T reg → TGF-β → deposizione di collagene→ Fibrosi→ ridotta IL-7 → ridotta rigenerazione di T cell → Infiammazione

Lymphoid Tissue Damage in HIV-1 Infection Depletes Naïve T Cells and Limits T Cell Reconstitution after Antiretroviral Therapy

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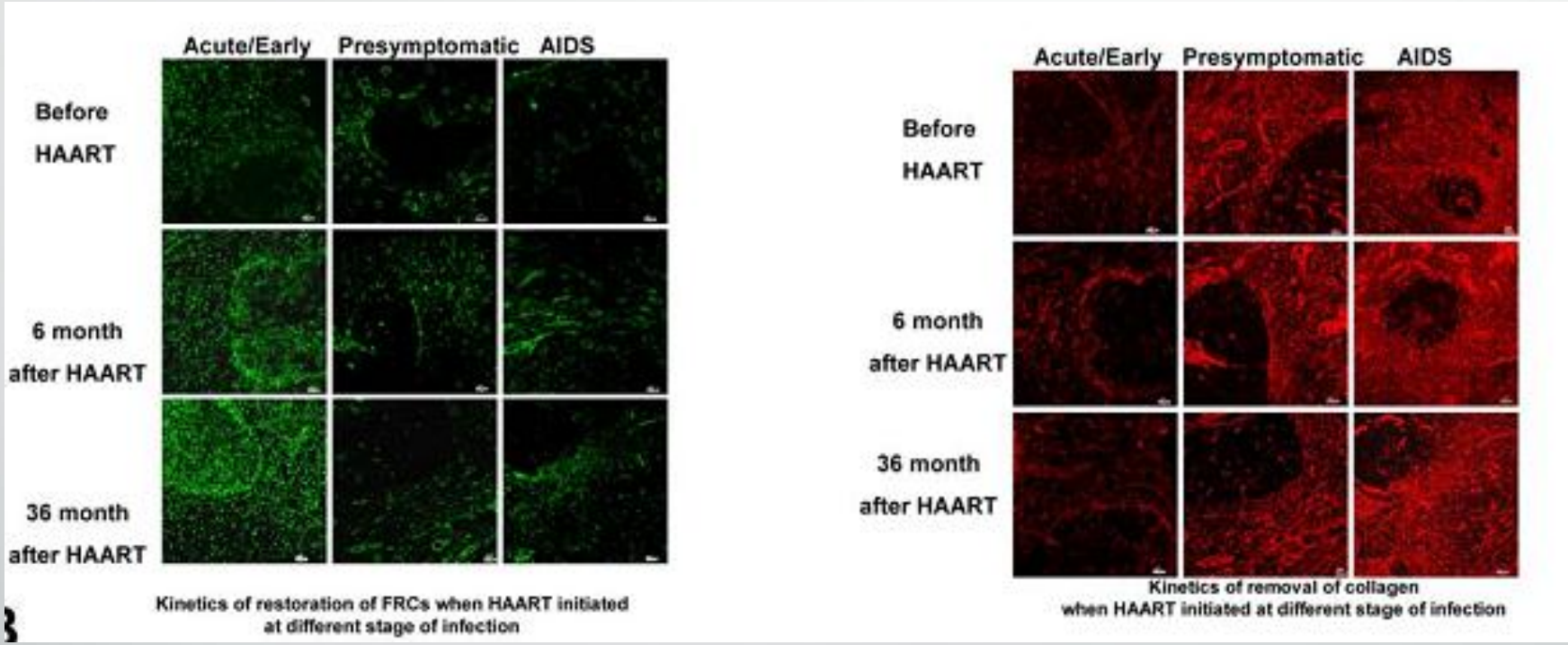
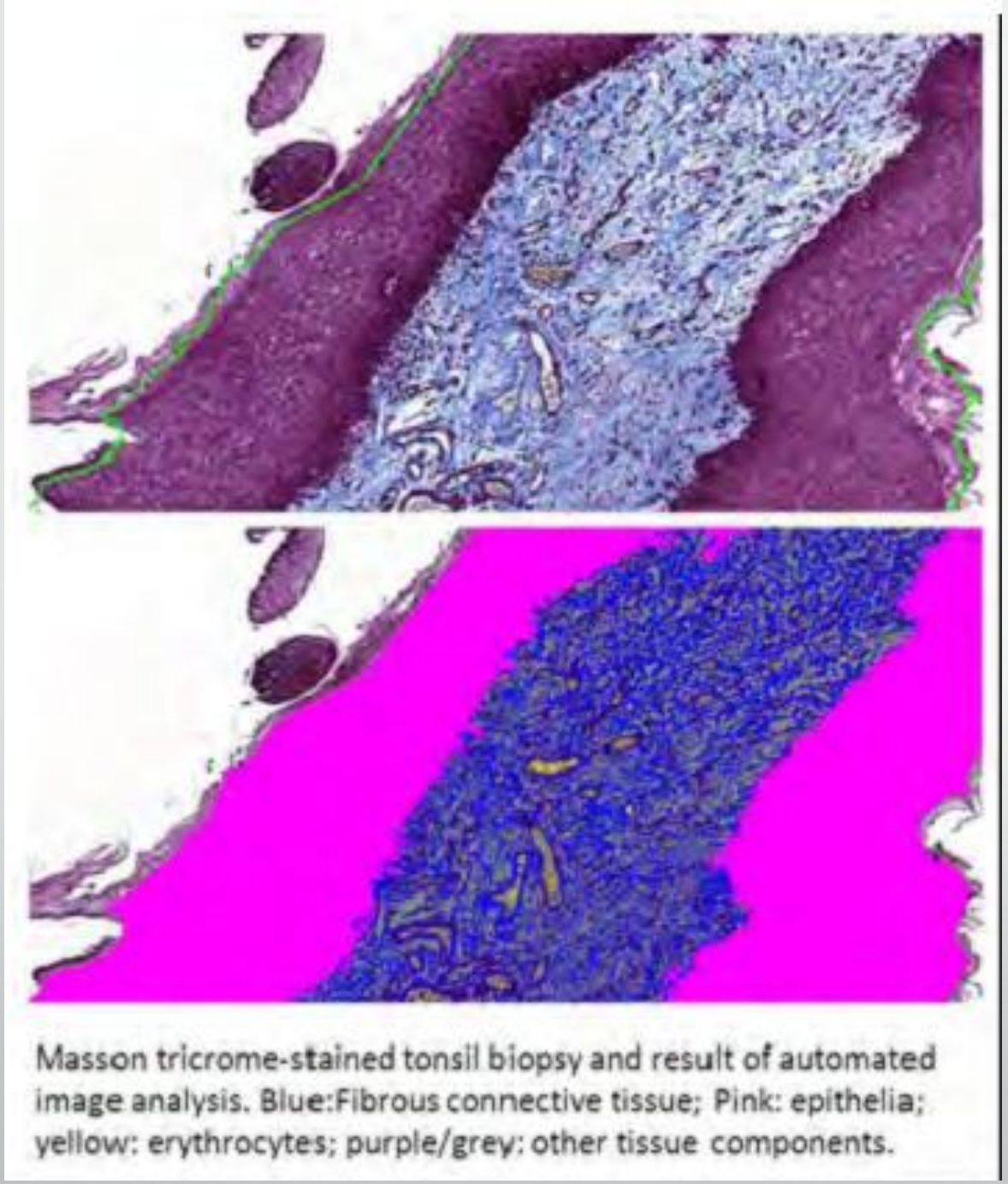


Un trattamento precoce corrisponde ad una minor deposizione di collagene a livello del tessuto linfoide e ad un aumento delle cellule T naive

PLoS Pathogens2012;8(1):e1002437

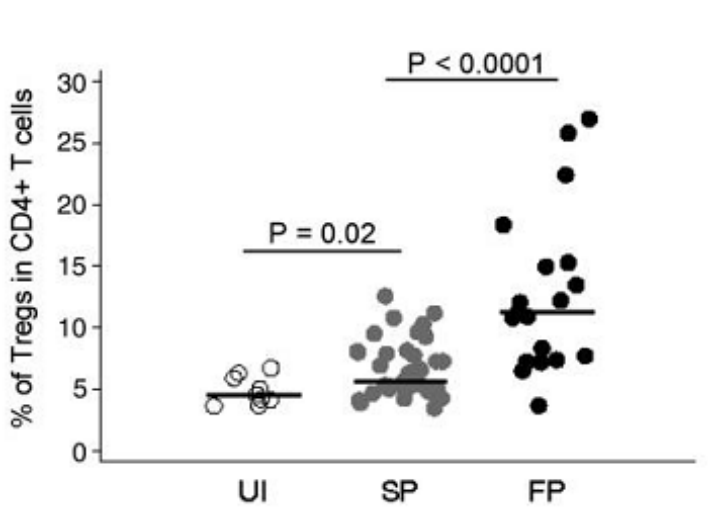


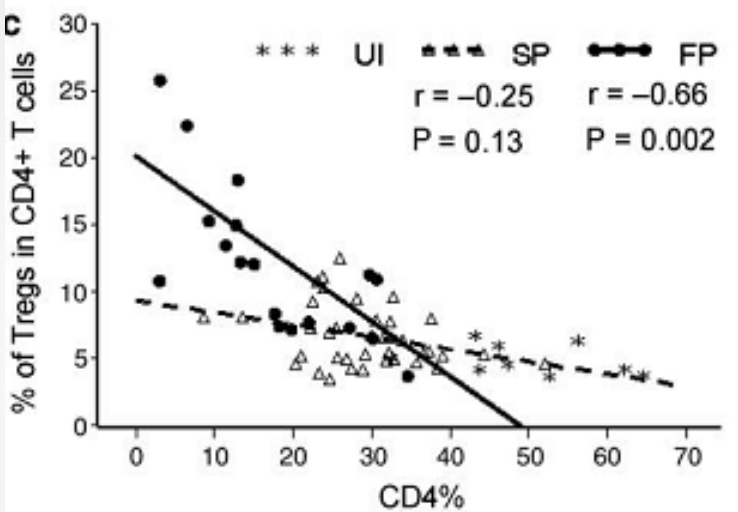
Masson tricrome-stained tonsil biopsy and result of automated image analysis. Blue:Fibrous connective tissue; Pink: epithelia; yellow: erythrocytes; purple/grey: other tissue components.

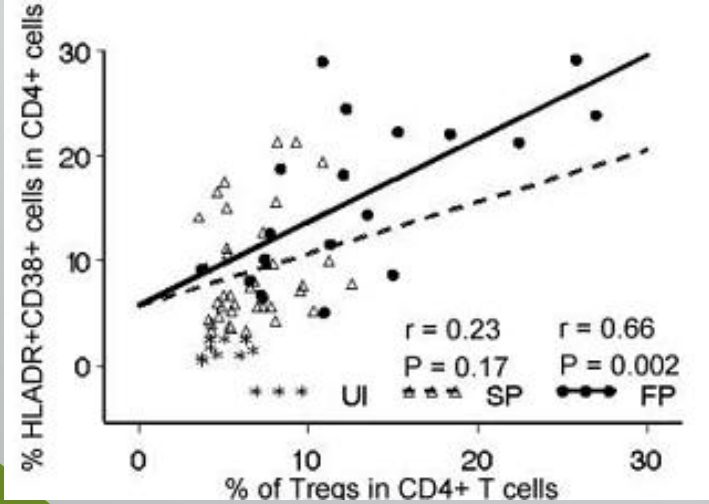
La bassa fibrosi a livello del tessuto linfoide si associa a una ridotta % di Treg e immuno attivazione

Possibile ruolo delle Treg nella patogenesi della fibrosi linfonodale nei pazienti HIV+

HIV e CD4 Treg





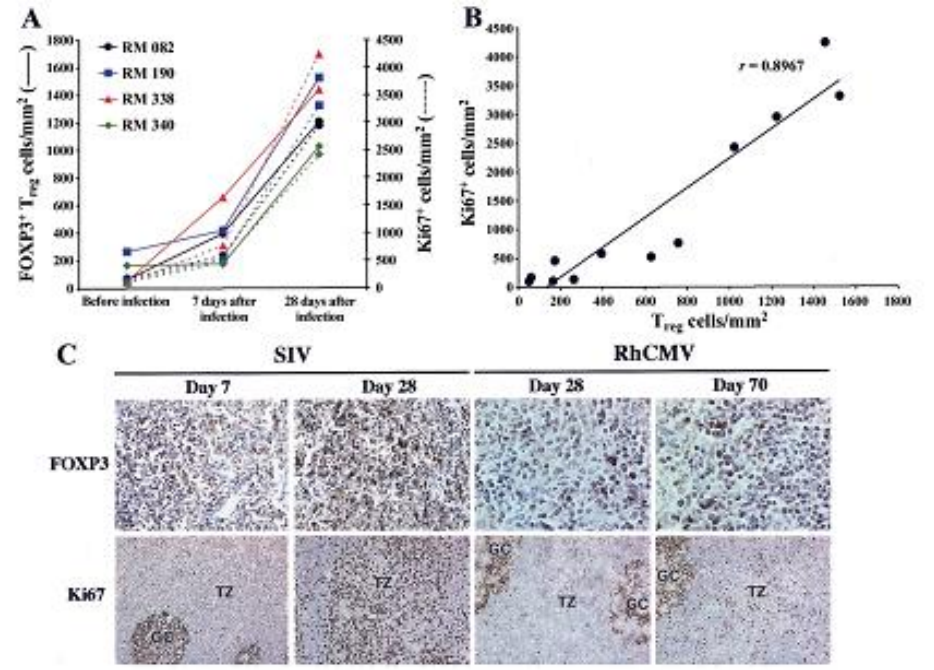


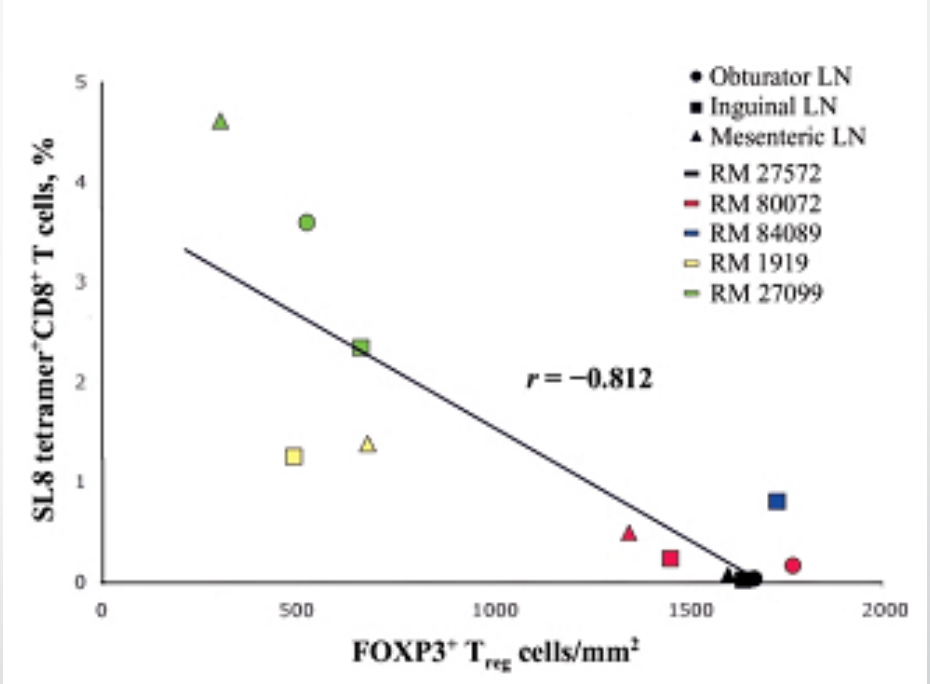
Nei pazienti con rapida progressione è presente una diretta correlazione tra l'immuno-attivazione e % di linfociti CD4 Treg

E' presente anche un aumento di linfociti T CD8 Foxp3+

Cao W. AIDS Res. and Hman Retroviruses, 2009:25;183-91

Treg e CTL nel modello animale (SIV)

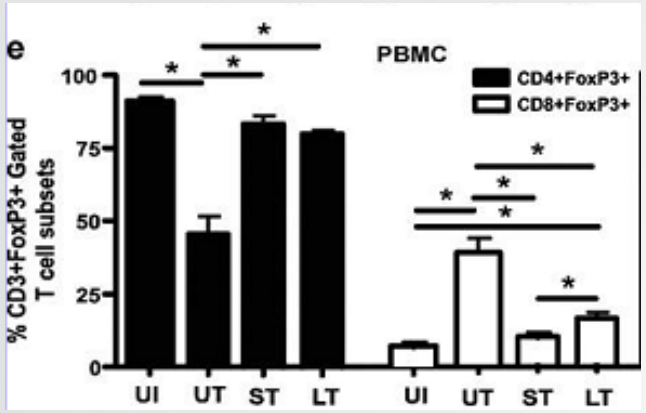


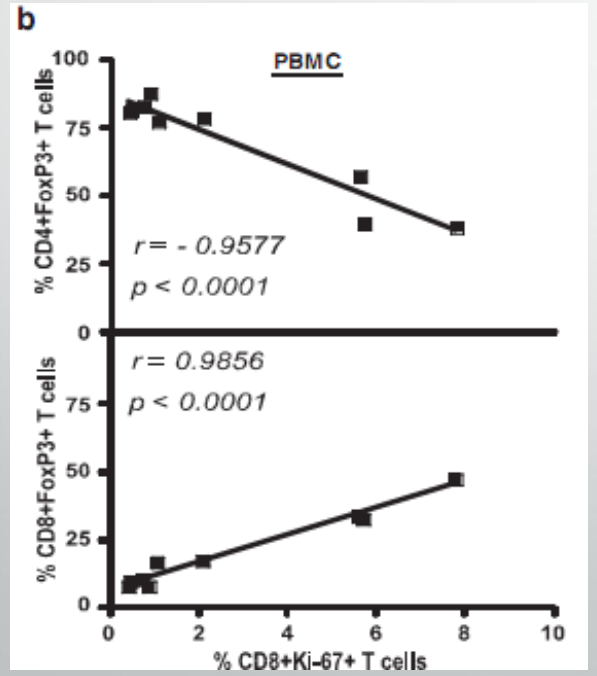


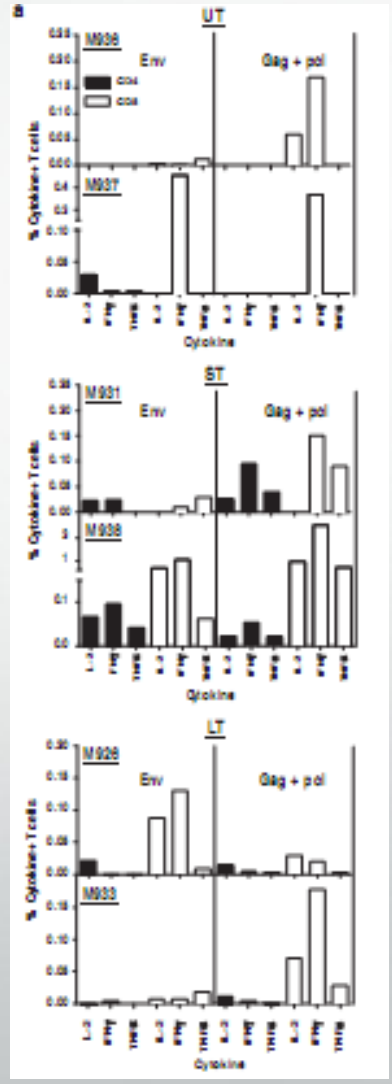
In RMs la precoce comparsa di linfociti T regolatori compromettono la risposta antivirale SIV-specifica cellulo-mediata

Estes JD JID 2006,193:703-12

HIV e CD8Treg/CTL







La terapia precoce, riduce il viral set-point e l'immunoattivazione.

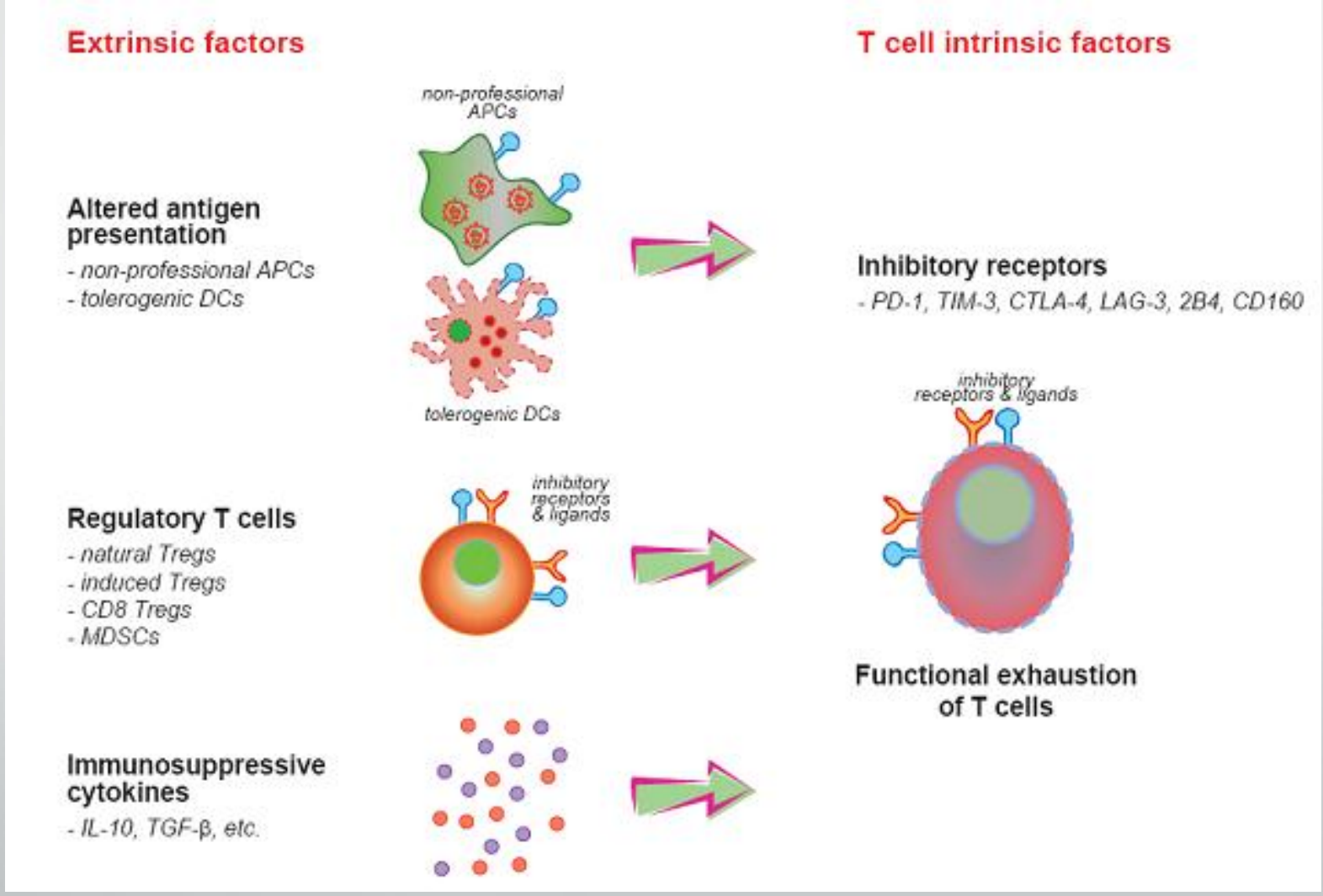
La riduzione precoce dalla replicazione virale previene l'espansione di linfociti T CD8Treg e preserva la risposta HIV specifica cellulo-mediata e umorale

Early Short-Term Antiretroviral Therapy Is Associated with a Reduced Prevalence of $CD8^{+}FoxP3^{+}$ T Cells in Simian Immunodeficiency Virus-Infected Controller Rhesus Macaques

Jeffy George,[1] Egidio Brocca Cofano,[2] Elizabeth Lybarger,[3] Mark Louder,[3] Bernard A.P. Lafont,[4] John R. Mascola,[3] Marjorie Robert-Guroff,[2] and Joseph J. Mattapallil[1]

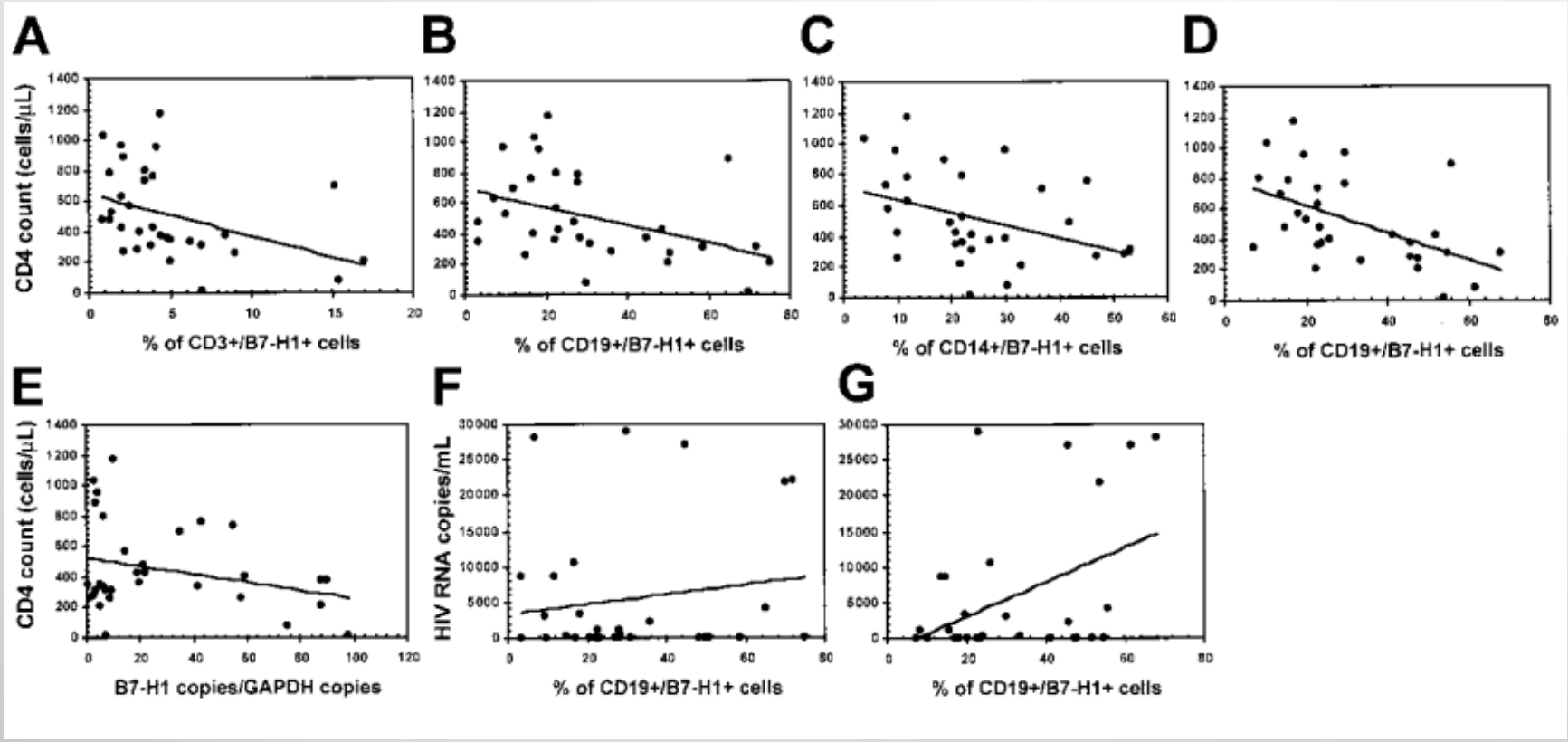
George J. AIDS Res and Human Retroviruses, 2011;27:763-75

Meccanismi di esaurimento del SI



JIN HT. BMB rep 2011;44:271-31

PD1/PD-L1 e HIV



Nell'infezione da HIV i livelli di espressione di PD1/PD-L1 correlano con la viremia e inversamente con la conta dei linfociti T CD4+

Trabattoni D. Blood, 2003;101:22514-20

TUPDA0106LB

The negative checkpoint receptor TIGIT marks exhausted T cells during SIV infection and correlates with SIV disease progression

G.M. Webb[1], G.M. Chew[2], T. Fujita[3], B.J. Burwitz[1], H.L. Wu[1], J.S. Reed[1], K.B. Hammond[1], S.G. Hansen[1], M. Maurer[4], A.J. Korman[4], L.C. Ndhlovu[2], J.B. Sacha[1]

[1]*Oregon Health & Science University, Vaccine & Gene Therapy Institute and Oregon National Primate Research Center, Beaverton, United States,* [2]*John A. Burns School of Medicine, University of Hawaii, Hawaii Center for HIV/AIDS, Department of Tropical Medicine, Honolulu, United States,* [3]*Tohoku University Graduate School of Medicine, Department of Microbiology and Immunology, Sendai, Japan,* [4]*Biologics Discovery California, Bristol-Myers Squibb, Redwood City, United States*

Background: During chronic viral infections, high antigenic load continually stimulates T cells resulting in T cell exhaustion. Exhausted T cells increase expression of negative checkpoint inhibitors such as PD-1, which raise the threshold for activation and contribute to suppressed immune responses. Another recently discovered immune checkpoint receptor, TIGIT, is up-regulated on T cells in neoplasms and chronic LCMV infection.
We hypothesize that TIGIT functions as a negative checkpoint receptor marking dysfunctional T cells during SIV infection, and that modulation of TIGIT would restore anti-SIV-specific T cell responses.

Methods: Spleen, lymph node (LN) and PBMCs from SIV-naïve and SIV-infected rhesus macaques (RMs) were examined for surface expression of TIGIT. In vitro cytokine production was assessed via intracellular cytokine staining. Proliferative capacity was determined through CFSE dilution assays in the presence of antibodies blocking TIGIT and PD-1 pathways (anti-TIGIT mAb and anti-PD-L1 mAb).

Results: TIGIT expression was significantly up-regulated on $CD8^+$ T cells derived from the spleen and LN, but not PBMC in SIV-infected animals. The frequency of $TIGIT^+$ $CD8^+$ T cells in the LN significantly correlated with SIV viral load, and TIGIT expression was driven primarily by g-chain cytokines such as IL-2. TIGIT was expressed on ~40% of SIV-specific $CD8^+$ T cells, even in animals with full cART suppression of viral replication. While Ki-67 expression did not differ between $TIGIT^+$ and $TIGIT^-$ $CD8^+$ T cells, $TIGIT^+$ $CD8^+$ T cells produced significantly more IFN-g compared to $TIGIT^+$ $CD8^+$ T cells. Single and dual blockade of TIGIT and/or PD-1 signaling pathways restored proliferative capacity of SIV-specific T cells in vitro.

Conclusions: TIGIT is a negative checkpoint receptor that marks a novel population of functionally exhausted SIV-specific $CD8^+$ T cells and is associated with SIV disease progression. The enhancement of virus-specific T cell proliferative responses in the presence of single or dual blockade of TIGIT and/or PD-1 suggests that targeting the TIGIT pathway is a viable therapeutic approach to reverse T cell dysfunction. Given the high sequence homology of rhesus and human TIGIT, this provides a platform to further investigate TIGIT, along with other checkpoint inhibitors, as potential targets for mediating a functional cure for HIV.

SIV/T Exhausted

Nel modello animale:
TIGIT risulta up-regolato sulle CD8 di derivazione dalla milza e dai linfonodi di RM SIV-infetti, indipendentemente dalla ART

% CD8+TIGIT+ nei linfonodi correla con il VL

Il-2 up-regola TIGIT

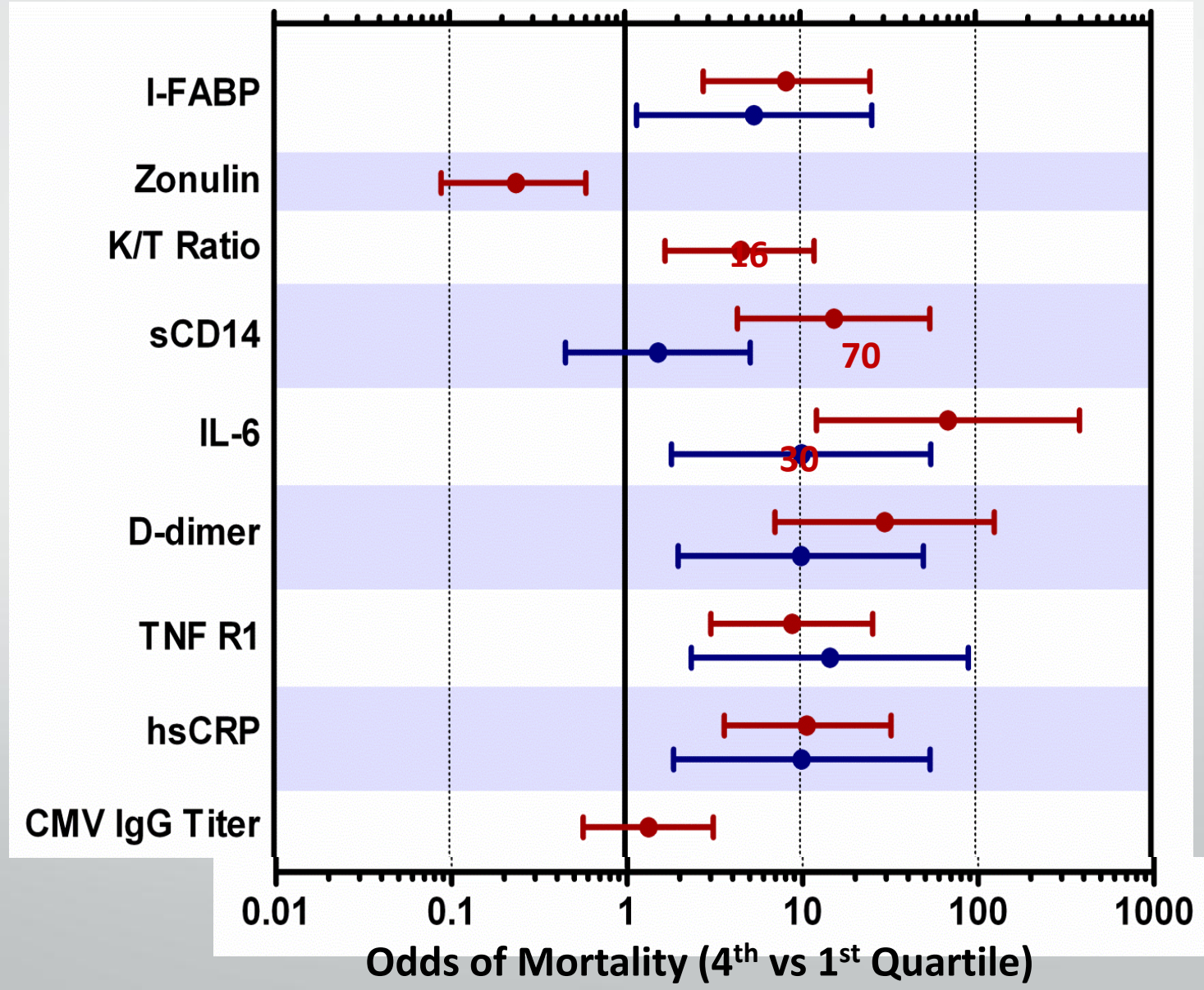
$TIGIT^+CD8+$ cells producono più INFγ rispetto alle $TIGIT^-CD8+$

Il singolo o doppio blocco di TIGIT e/o PD1, induce il recupero della capacità replicativa delle T CD8 SIV-specifiche

Immunoattivazione: conseguenze cliniche

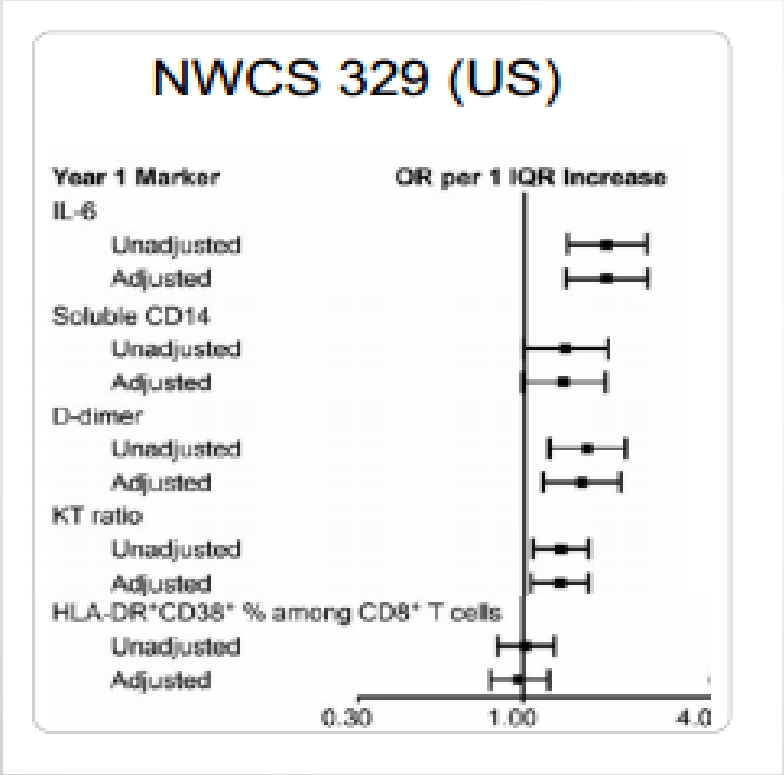
SOCA
(median nadir CD4=30)

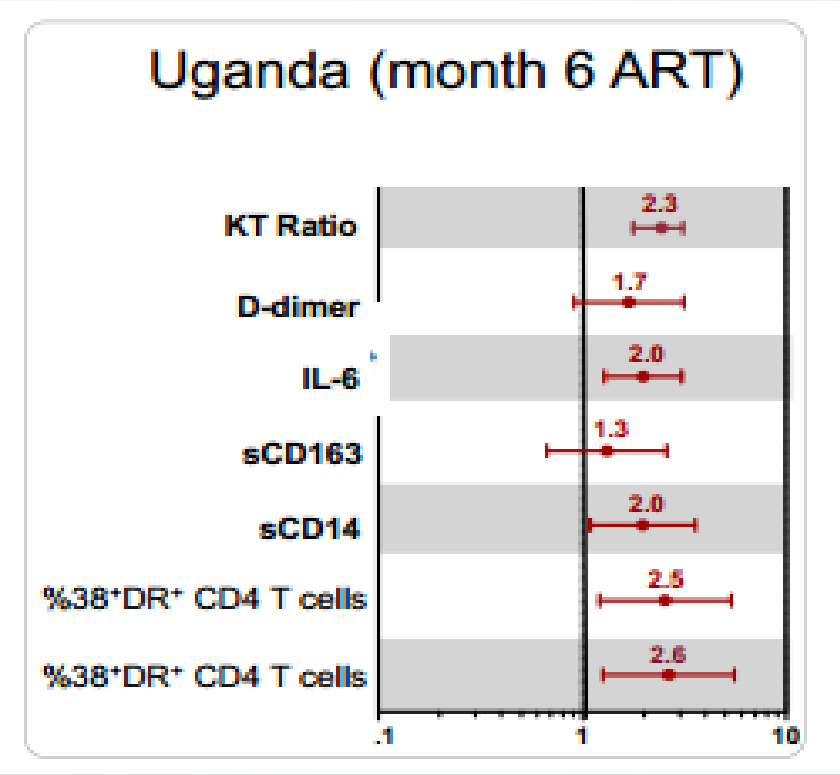
SCOPE
(median nadir CD4=88)



Hunt, CROI 2012

Infiammazione e mortalità nei RLS

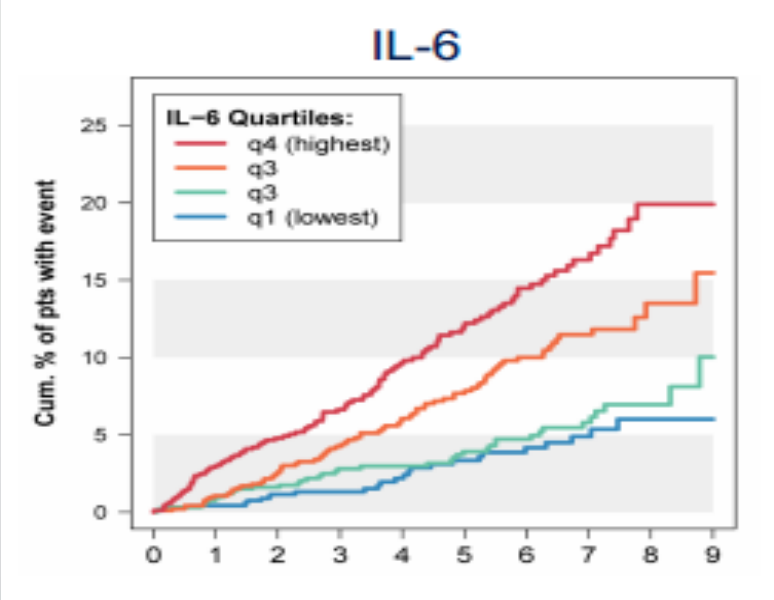


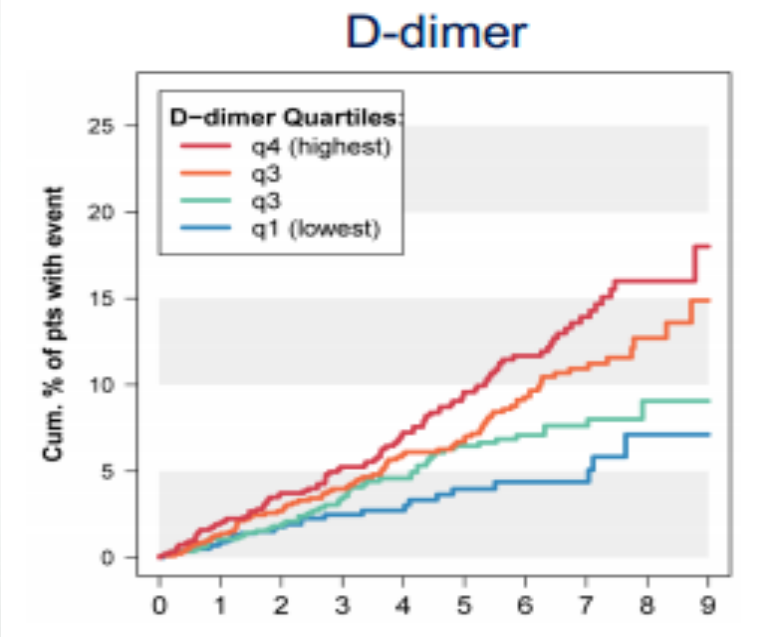


Alterazioni dell'immunità innata presentano un impatto sulla mortalità nei paesi africani

Tenorio JID 2014; Lee CROI 2015

Marcatori dell'infiammazione e mortalità





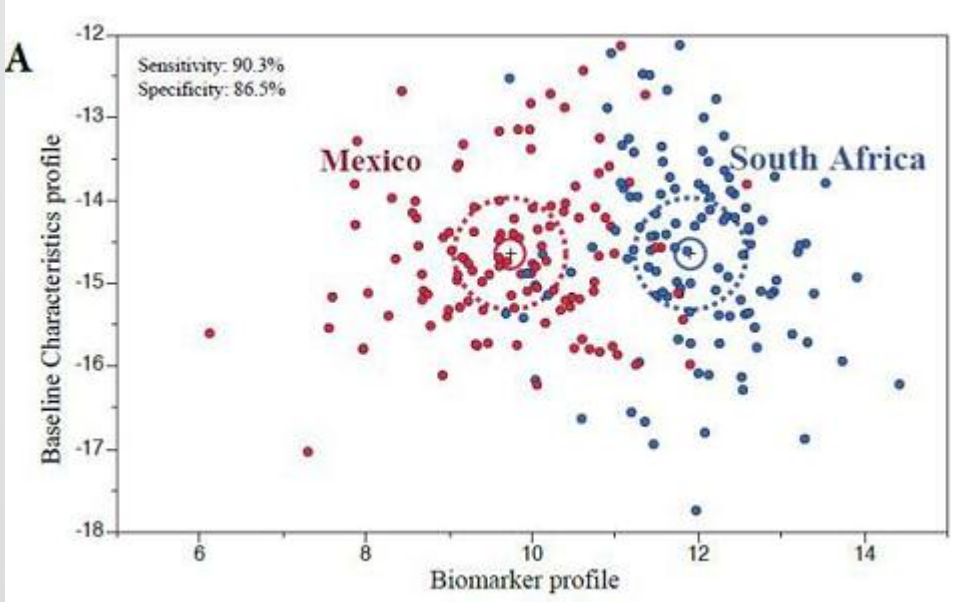
La singola misurazione dell'IL6 o del D-dimero predicono la morbidità e mortalità per i successivi 10 anni
Dato solo parzialmente modificato dal fumo e dall'obesità

CROI 2015

	Patients group					
	1	2	3	4	5	6
CD4 count						
CD57+ NK cell count						
%Treg cell						
%activated Treg cell						
CD57+ CD4+ T cell count						
CD57+ CD28-CD4+ T cell count						
%CD57+ CD4+ T cell						
%CD57+ CD28-CD4+ T cell						
%CD57+ CD28-CD27-CD4+ T cell						
CD57+ CD28-CD27-CD4+ T cell count						
CD57+ CD8+ T cell count						
CD57+ CD28-CD8+ T cell count						
CD57+ CD28-CD8+CD27- T cell count						
%CD57+ CD8+ T cell						
%CD57+ CD28-CD8+ T cell						
%CD57+ CD28-CD27-CD8+ T cell						
% CD57+ NK cell						

Netta correlazione tra immunoattivazione e la % CD4, % Treg, cellule T ed NK senescenti

Contrariamente non vi erano correlazioni con viremia residua, MT e co-infezioni



Nell'analisi multivariata:
I pazienti messicani hanno un più alto valore di IFNγ, IL-8 e IP-10 mentre i pazienti del Sud Africa hanno più elevati livelli di vit D, fibrinogeno, LTB4, P-selecina, proteina S e sCD40L

Biomarkers from CADIRIS study based upon country of origin

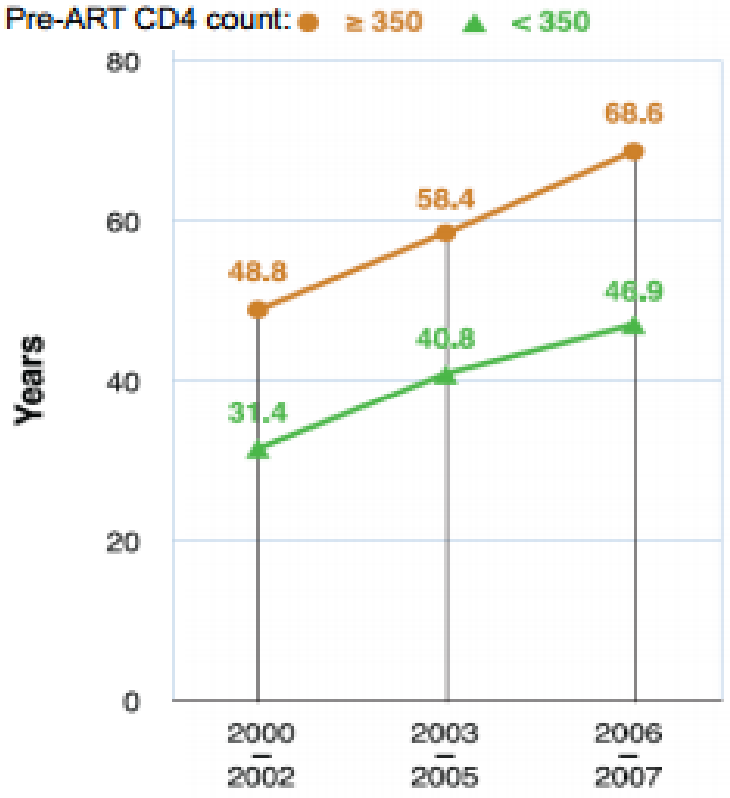
	Mexico	South Africa	P-value
Fibrinogen (mg/dL)	723 [448, 1300]	1229 [715, 1945]	<0.0001
IFN-γ (pg/mL)	4.09 [2.20, 7.41]	2.19 [1.17, 5.58]	0.034
IL-8 (pg/mL)	11.80 [7.77, 18.13]	5.79 [3.40, 8.31]	<0.0001
IP 10 (pg/mL)	2575 [439, 1780]	1691 [999, 2693]	<0.0001
Leukotriene B4 (pg/mL)	10.20 [10.20, 37.17]	47.84 [16.50, 76.57]	<0.0001
P-selectin (ng/mL)	48.49 [37.2, 64.71]	64.65 [50.05, 82.57]	<0.0001
% Protein S	3550 [3037, 3960]	4376 [3658, 5430]	<0.0001
Vitamin D (ng/mL)	7.72 [4.44, 13.36]	10.42 [6.9, 17.09]	0.026
sCD40 L (pg/ml)	586 [227, 1096]	1157 [870, 1716]	<0.0001

A) Sparse Canonical Correlation: The x-axis gives the score in the biomarker profile while the y-axis gives the score in the baseline characteristics. Data on biomarkers were inputted after logaritimic transformation. Data points were labeled based on the two diagnostic classes South Africa or Mexico. Small circles denote 95% confidence region estimated to contain the mean of a group. Ellipses denote the regions estimated to contain 50% of the class clusters.

B) Biomarkers (Medians and IQR) that differed significantly by country when adjusted for baseline characteristics.

Il recupero immunologico: significato clinico

Life Expectancy in the Era of cART



- Life expectancy for 20 year old on or starting ART in North America
- 23,000 person-years FU
- 1,622 deaths
- Majority of HIV+ around the world still starting ART <350
- May overestimate life expectancy
  - Excludes those out of care
  - "Survivor bias" for older people who survived 1980s and 1990s

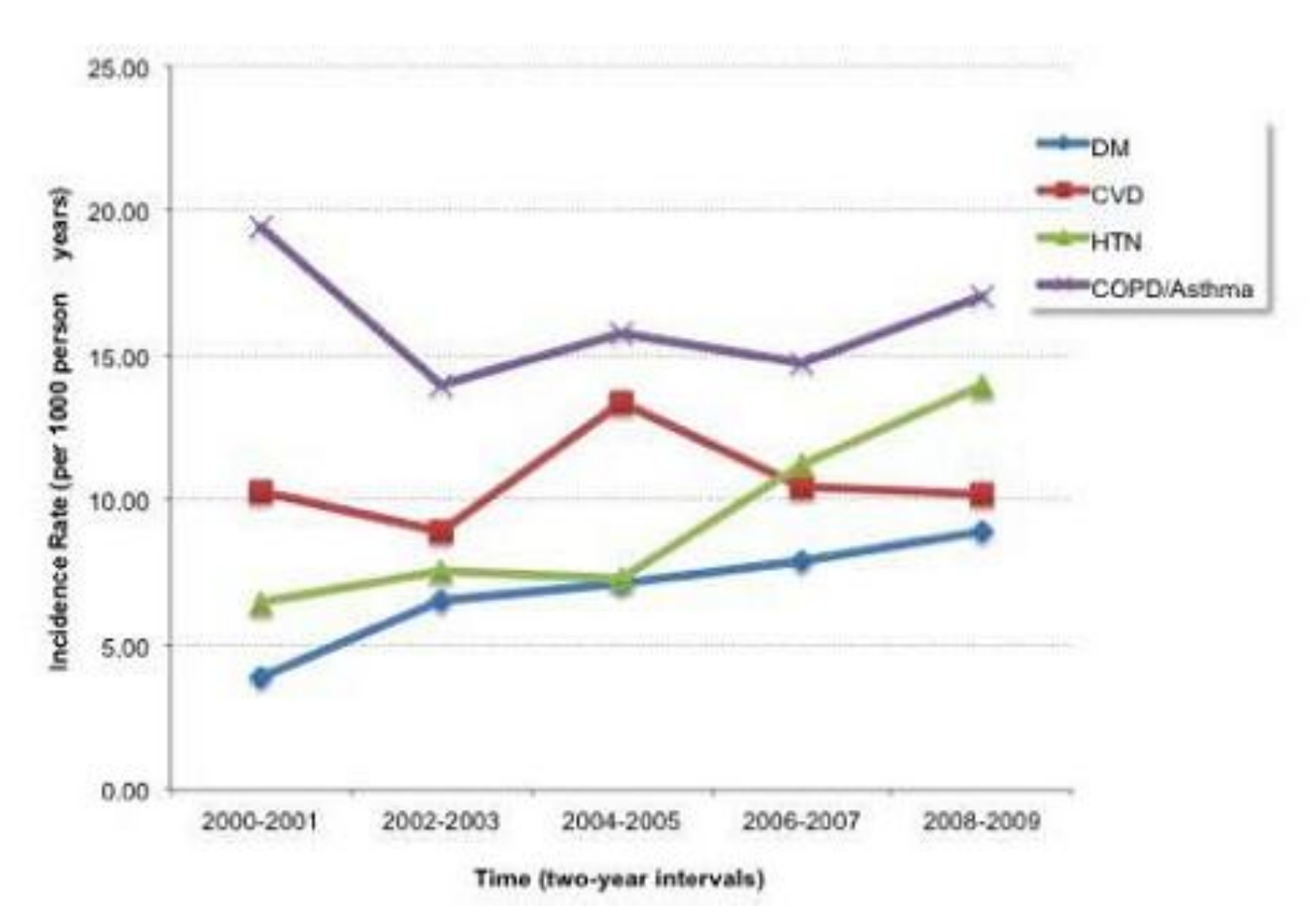
Samji for NA-ACCORD, *PLoS One* 2013

Life expectancy improving but important gap persists

Immunoattivazione e malattie specifiche

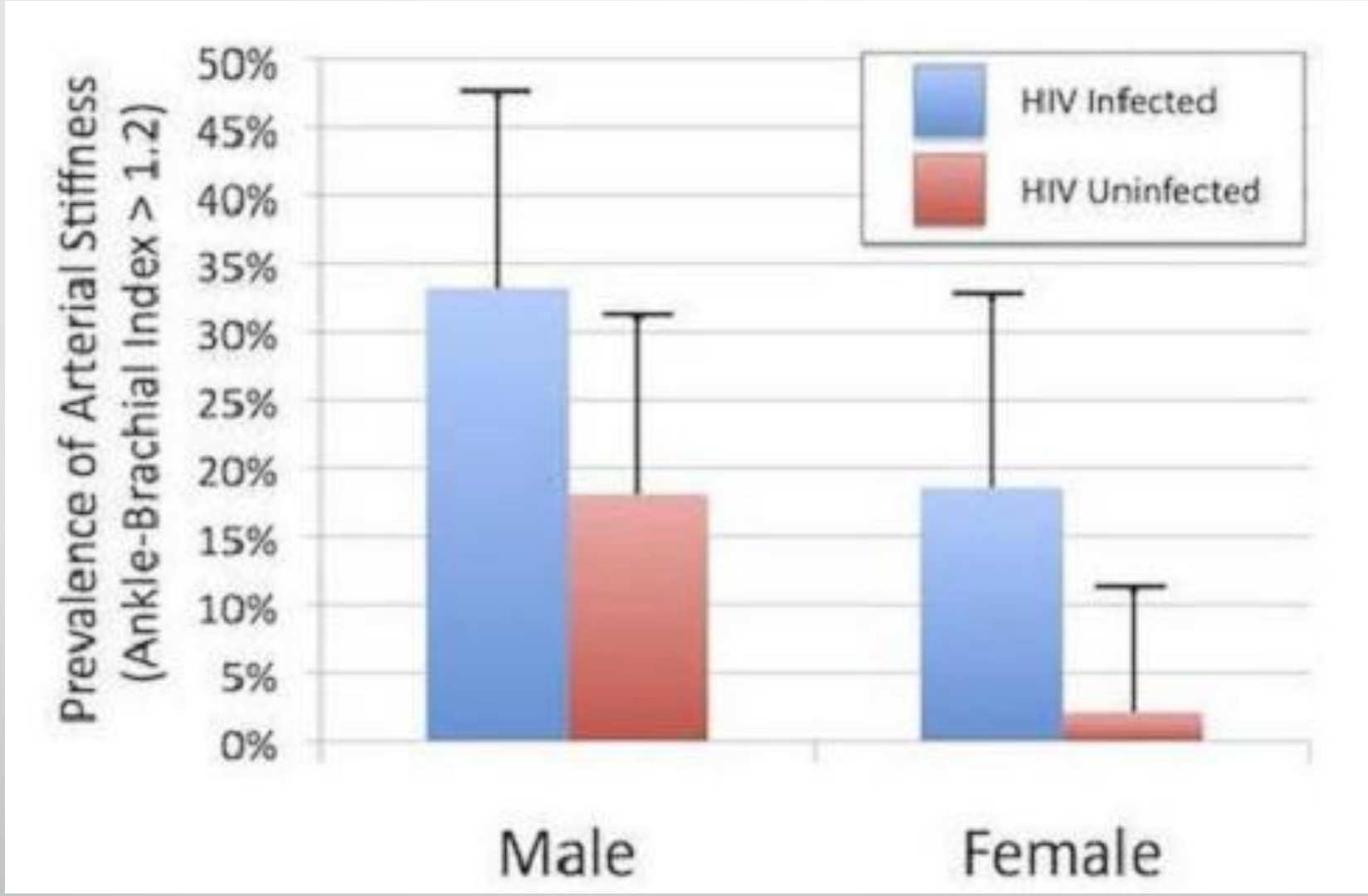
- Mortalità (Kuller PLoS M 2008; Tien JAIDS 2010; Justice CID 2012; Hunt JID 2014)
- Malattie cardiovascolari (Duprez, Atherosclerosis 2009)
- Cancro (Breen Cancer Epi Bio Prew 2010; Brges AIDS 2013)
- Epatopatie (Musselwhite AIDS 2011)
- Diabete tipo II (Brown Diabetes Care 2010)
- Alterazioni cognitive (Burdo AIDS 2013 Letendre CROI 2012)
- Frailty (Erlandson JID 2013)

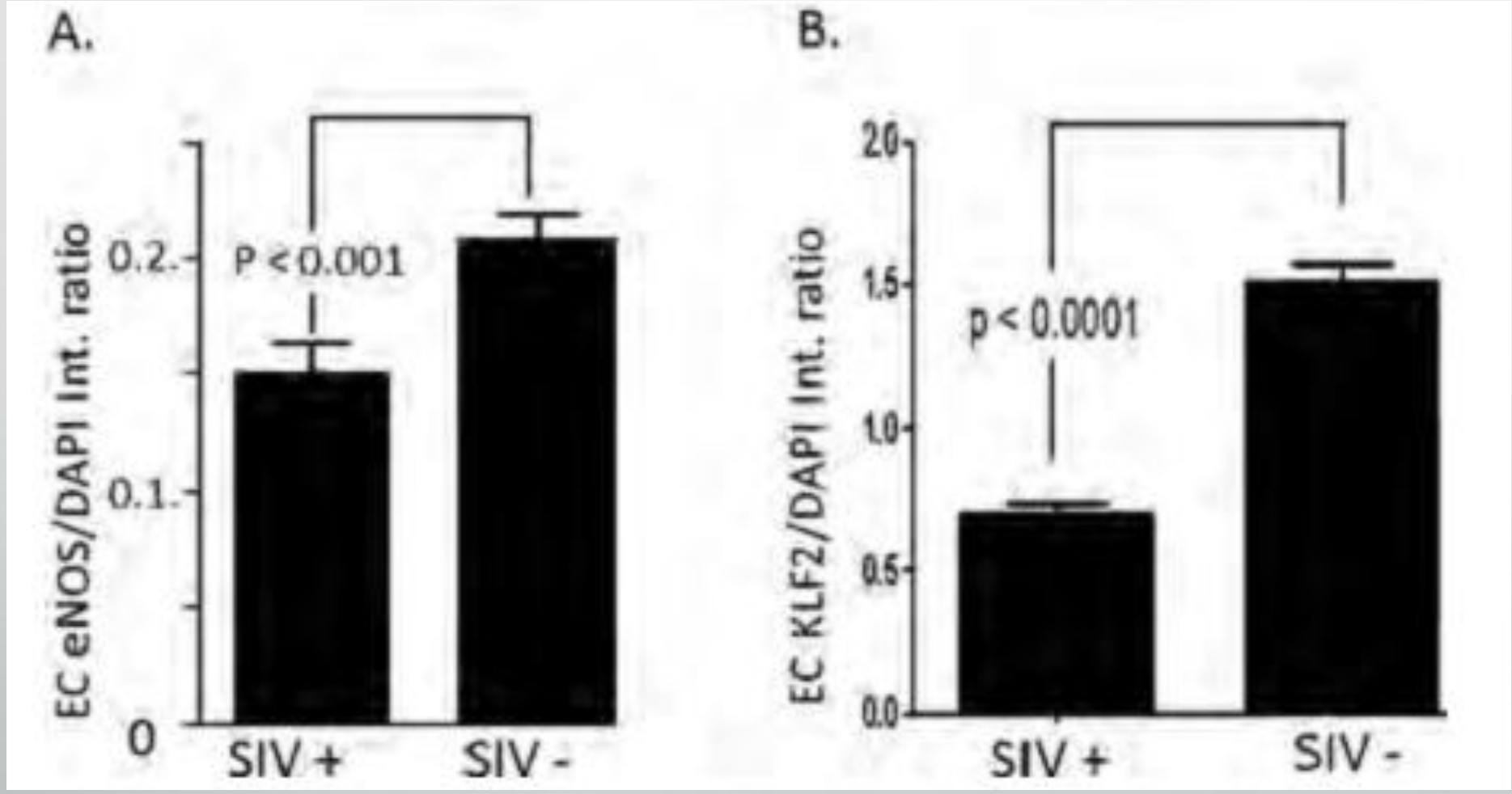
L’infiammazione predice specifiche patologie nella popolazione HIV in terapia

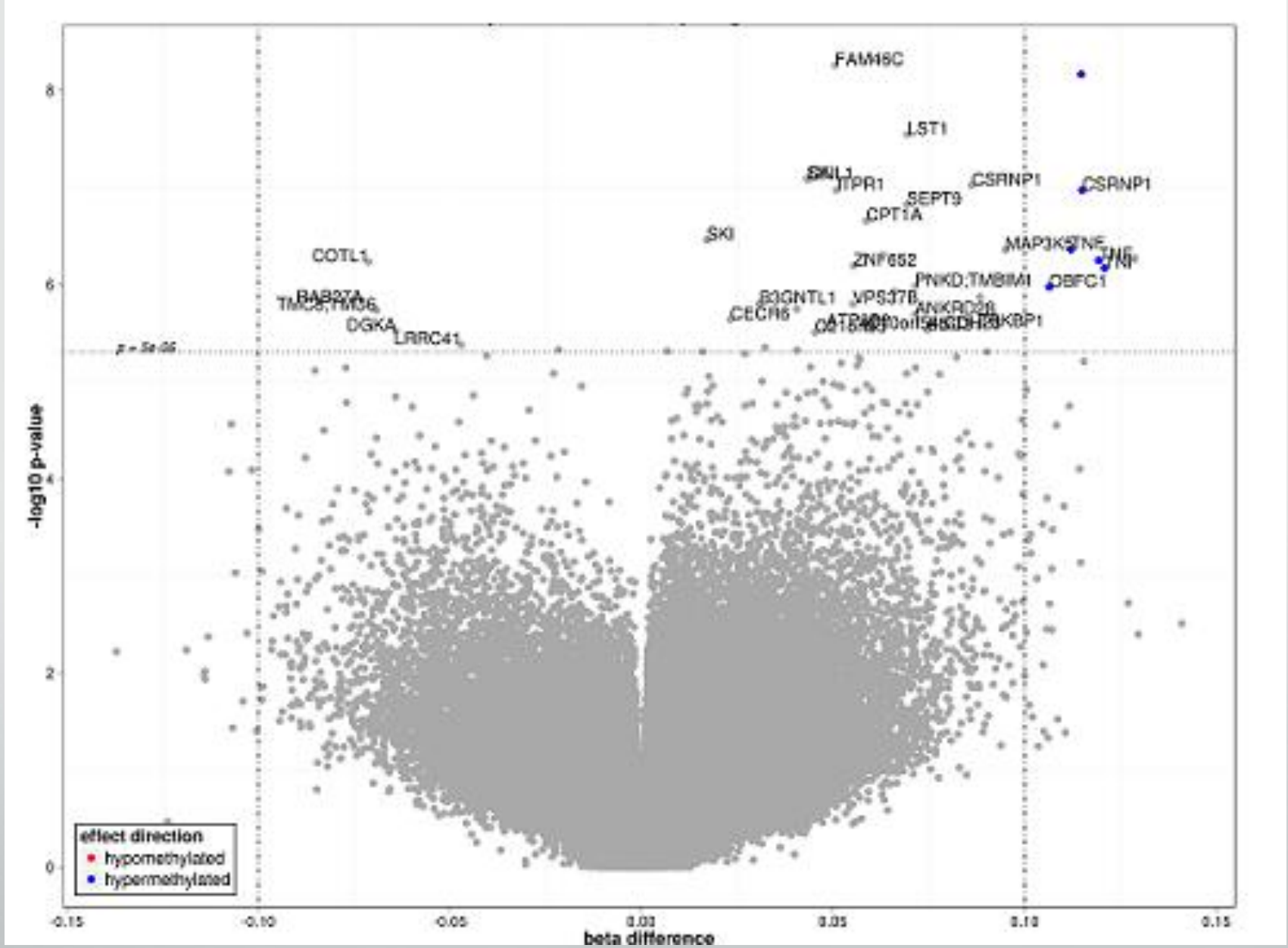


Registro canadese
8620 pz HIV+ nadir CD4<200 VL 80.000

Analisi tra il 2000-2009


Prevalence of Arterial Stiffness
(Ankle-Brachial Index > 1.2)
50%
45%
40%
35%
30%
25%
20%
15%
10%
5%
0%
HIV Infected
HIV Uninfected
Male
Female


A.
EC eNOS/DAPI Int. ratio
0.2
0.1
0
P < 0.001
SIV +
SIV -
B.
EC KLF2/DAPI Int. ratio
2.0
1.5
1.0
0.5
0.0
p < 0.0001
SIV +
SIV -


FAM46C
LST1
JTPR1
CSRNP1
CSRNP1
SEPT9
CPT1A
SKI
COTL1
ZNF652
PNKD;TMBIM4
DBFC1
BOGNTL1
VPS37B
ANKRD28
CECR6
OGKA
LRRC41
-log10 p-value
beta difference
effect direction
hypomethylated
hypermethylated

Terapia antiretrovirale precoce: quali vantaggi

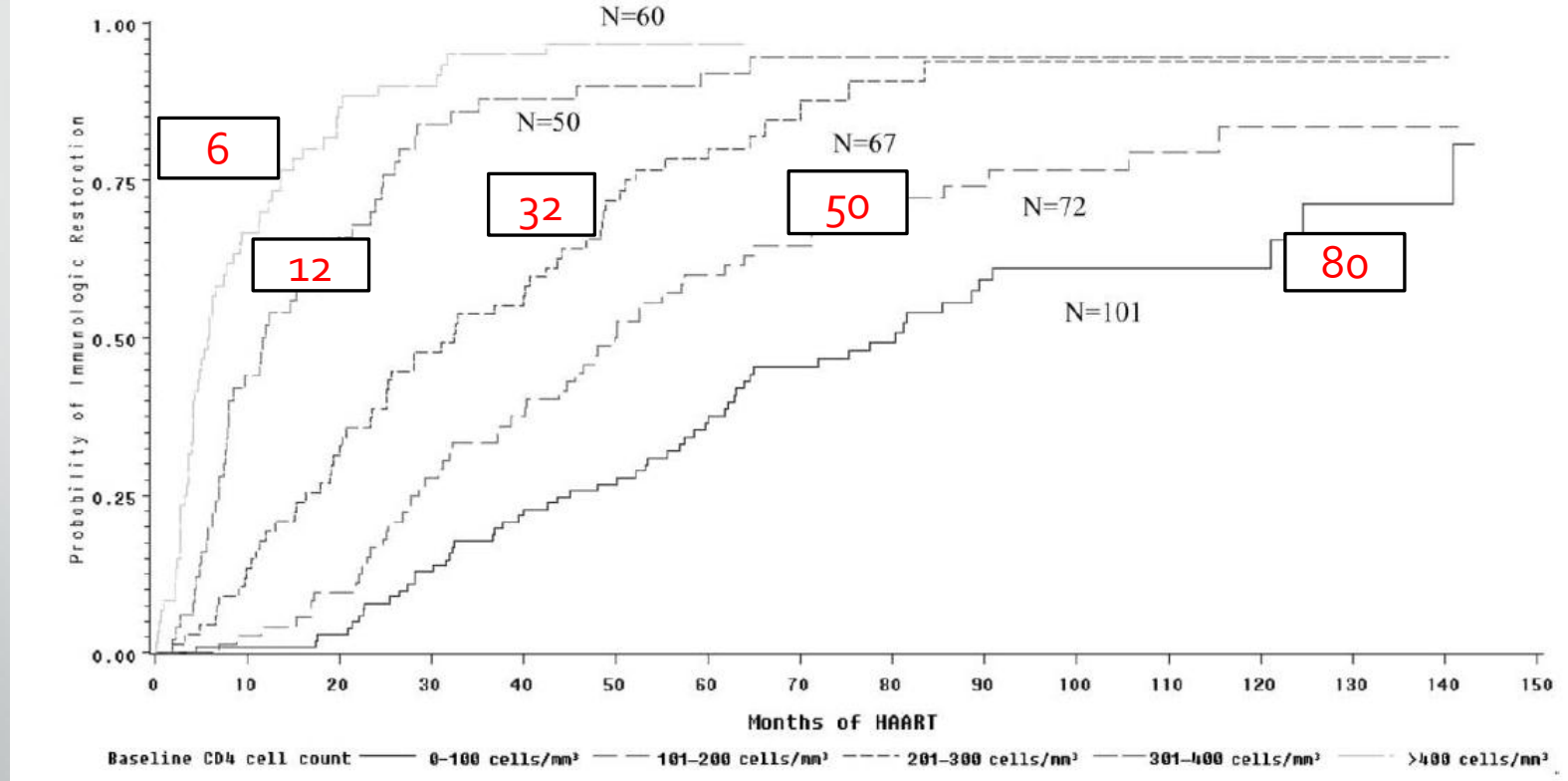
MAJOR ARTICLE HIV/AIDS

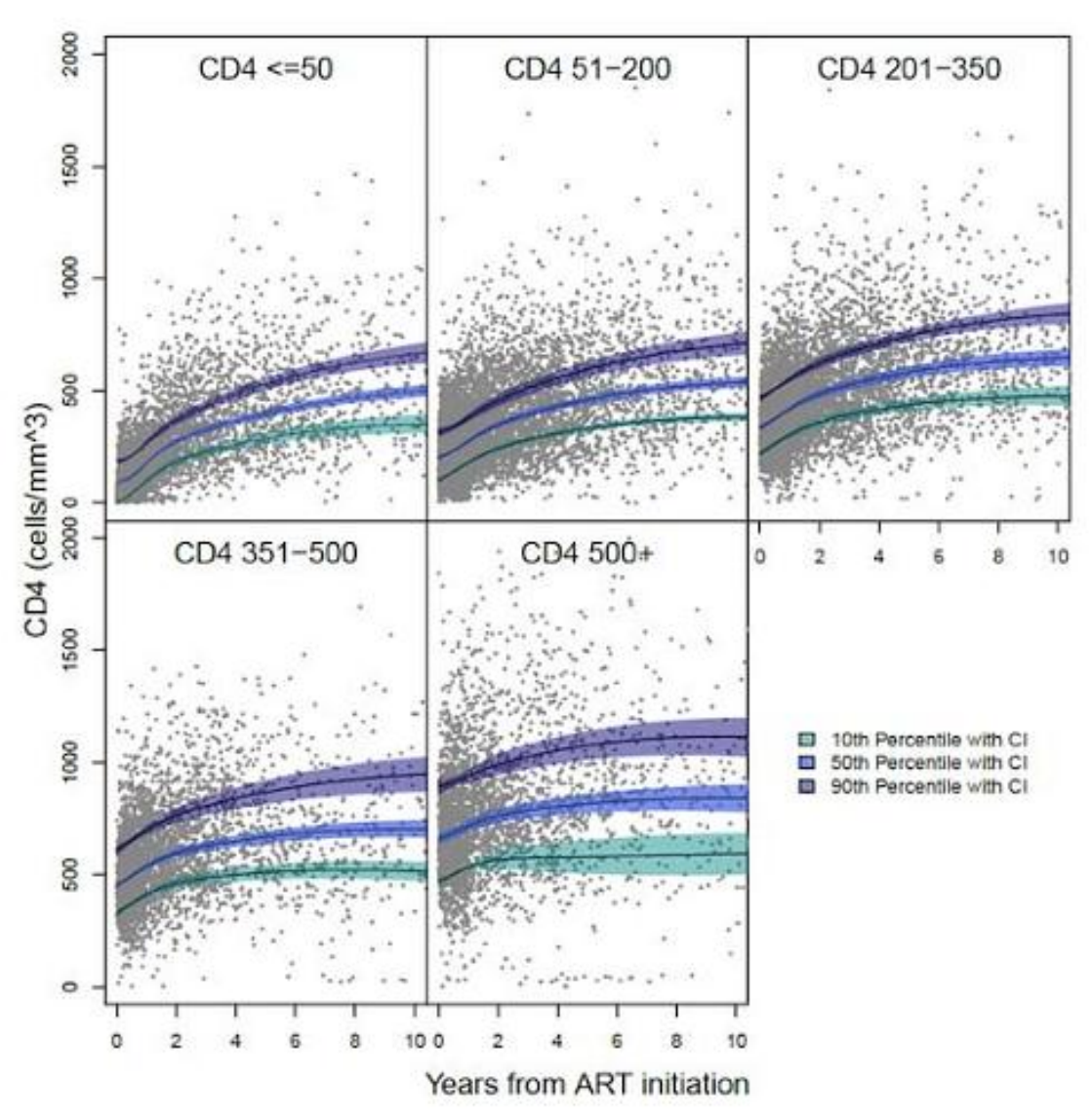
Incomplete Peripheral CD4+ Cell Count Restoration in HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment

Colleen F. Kelley,[1,a] Christina M. R. Kitchen,[2,a] Peter W. Hunt,[3] Benigno Rodriguez,[4] Frederick M. Hecht,[3] Mari Kitahata,[5] Heide M. Crane,[5] James Willig,[6] Michael Mugavero,[6] Michael Saag,[6] Jeffrey N. Martin,[3] and Steven G. Deeks[3]

[1]Emory University, Atlanta, Georgia; [2]University of California at Los Angeles, Los Angeles, and [3]University of California, San Francisco; [4]Case Western Reserve University, Cleveland, Ohio; [5]University of Washington, Seattle; and [6]University of Alabama at Birmingham, Birmingham

Il nadir dei CD4 influenza il tempo necessario per la normalizzazione dei CD4



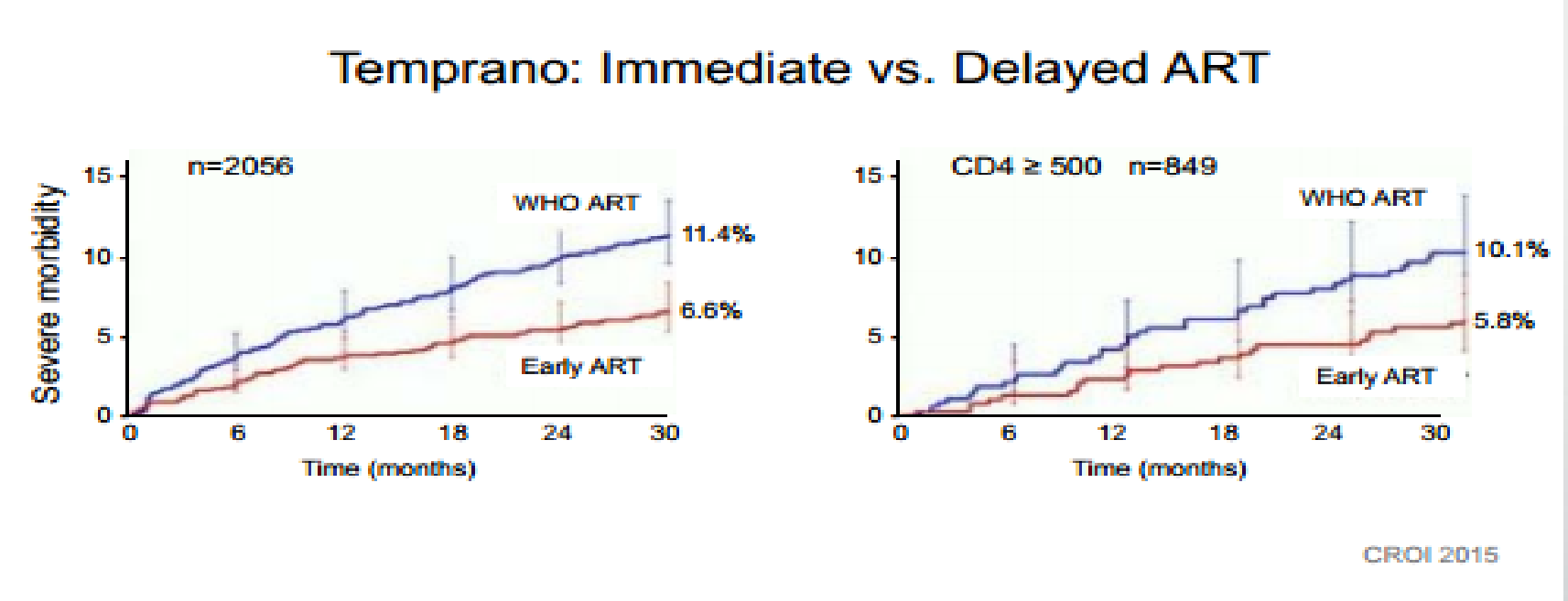


75281 pazienti in ART tra il 1996 e il 2012

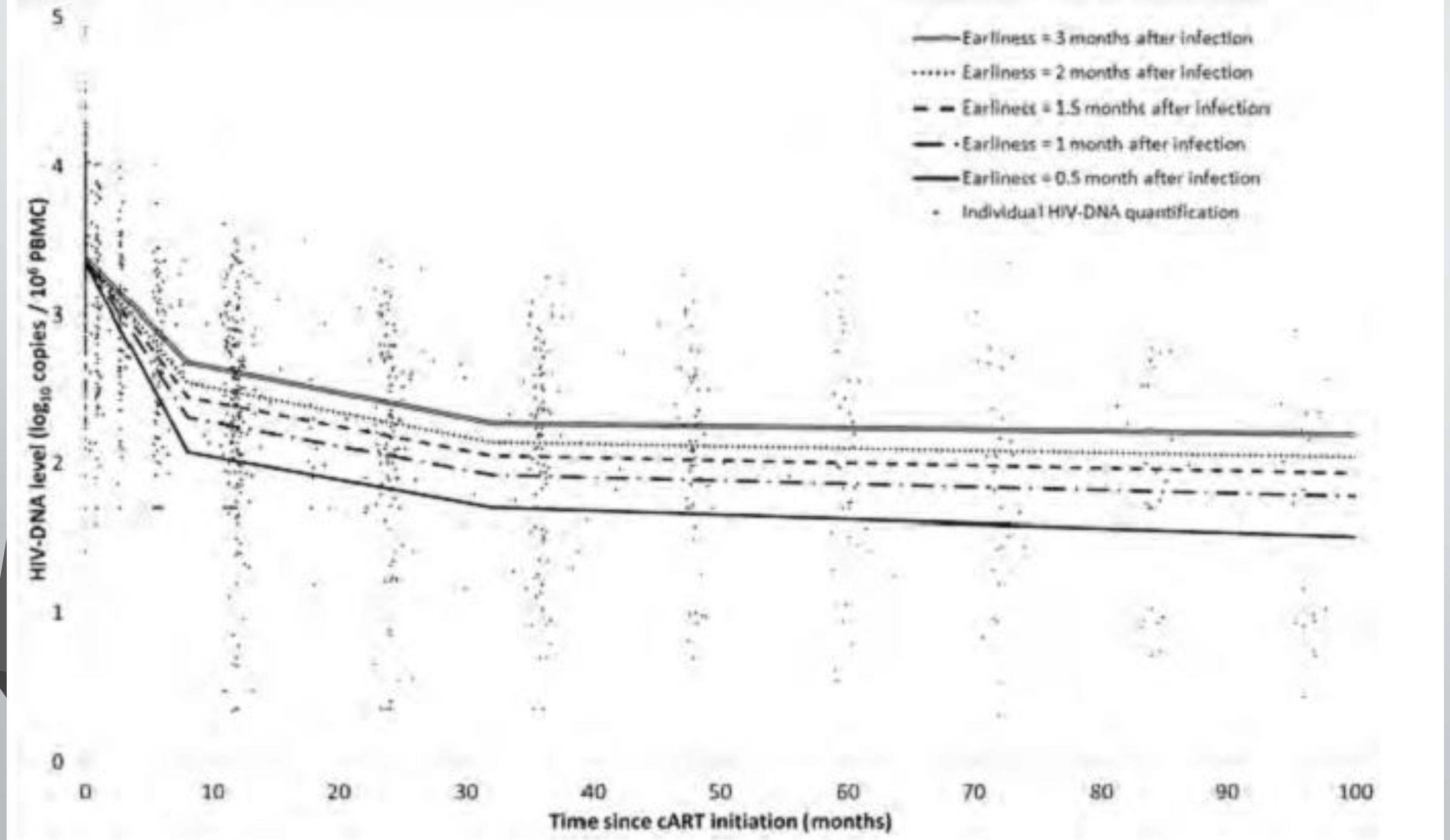
Il massimo recupero dei CD4 (>500 cell/mm3) è in parte determinato dal valore dei CD4 alla partenza.

Il valore di CD4>500 è raggiungibile anche per nadir molto bassi, anche se si riduce nettamente la probabilità di raggiungerlo

I benefici della ART precoce



In RLS, la ART precoce ha un significativo benefico effetto sulla morbidità e sul valore dei CD4


Earliness = 3 months after infection
Earliness = 2 months after infection
Earliness = 1.5 months after infection
Earliness = 1 month after infection
Earliness = 0.5 month after infection
Individual HIV-DNA quantification
HIV-DNA level (log10 copies / 10^6 PBMC)
0
1
2
3
4
5
0
10
20
30
40
50
60
70
80
90
100
Time since cART initiation (months)

Median biomarker values among 327 SUN Study patients who had viral loads < 200 copies/mL when biomarker levels were done by years of ARV experience compared to median values among 40 ARV naives and 30 HIV-negative matched controls

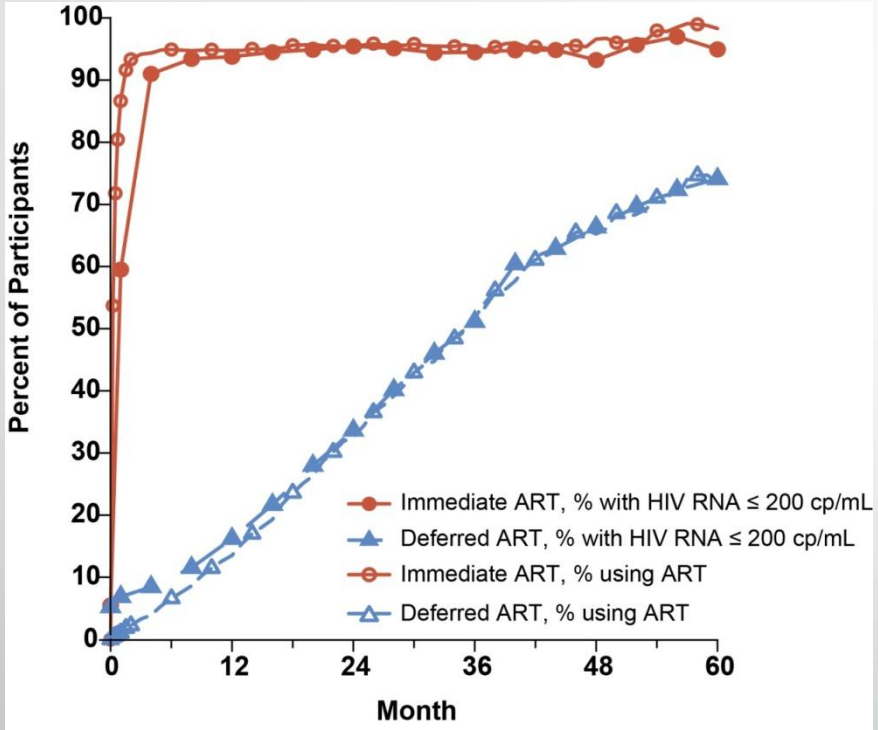
Year	N	RANTES	p: naives	p: controls	TNF-α	p: naives	p: controls	MIP-1α	p: naives	p: controls	MIP-1β	p: naives	p: controls	IL-6	p: naives	p: controls	IL-7	p: naives	p: controls	IL-15	p: naives	p: controls	MCP-1	p: naives	p: controls
1	56	13375	↑	↑	9.96	·	↑	824	·	↑	247	↑	↑	0.00	·	↑	4.35	↓	↑	6.02	·	↑	371	·	↑
2	76	14037	↑	↑	19.89	·	↑	938	·	↑	256	↑	↑	1.81	·	↑	5.82	↓	↑	5.51	·	↑	361	·	↑
3	78	14330	↑	↑	18.78	·	↑	891	·	↑	254	↑	↑	3.24	·	↑	5.89	↓	↑	6.00	↑	↑	333	·	↑
4	70	13913	↑	↑	14.41	·	↑	833	·	↑	241	↑	↑	0.00	·	↑	2.05	↓	·	2.73	·	↑	305	·	↑
5	62	14635	↑	↑	0.00	·	↑	904	·	↑	267	↑	↑	0.00	·	↑	2.69	↓	·	5.69	·	↑	345	·	↑
6	80	12467	↑	↑	0.00	·	↑	704	·	↑	239	↑	↑	0.00	·	↑	0.00	↓	↓	0.00	·	↑	275	·	↑
7	100	12975	↑	↑	8.04	·	↑	796	·	↑	239	↑	↑	0.00	·	↑	0.00	↓	·	1.08	·	↑	316	·	↑
8	76	12236	↑	↑	8.94	·	↑	660	↓	↑	222	·	↑	0.00	·	↑	0.00	↓	·	0.00	·	↑	235	·	↑
9	62	13457	↑	↑	0.00	·	↑	692	↓	↑	228	·	↑	0.00	·	↑	0.00	↓	·	0.00	·	↑	299	·	↑
10	128	13450	↑	↑	7.00	·	↑	645	↓	↑	234	·	↑	0.00	↓	↑	0.00	↓	·	0.00	↓	↑	259	↓	↑
Naives		N=40	10087			11.43			795			198			5.49			14.99			4.31			370		
Controls		N=30	9868			0.00			256			147			0.00			1.45			0.00			146		

Legend: ↑=p-value indicates significantly higher ($p<0.05$) median value compared to naives or controls; ↓=p-value indicates significantly lower ($p<0.05$) median value compared to naives or controls; · = non-significant difference compared to naives or controls. Statistical test used: Kruskal-Wallis test of medians.

Dopo circa 10 anni di ART, i livelli dei principali markers infiammatori rimanevano elevati nei HIV+ rispetto a controlli negativi. Non differenze significative rispetto ai diversi farmaci antiretroviirali

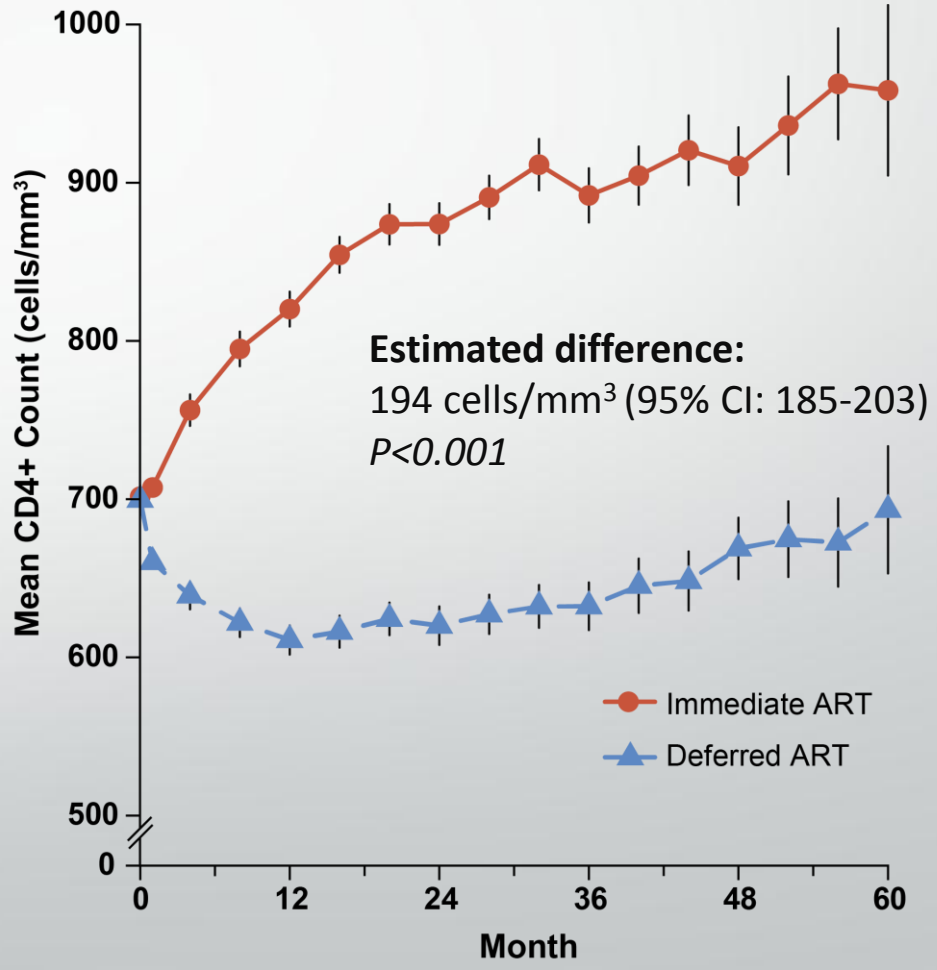
START Study

Percent on ART and Percent With HIV-RNA ≤ 200 copies/mL



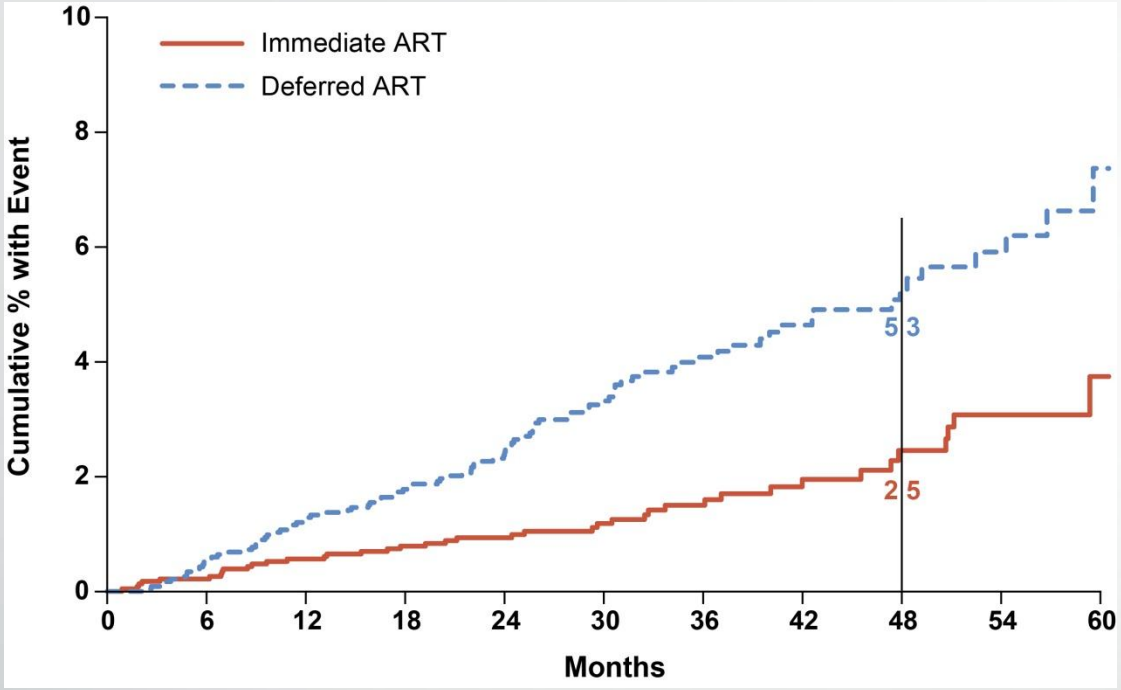
Deferred Arm:
Median time to ART 3 years (IQR 1.6−4.8)

Mean CD4+ Count During Follow-up



Babiker et al. IAS 2015; Vancouver, Canada. Slides MOSY0302.

Primary Endpoint (AIDS and non-AIDS events)

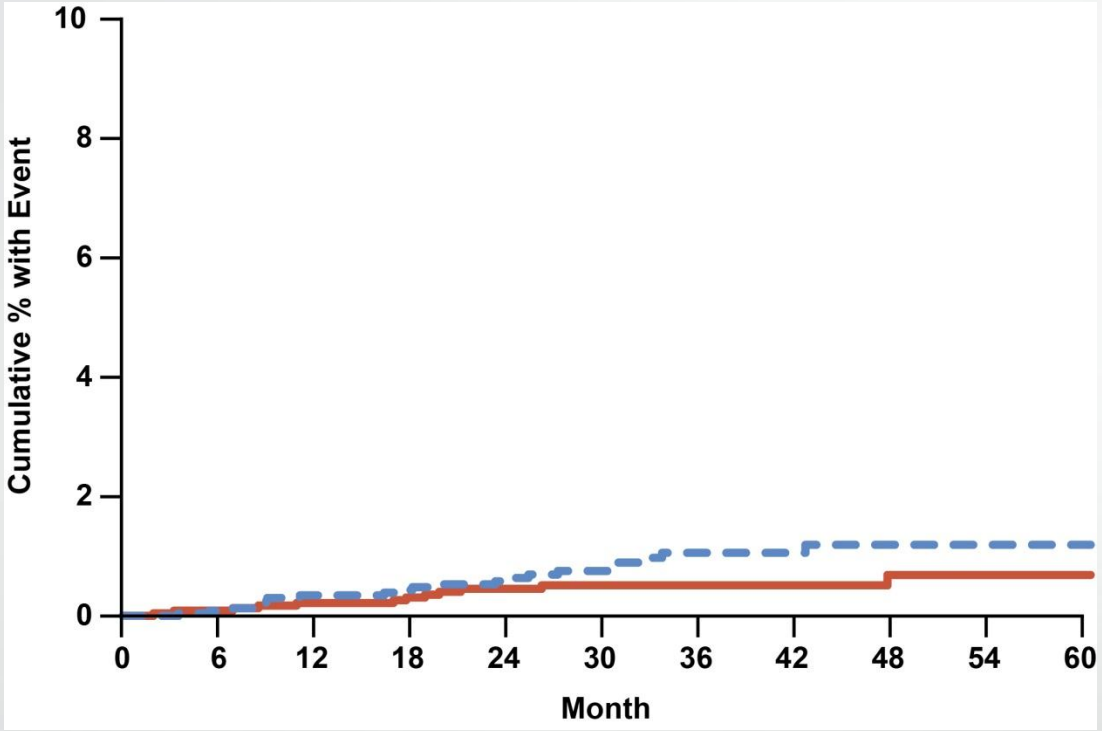


	No. of Participants	
Type of event	Imm. ART	Def. ART
Serious AIDS	14	50
Serious non-AIDS	29	47
Total*	**42**	**96**

**One participant in each group had both a Serious AIDS and a Serious Non-AIDS Event*

Babiker et al. IAS 2015; Vancouver, Canada. Slides MOSY0302.

Deaths



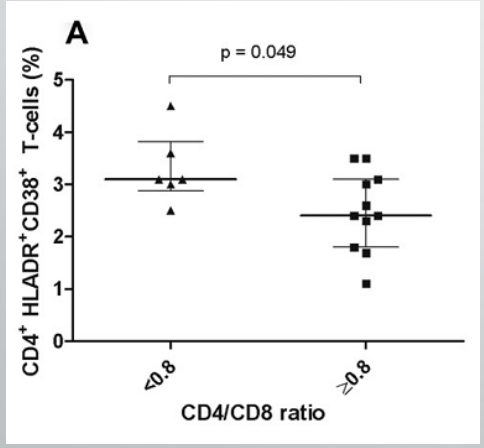
	Immediate ART	Deferred ART
No. with Event	**12**	**21**
Rate/100PY	0.17	0.30
HR (imm/Def)	0.58 (95% CI: 0.28-1.17, p=0.13)	

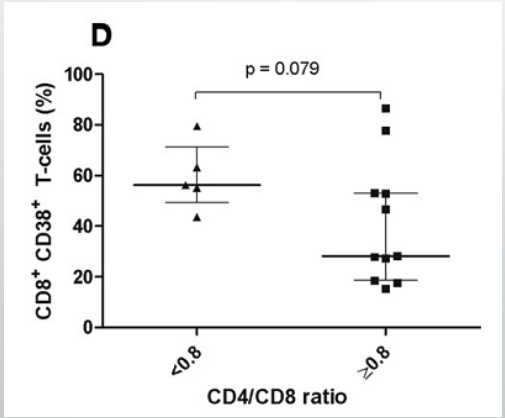
Babiker et al. IAS 2015; Vancouver, Canada. Slides MOSY0302

The CD4/CD8 ratio in HIV-infected subjects is independently associated with T-cell activation despite long-term viral suppression

Table 2 Multivariate analyses: independent associations of activated and senescent T cells (dependent variable) with CD4/CD8 ratio (independent variable), adjusting by accumulated ART exposure and nadir CD4.

Dependent variable	Coefficient	Std. error	*p*
$CD4^+HLADR^+CD38^+$ T-cells	−1.197	0.387	**0.020**
$CD4^+HLADR^+$ T-cells	−7.082	9.659	0.476
$CD4^+CD38^+$ T-cells	−3.143	2.523	0.235
$CD4^+CD57^+$ T-cells	−0.799	0.632	0.228
$CD8^+HLADR^+CD38^+$ T-cells	−1.257	1.313	0.357
$CD8^+HLADR^+$ T-cells	−7.490	3.247	**0.040**
$CD8^+CD38^+$ T-cells	−19.169	14.793	0.219
$CD8^+CD57^+$ T-cells	−1.945	2.233	0.400



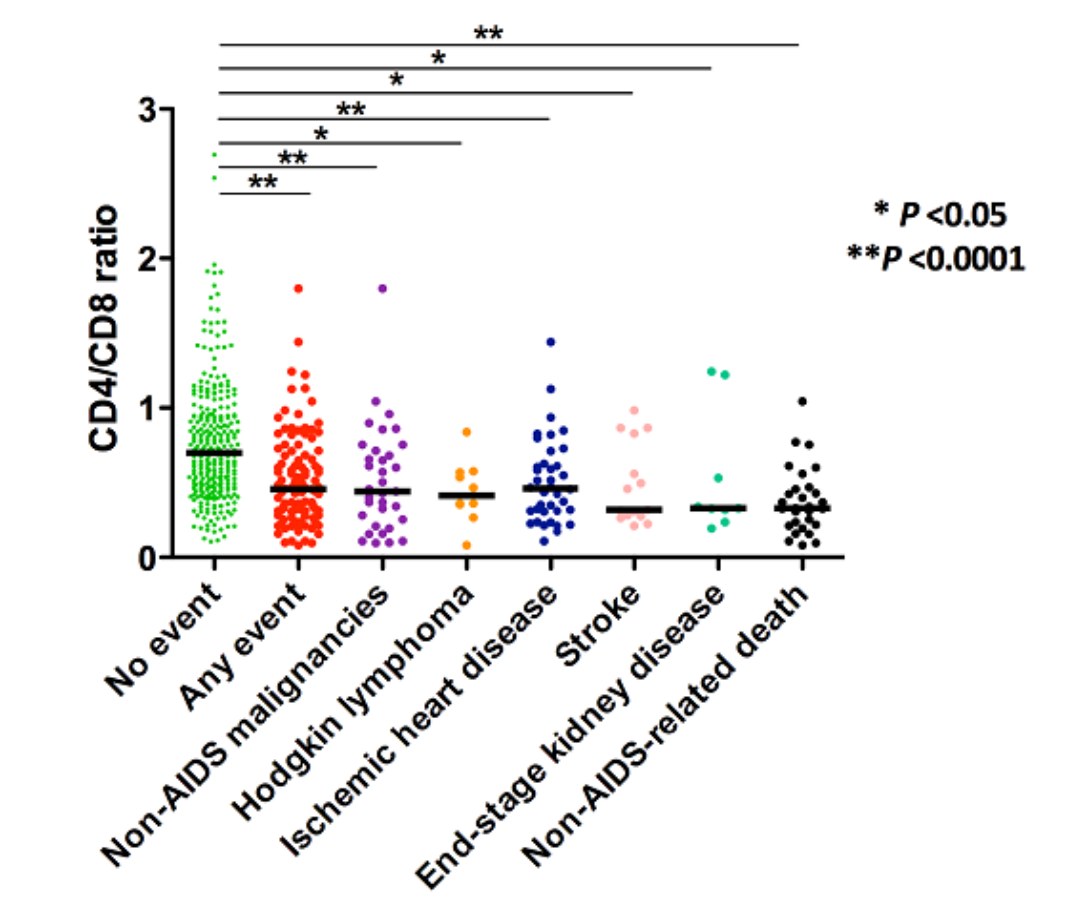


Significativa inversa associazione tra il rapporto CD4/CD8 e i marcatori di immunoattivazione

Serrano-Villar S. J.Infect 2013;66:57-66

Increased Risk of Serious Non-AIDS-Related Events in HIV-Infected Subjects on Antiretroviral Therapy Associated with a Low CD4/CD8 Ratio

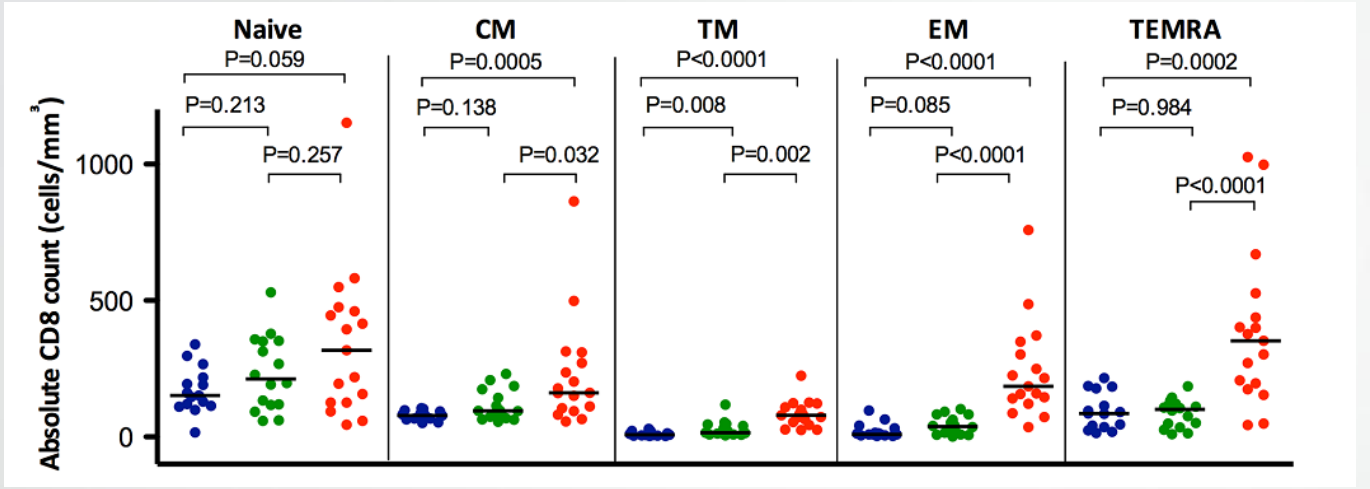
Serrano-Villar S PLoS One 2014:9(1):85798

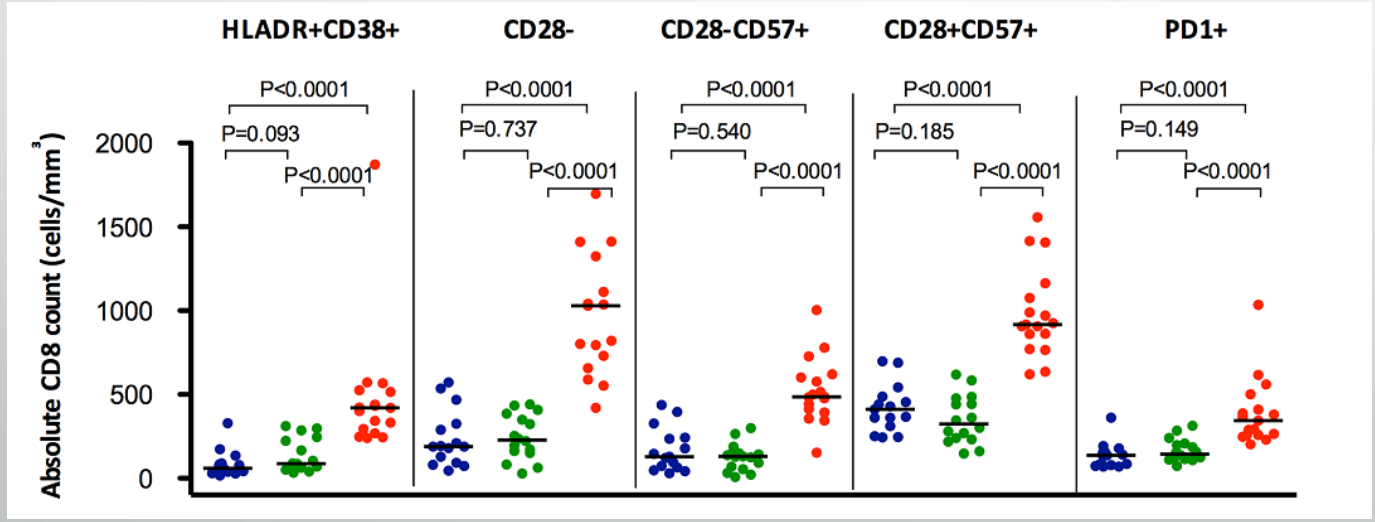


Il rapporto CD4/CD8 correla con l'insorgenza di non AIDS–comorbidità più dello stesso valore dei CD4, dei CD8 o del nadir

HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality

HIV-
HIV+ ART+ CD4 T cells >500, CD4/CD8 ≥1
HIV+ ART+ CD4 T cells >500, CD4/CD8 ≤0.4



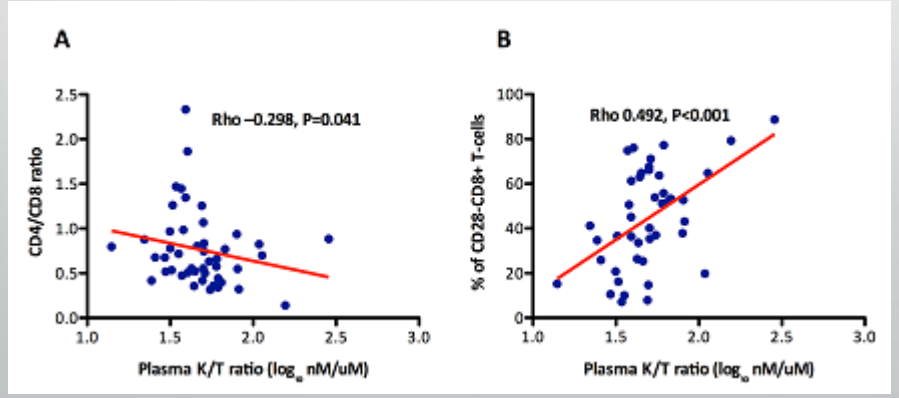


Serrano-Villar S. PLOS Pathogens;2014:10;e1004078

HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality

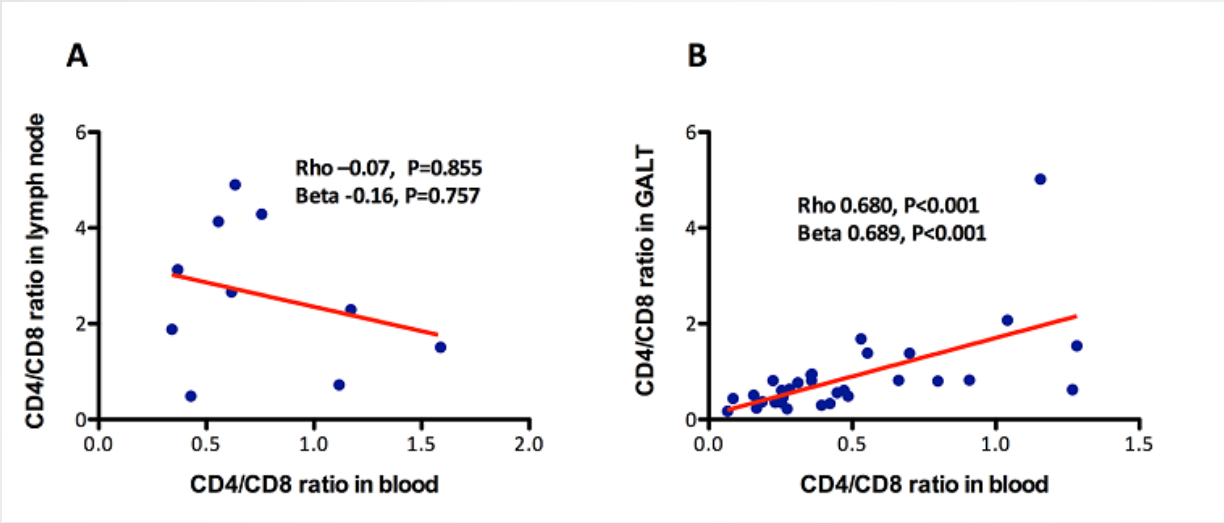
Table 3. Correlations with biomarkers of innate immune activation and epithelial integrity in SOCA cohort.

All subjects (n = 192)	CD4 Rho (P value)	CD8 Rho (P value)	CD4/CD8 ratio Rho (P value)
KT ratio	**−0.225 (0.003)**	0.127 (0.095)	**−0.336 (<0.001)**
sCD14	**−0.228 (0.002)**	0.028 (0.708)	**−0.232 (0.002)**
hs-CRP	**−0.148 (0.049)**	−0.071 (0.345)	**−0.152 (0.043)**
D-dimers	−0.139 (0.063)	−0.05 (0.469)	−0.141 (0.060)
Interleukin-6	**−0.158 (0.035)**	−0.035 (0.639)	**−0.162 (0.031)**
IFABP	−0.138 (0.067)	0.015 (0.841)	−0.146 (0.052)
Zonulin	0.111 (0.142)	−0.048 (0.523)	0.108 (0.155)
Subjects with CD4≥500 cells/mm^3 (n = 49)			
KT ratio	−0.209 (0.163)	0.252 (0.091)	**−0.298 (0.041)**
sCD14	**−0.394 (0.007)**	−0.011 (0.943)	−0.155 (0.303)
hs-CRP	−0.063 (0.673)	−0.023 (0.876)	−0.039 (0.799)
D-dimers	−0.004 (0.980)	−0.095 (0.537)	0.015 (0.919)
Interleukin-6	−0.152 (0.313)	−0.097 (0.521)	−0.012 (0.930)
IFABP	−0.144 (0.337)	0.144 (0.338)	−0.223 (0.134)
Zonulin	0.066 (0.664)	−0.048 (0.526)	−0.087 (0.569)



Serrano-Villar S. PLOS Pathogens;2014:10;e1004078

HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality



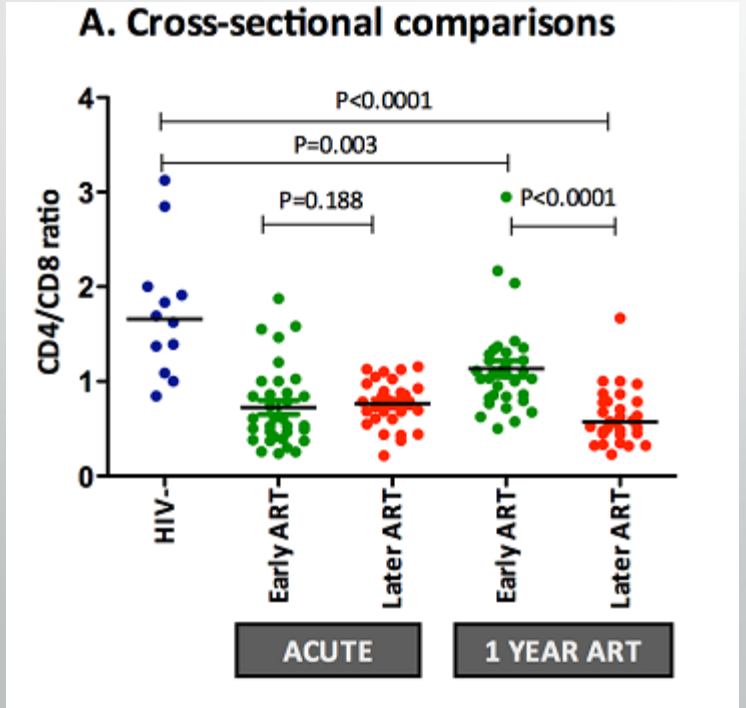


Table 5. Conditional logistic regression analysis: Predicted morbidity and mortality by the CD4+ and CD8+ T cell counts and the CD4/CD8 ratio in the Madrid cohort and SOCA cohort mortality nested studies.

	Beta	Std. error	P value
Madrid cohort (N = 66) (all subjects CD4≥500 cells/mm³)			
CD4+ T cells			
Unadjusted	−1.86	2.85	0.514
Adjusted by ART duration	−0.66	3.76	0.859
CD8+ T cells			
Unadjusted	2.80	1.12	0.013
Adjusted by ART duration	2.29	1.16	0.048
CD4/CD8 ratio			
Unadjusted	−6.23	2.48	0.012
Adjusted by ART duration	−5.08	2.53	0.045
SOCA cohort (N = 192)			
CD4+ T cells			
All subjects	−1.52	0.58	0.009
Subjects with CD4≥500 cells/mm³*	−4.09	6.43	0.525
CD8+ T cells			
All subjects*	0.28	0.33	0.392
Subjects with CD4≥500 cells/mm³*	2.37	2.05	0.246
CD4/CD8 ratio			
All subjects*	−1.38	0.55	0.012
Subjects with CD4≥500 cells/mm³*	−5.04	3.88	0.194

Serrano-Villar S. PLOS Pathogens;2014:10;e1004078

In un numeroso gruppo di virologicamente ART soppressi HIV pazienti con CD4>500 si dimostra

- persistente elevato valore di CD8
- basso rapporto CD4/CD8

Un negativo rapporto CD4/CD8 in corso di ART dovrebbe essere considerato un marker di infiammazione e alterata funzione del SI

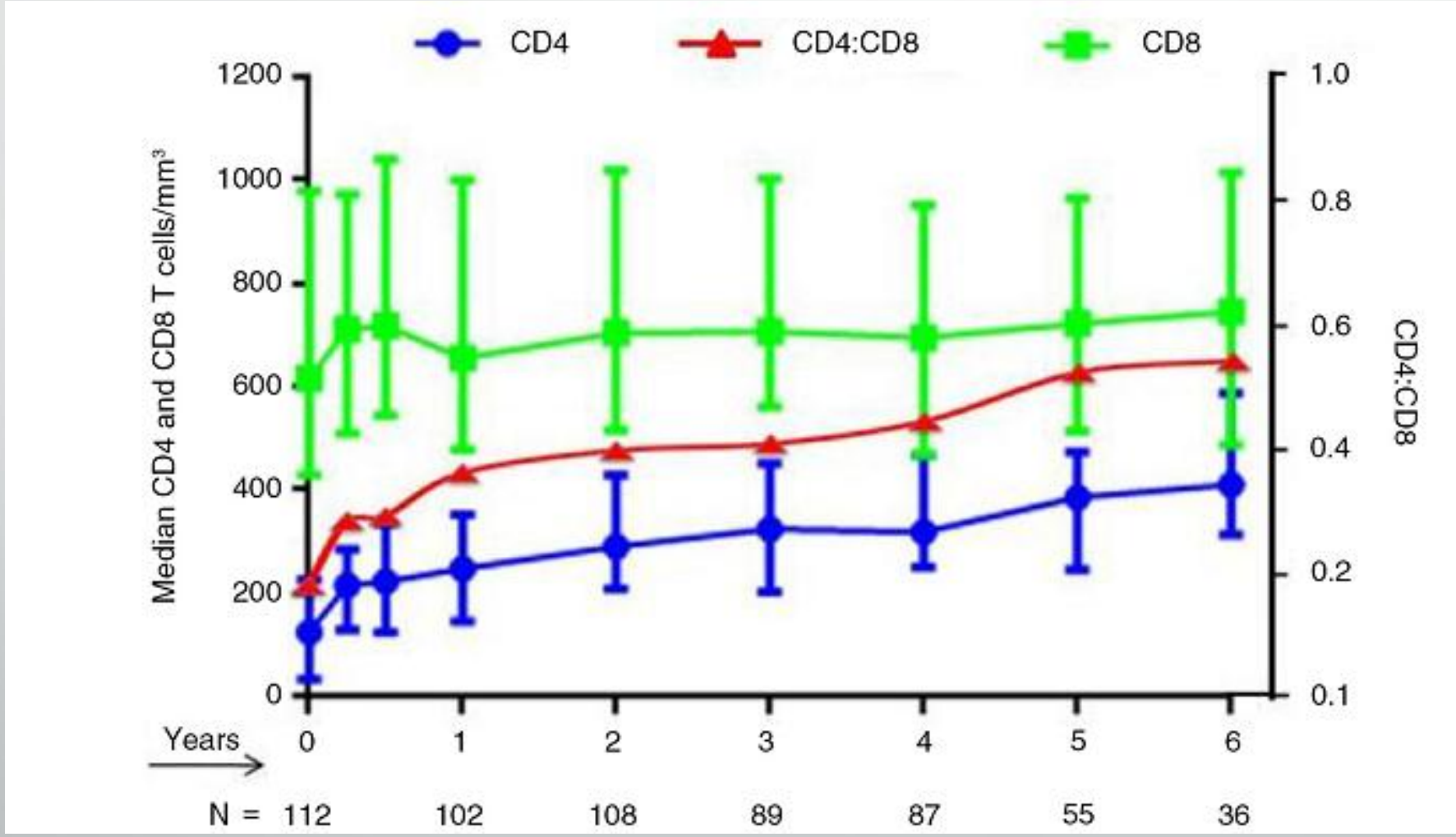
Monitorare il rapporto ha un'importanza clinica e non solo speculativa

Una ART efficace al pari di azzerare la replicazione virale dovrebbe normalizzare i CD4 e il rapporto CD4/CD8

- una ART più precoce si associa ad una più rapida normalizzazione del rapporto
- il basso valore del rapporto si associa in modo indipendente alla mortalità e all'insorgenza di non AIDS-comorbidità

Serrano-Villar S. PLOS Pathogens;2014:10;e1004078

CD4/CD8


CD4
CD4:CD8
CD8
Median CD4 and CD8 T cells/mm³
CD4:CD8
1200
1000
800
600
400
200
0
1.0
0.8
0.6
0.4
0.2
0.1
Years
0
1
2
3
4
5
6
N = 112
102
108
89
87
55
36

Infezione acuta: quali novità

– Comparison of immunological reconstitution in individuals who start ART during primary infection (PHI) vs in Chronic Infection (CI)

me nce art RT	Median CD4 count (cells/mm³)			Median CD4%			Median CD4:CD8 ratio			Presence of OIR*		
	PHI	CI	P-value	PHI	CI	P-value	PHI	CI	P-value	PHI	CI	P-value
Year	743	600	<0.0001	35	26	<0.0001	0.95	0.52	<0.0001	51% (19/37)	25% (39/153)	0.002
ears	850	779	0.005	39	33	<0.0001	1.05	0.78	<0.0001	73% (27/37)	46% (73/158)	0.003
ears	966	874	0.02	38	33	0.01	1.09	0.85	0.04	85% (23/27)	53% (38/72)	0.003

* OIR=optimal immune reconstitution defined by CD4 count =800 cells/mm³ or CD4% = 40% or CD4:CD8 ratio = 1.00

27 patients in PHI cohort had more than ten years of follow-up since treatment initiation

I benefici della ART nella AHI

	Week 0		Weeks Since HIV Diagnosis (*P* compared to day 0)					
	HIV- (N=109)	HIV+ (N=78)	2 (N=77)	12 (N=75)	24 (N=75)	36 (N=71)	48 (N=62)	96 (N=32)
I-FABP (pg/mL)	701	984 *P*=0.0115	2746 *P*<0.0001	2625 *P*<0.0001	1837 *P*<0.0001	2287 *P*<0.0001	2797 *P*<0.0001	2254 *P*<0.0001
sCD14 (µg/mL)	0.78	1.53 *P*<0.0001	1.32 *P*=0.0046	1.17 *P*<0.0001	1.15 *P*<0.0001	1.05 *P*<0.0001	1.04 *P*<0.0001	1.16 *P*<0.0001
CRP (µg/mL)	0.26	1.35 *P*<0.0001	0.77 *P*=0.0009	0.51 *P*=0.0001	0.45 *P*<0.0001	0.41 *P*<0.0001	0.36 *P*=0.0001	0.49 *P*=0.0047
D-dimer (µg/mL)	0.17	0.28 *P*<0.0001	0.23 *P*=n.s.	0.15 *P*<0.0001	0.16 *P*<0.0001	0.16 *P*<0.0001	0.17 *P*<0.0001	0.18 *P*=0.0004
Hyaluronic acid (ng/mL)	9.00	18.39 *P*<0.0001	10.21 *P*=0.0030	12.55 *P*<0.0001	18.89 *P*=n.s.	16.29 *P*=0.0410	15.94 *P*=0.0019	11.94 *P*=0.0043

MT, fibrosi, coagulazione e infiammazione si riducono a valori molto più bassi di quelli di pazienti in terapia ART cronica

Rimangono elevati rispetto a controllo HIV-, con l'eccezione del D-dimero, anche se la terapia è iniziata al Fiebig 1

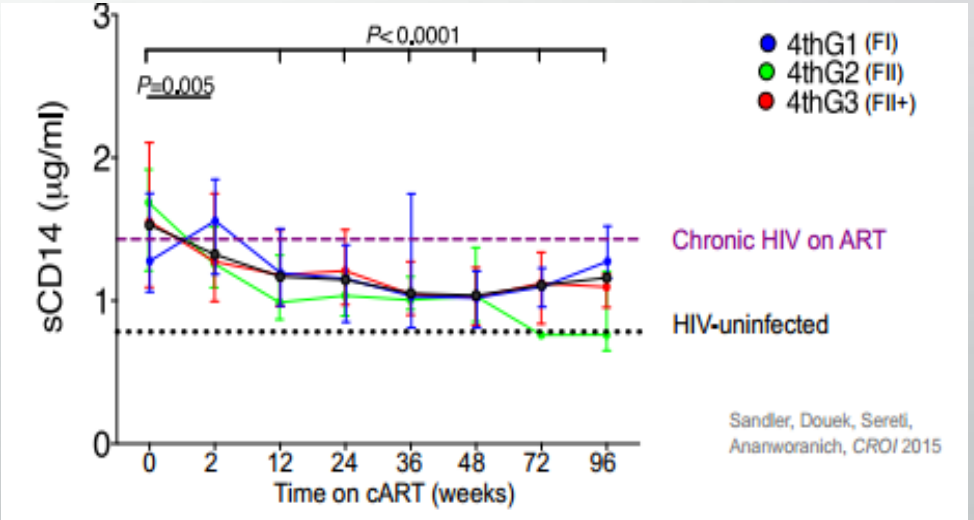


Table 1. Characteristics of Subjects during Acute HIV Infection, Stratified by Colonic HIV RNA

Characteristic	Colonic HIV RNA ≥50 copies/mg [N=32]	Colonic HIV RNA <50 copies/mg [N=10]	p
	Median (IQR)	Median (IQR)	
Age (years)	29 (24 – 31)	28 (25-42)	**0.62**
Time since HIV exposure (days)	16 (14 – 22)	11 (8 – 16)	**0.02**
Peripheral Blood			
CD4 (cells/mm^3)	391 (339 – 564)	491 (311 – 565)	0.67
CD8 (cells/mm^3)	624 (409 – 1105)	421 (271 – 500)	**0.03**
CD4:CD8 ratio	0.56 (0.38 – 1.17)	1.14 (1.12 – 1.45)	**0.01**
HLA-DR/CD38 expression on CD4 (%)	2.6 (1.8 – 4.3)	1.9 (1.4 – 2.4)	0.06
HLA-DR/CD38 expression on CD8 (%)	14.3 (9.5 – 16.9)	7.6 (5.7 – 11.7)	**0.01**
Ki-67 expression on CD4 (%)	1.8 (1.3 – 3.0)	1.8 (1.2 – 2.8)	0.92
Ki-67 expression on CD8 (%)	2.9 (2.2 – 5.5)*	1.6 (1.3 – 2.8)	**0.01**
HIV-RNA (log_{10}copies/mL)	5.6 (5.4 – 6.6)	4.3 (3.4 – 5.4)	**<0.01**
Total HIV DNA (copies/10^6 PBMC)	145 (9 – 1056)	8 (0 – 74)	**0.05**
Integrated HIV DNA (copies/10^6 PBMC)	2 (0 – 69)	0 (0 – 0)	**0.01**
IP-10 (pg/mL)	477.2 (205.6 – 727.2)	148.5 (68.3 – 350.9)	**0.02**
TNF-RII (pg/mL)	1062.1 (739 – 1771.4)*	649.3 (581.1 – 793)	**<0.01**
Neopterin (pg/mL)	2418.1 (1746.5 – 3137)	1367.6 (866.3 – 1910.3)	**0.01**
Colon			
CD4 (x10^6 cells/gm)	6.7 (2.2 – 9.9)	14.1 (7.6 – 18.2)	**0.05**
HLA-DR/CD38 expression on CD4 (%)	2.6 (2.0 – 3.4)	2.4 (0.9 – 3.4)	0.22
HLA-DR/CD38 expression on CD8 (%)	8.9 (5.5 – 13.4)	4.5 (3.2 – 6.0)	**0.01**
Ki-67 expression on CD4 (%)	8.5 (3.8 – 11.1)	2.4 (1.9 – 3.2)	**<0.01**
Ki-67 expression on CD8 (%)	13.5 (8.2 – 20)*	3.9 (3.4 – 8.2)	**<0.01**
Total HIV DNA (copies/10^6 cells)	477 (68 – 1610)	0 (0 – 29)	**0.01**
Integrated HIV DNA (copies/10^6 cells)	116 (0 – 425)	0 (0 – 0)	**0.03**
Cerebrospinal fluid			
HIV-RNA (log_{10}copies/mL)	3.9 (3.1 – 4.5)	1.8 (1.7 – 2.1)	**<0.01**

Characteristics of Subjects during Acute HIV Infection, Stratified by Colonic HIV RNA

Durante infezione acuta, l'evidenza di HIVRNA nel colon è molto comune e correla con HIV RNA nel sangue e nel CSF.

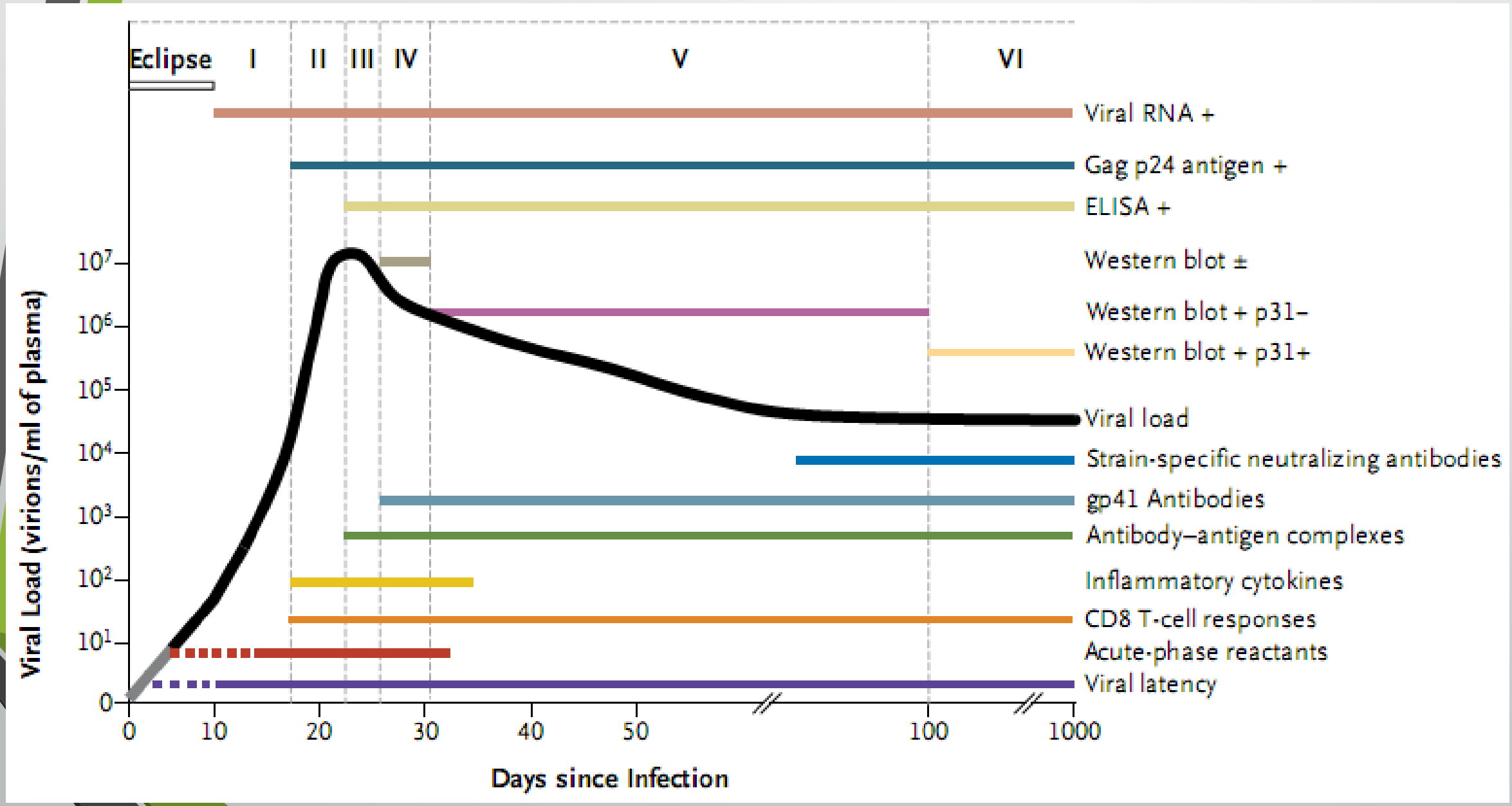
I pazienti con positività per HIV RNA nel colon durante AHI presentano una riduzione dei CD4 nel colon, infiammazione periferica e attivazione dei CD8 nel colon come nella periferia

I pazienti con negatività per HIV RNA nel colon sono in una fase più precoce dell'AHI

		Group 1 (HIV+, started ART during PHI)		Group 2 (HIV+, started ART during CHI)		Group 3 (HIV-negative)			
		Median	IQR	Median	IQR	Median	IQR	p-value	Results
Markers of immune senescence	Naïve/Memory T cell ratio	1.86	0.96 - 2.6	0.71	0.57 - 1.6	0.47	0.30 - 1.8	0.0360	G1 > G2/G3
	Terminally differentiated CD8+T cells (%CD27-CD28-)	19.1	15.2 - 32.0	39.1	30.0 - 44.8	22.7	9.4 - 34.1	0.0386	G2 > G1/G3
	Senescent CD4+ T cells (%CD28-CD57+)	1.54	1.2 - 1.9	3.29	1.7 - 5.6	1.12	0.89 - 2.6	0.0057	G2 > G1/G3
	Marker of vaccine response (%CD4+CD28+)	53.8	53.1 - 68.4	37.3	29.4 - 47.4	50.7	40.2 - 56.8	0.0006	G1 > G2
	Telomere lengths in CD8+ cells (relative to 1301 cells)	13.8	13.0 - 14.4	11.8	8.0 - 13.1	13.7	12.0 - 15.2	0.0116	G2 < G1/G3
Markers of immune activation / coagulation	Plasma CRP (mg/L)	0.36	0.19 - 0.95	0.24	0.13 - 0.32	0.22	0.13 - 0.60	0.2008	NS
	Plasma Fibrinogen (mg/dL)	207	165 - 444	196	180 - 325	175	147 - 247	0.3550	NS
	Plasma IL-1β (ng/mL)	0.26	0.18 - 0.32	0.23	0.08 - 0.34	0.23	0.15 - 0.38	0.5139	NS
	Plasma IL-6 (ng/mL)	0.93	0.54 - 1.2	1.02	0.76 - 1.6	0.90	0.62 - 1.0	0.2395	NS
	Plasma TNF-α (ng/mL)	6.18	5.0 - 8.2	6.38	5.7 - 8.8	5.00	4.2 - 5.4	0.9006	NS
	Monocytes (% of CD3- lymphocytes)	2.50	2.1 - 4.8	2.23	1.4 - 7.3	1.17	0.55 - 1.7	0.0300	G1/G2 > G3
	Serum sCD14 (ng/mL)	2035	1848 - 2280	1930	1805 - 2055	1510	1395 - 1605	0.0003	G1/G2 > G3
	Activated CD8+ T cells (% CD38+HLA-DR+)	1.45	1.2 - 2.5	1.80	1.2 - 2.6	1.11	0.60 - 1.4	0.0433	G1/G2 > G3
	Th17/Treg ratio	0.36	0.24 - 0.43	0.33	0.28 - 0.44	0.46	0.41 - 0.71	0.0145	G1/G2 < G3

L'inizio della ART nella fase acuta si traduce in una normalizzazione dei markers immunologici di senescenza del SI

CROI 2015


Eclipse
I
II
III
IV
V
VI
Viral RNA +
Gag p24 antigen +
ELISA +
Western blot ±
Western blot + p31–
Western blot + p31+
Viral load
Strain-specific neutralizing antibodies
gp41 Antibodies
Antibody–antigen complexes
Inflammatory cytokines
CD8 T-cell responses
Acute-phase reactants
Viral latency
Viral Load (virions/ml of plasma)
10^7
10^6
10^5
10^4
10^3
10^2
10^1
0
0
10
20
30
40
50
100
1000
Days since Infection

PTC: quali novità

Virologic and immunologic correlates of viral control post-ART interruption in SIV-infected rhesus macaques

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Background: Antiretroviral therapy (ART) does not eradicate HIV and the virus rebounds upon treatment interruption. Recently, a sustained control of HIV replication in the absence of ART has been achieved in a subset of patients starting ART early after infection, defined as post-ART treatment controllers (PTC). Unfortunately, the virologic and immunologic determinants of post-ART control of HIV replication are still unclear, particularly in tissues. Here, we used the well-established model of SIV-infection in rhesus macaques (RMs) to investigate the existence of PTC in this model and the features associated with post-ART SIV control.

Methods: 15 RMs (B*08- and B*17-) were infected (i.v.) with SIV_{mac239}. All 15 animals initiated a 5-drug ART regimen 60 days after infection, which was maintained for seven months. ART was then interrupted and RMs monitored for eight additional months. Blood (PB), lymph node (LN), and colorectal (RB) biopsies were collected throughout the study. Quantitative assessment of total SIV-DNA and RNA was performed on purified blood CD4 T cells and mucosal tissues by quantitative PCR; immunological parameters were determined by flow cytometry.

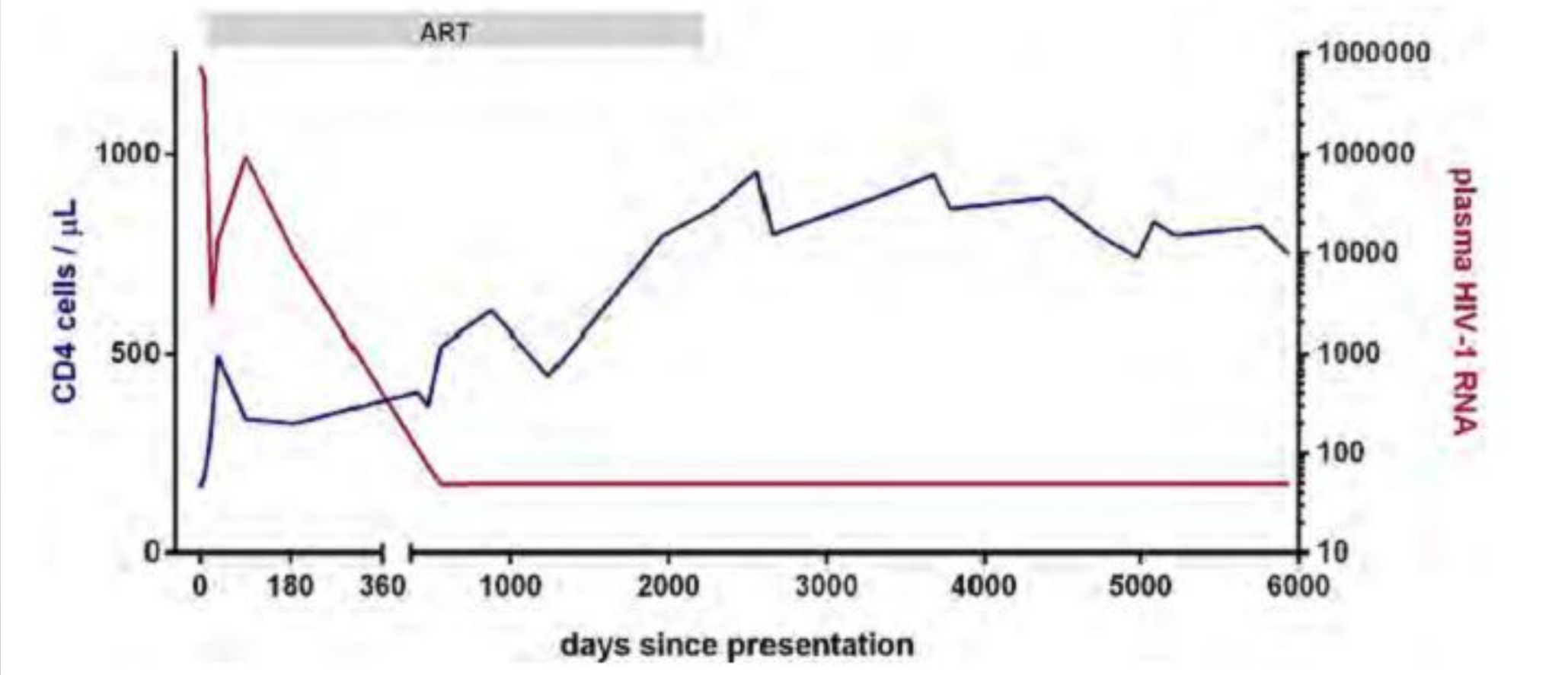
Results: ART suppressed SIV-RNA to <60 copies/mL in all RMs. After ART interruption, 6 RMs controlled SIV viremia at $<10^3$ copies/mL up to 8 months off-ART (PTC), while 9 RMs rebounded to pre-ART levels (non-controllers, NC). At pre-ART, PTC had significantly lower plasma viremia and SIV-DNA content, as well as higher CD4 T cell counts as compared to NC. Levels of intestinal CD4 T cells were similar, but PTC had higher frequencies of Th17 cells than NC. On-ART, PTC had significantly lower levels of residual plasma viremia (3 copies/mL, limit of detection) and SIV-DNA content (both in blood and colorectum). After ART interruption, SIV-DNA content rapidly increased in NC while it progressively decreased in PTC. Finally, in PTC control of SIV rebound associated with higher CD4 T cell levels and reduced immune activation in PB and RB during the entire off-ART period.

Conclusions: Lower set point viremia, reduced cell-associated SIV-DNA, and preserved Th17 cell homeostasis associate with improved virologic response to ART and sustained viral control post-ART interruption in SIV-infected RMs.

15 RM infetti
ART (5 farmaci) da 60g→6m

Dopo interruzione ART: 6PTC, 9NC

PTC:
↓VL e SIV-DNA pre-ART
↑CD4 pre-ART
↑Th17 intestinali pre-ART
↓Viremia residua e SIV DNA in ART (sangue e colon)
↑CD4 e ↓immunottivazione dopo interruzione di ART



Ridotti livelli di HIV DNA associati a TCD4 HIV specifici e TCD8 CTL con attività inibitoria anti-virale si associano a prolungata aviremia dopo interruzione delle ART

HIV-1 virological remission for more than 11 years after interruption of early initiated antiretroviral therapy in a perinatally-infected child

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Background: Durable HIV-1 remission after interruption of combined antiretroviral therapy (cART) has been reported in some adults who started cART during primary HIV-1 infection. The *in utero* HIV-1-infected «Mississippi child», exhibited transient viral control after interrupting very early-initiated cART. However viremia rebounded 27 months later, leaving unclear the possibility of obtaining long-term post-treatment remission in vertically-infected children. Here we report the case of a perinatally-HIV-1-infected adolescent who shows unprecedented virological remission more than 11 years after cART discontinuation.

Methods: HIV-RNA and CD4+ T-cell counts have been monitored since birth. Ultrasensitive HIV-RNA, PBMC-associated HIV-DNA, flow-cytometry-assessed frequency of HIV-specific CD8+ T-cells, CD8+ T-cell mediated HIV-suppression, reactivation of the CD4+ T-cell reservoir were evaluated after 10 and 11 years of control off therapy. Plasma concentrations of antiretrovirals were determined by tandem mass spectrometry.

Results: One infant born from a woman with uncontrolled HIV-1 viremia received zidovudine-based prophylaxis during 6 weeks. HIV-RNA and DNA were not detected 3 and 14 days after birth. HIV-DNA was detected at 4 weeks of age. HIV-RNA reached a peak of 2.1×10^6 copies/ml at 3 months of age when cART (zidovudine, lamivudine, didanosine, ritonavir) was initiated. HIV-RNA was undetectable one month later and remained below assay-detection limits while on cART, except at 15 and 21 months of age. Between 5.8 and 6.8 years of age cART was discontinued by the family. HIV-RNA was undetectable at 6.8 years of age and cART was not resumed. HIV-RNA has remained < 50 copies/ml through 18.3 years of age, except for one blip (515 copies/ml). CD4+ T-cell counts remained stable. After 11 years of control off therapy (confirmed by undetectable plasma concentrations of antiretrovirals), HIV-RNA was below 4 copies/ml and HIV-DNA was 2.2 Log copies/10^6 PBMC. Low levels of HIV-RNA and p24 were detected upon activation of CD4+ T-cells with PHA. HLA genotype showed homozygosity at several loci (A*2301-;B*1503/4101;C*0210/0802;DRB1*1101-;DQB1*0602-). HIV-specific CD8+ T-cell responses and T-cell activation were very weak. HIV-1 western blot was positive with absence of antibodies against gp110 and p18.

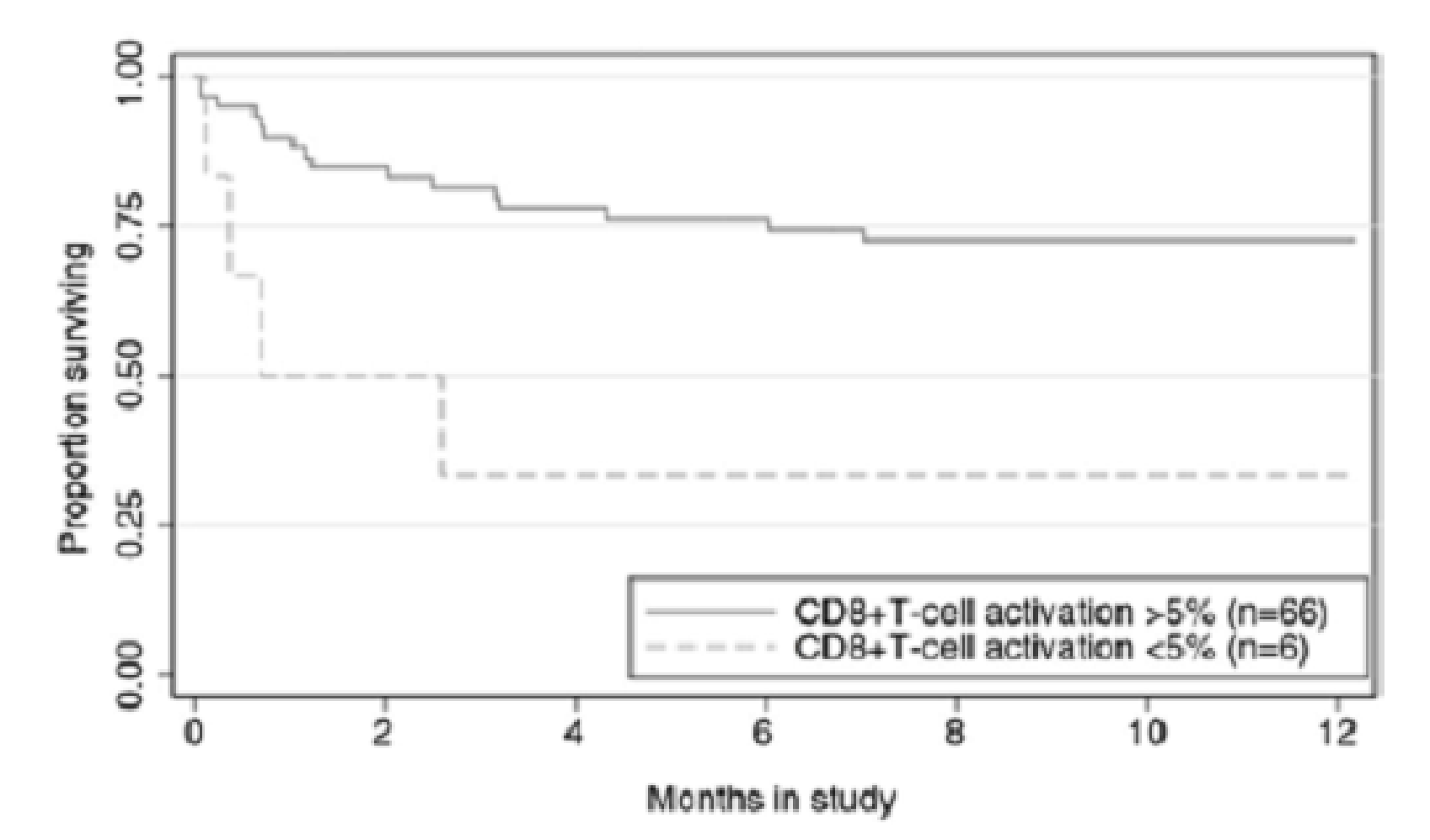
Conclusions: This case provides first-time evidence that very long-term HIV-1 remission is possible in perinatally-infected-early-treated children, with similar characteristics as reported in adult post-treatment controllers.

Persistente assenza di spontanea replicazione virale dopo 11 anni di interruzione dalla ART iniziata a tre mesi di vita e interrotta circa a 6 anni

Attivazione cellule T e risposta CD8 HIV specifica debole

Stimolazione in vitro dei CD4 con PMA debole produzione di p24 e HIV RNA

CD8 attivati e mortalità in pediatria



TUPEA093

Robust HIV-specific T cells in post-treatment controllers from the VISCONTI cohort

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Presenting author email: brigitte.autran@psl.aphp.fr

Background: Post-Treatment-Controllers (PTCs) represent models of functional HIV remission with an exceptional HIV control years after interruption of an early-initiated antiretroviral therapy. The enrichment in the HLA-B35 allele, associated with symptomatic primary-infection and poor prognosis, instead of the protective HLA alleles reported in Elite Controllers (ECs) questions the role mechanism of this HIV control and the role of anti-HIV T cell responses, particularly those driven by HLA-B35. We therefore compared the PTCs HIV-specific CD4 and CD8 T cells to those from continuously early-treated patients (CETs) and ECs.
Methods: We included 12 PTCs from the VISCONTI study*, half HLA-B35+, 10 CETs under a cART initiated within 10 weeks post-infection and 8 ECs from the ANRS-Co15 cohort. Multiparametric flow-cytometry assessed HIV-specific IFNg, IL2, TNFα, MIP1β or CD40L producing CD4 and CD8 T cell stimulated with HIV-p24 protein and peptides. The cell-associated HIV-DNA was measured in PBMCs and naïve and memory sorted resting CD4 T cell subsets.
Results: High frequencies of HIV-p24 specific CD4+ cellswere observed in PTCs and did not differ from the ECs or CETs ones. A third of these PTCs HIV-p24 specific CD4 cells were highly polyfunctional producing 2, 3 and 4 functions, similarly to from CETs and ECs. HLA-B35 did not influence these results. In contrast frequencies of PTCs CD8+ cells producing against HIV-p24 peptides IFNg (p=0.015) or MIP1b (p=0.001) were lower than ECs but equivalent to CETs ones, without differences in poly-functionality between the 3 groups. Among the functions tested here-in there were 20-fold less IFN-g producing HLA-B35+ CD8 T cells than HLA-B35- ones (0.006% versus 0.130%, p=0.041) against HIV-p24 peptides.
Conclusions: The model of HIV remission represented by VISCONTI PTCs is characterized by robust polyfunctional HIV-specific CD4+ T cells similar to those from Elite Controllers and from continuously early-treated patients, independently from the HLA-B35 allele which negatively impacts IFN-g producing CD8 T cells. These results illustrate differences between ECs and PTCs linked to HLA background and suggest early initiation of treatment allows maintenance of robust HIV-p24 specific CD4 T cells in PTCs.

Valutata funzione cellulare HIV specifica in

12 PTCs
10 CETs
8ECs

CD4 HIV-specifici polifunzionali
PTCs=CETs=Ecs

HLAB35 non influenzava il risultavo

CD8 HIV-specifici MIP1β e INFγ+
ECs↓PTCs=CETs non differenza sulla polifunzionalità

Nel modello dei PTCs viene mantenuta una robusta polifunzionalità delle cellule T indipendentemente dall'HLA

52 **Post-Treatment Controllers Have Particular NK Cells With High Anti-HIV Capacity: VISCONTI Study**

Daniel Scott-Algara[1]; Céline Didier[1]; Vincent Arnold[1]; Jean-Saville Cummings[1]; Faroudy Boufassa[2]; Olivier Lambotte[5]; Laurent Hocqueloux[4]; Asier Sáez-Cirión[1]; Christine Rouzioux[3]

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Background: The association of T-cells with the control of HIV infection has been well documented. In addition, several studies pointed out the role on innate immune responses in the resistance or delay to HIV disease progression. The recent description (VISCONTI study) of a group of patients, so-called post-treatment controllers (PTCs), who remains with undetectable viral load after treatment interruption despite poor CD8+T cell responses, led us to study NK cells and their relationship to HIV control.

Methods: 14 patients enrolled in the French ANRS VISCONTI cohort were studied. PTCs' results were compared to those of Viremic patients, (VIR), natural HIV controllers (HIC), patients on ARV since acute infection (ARV) and normal blood donors (controls). All phenotypic studies were done on fresh whole blood. CD107a expression on NK cells and the ICS-based assay were done on PBMC after stimulation. NK cells were tested for their capacity to inhibit HIV infection (measured by intracellular or supernatant p24) in autologous CD4 T cells. Results obtained were compared using Wilcoxon rank sum test or by the Fisher exact test

Results: Visconti patients showed significantly higher expression of CD158a, CD158b (KIR2DL2/DL3), PTC showed significantly higher expression of CD158a, CD158b (KIR2DL2/DL3), CD158b/DX9 (KIR3DL1/3DS1) and NKG2A receptors ($p<0.002$, $p<0.001$, $p<0.0.01$, $p<0.001$, respectively) with lower expression of CD160 and NKp46 ($p<0.001$, $p<0.03$, respectively) receptors compared to others groups. Activation of NK cells from PTC and ARV, measured by CD69 marker, was similar to normal donors but lower than in HIC ($p<0.006$) or VIR patients ($p<0.001$). Functional studies when NK cells were stimulated by K562 cell line showed normal levels of degranulation (CD107a marker) but higher IFN-γ production for PTC than for other groups ($p<0.01$). NK cells from PTCs, but not from other individuals ($p<0.001$), were able to reduce p24 levels when co-cultured with in vitro-infected autologous CD4 T cells.

Conclusions: NK cells from PTC had a different NK cell repertoire and higher potential to produce IFN-γ compared to HIC, Viremic patients or normal donors, but similar to those treated since primary infection. More importantly, PTC NK cells showed high capacity to control in vitro HIV infection on autologous CD4 T cells. Our results suggest that preserving NK cell phenotype and function during acute infection is important to control HIV and identify NK cells as possible important agents in the durable remission of PTC.

T cell	Biomarker	Association with time to rebound* (HR [CI]; p-value)
CD4+	PD-1	1.46 [1.06-1.85]; p=0.016
	Tim-3	1.36 [1.16-1.60]; p=0.009
	Lag-3	1.08 [1.02-1.15]; p=0.007
CD8+	Tim-3	1.15 [1.06-1.26]; p=0.001

Table 1: T cell exhaustion markers pre-therapy are associated with time to viral rebound at TI. *Multivariable Cox model adjusting for the biomarker and Total HIV-1 DNA. HR: Hazard ratio; CI: 95% confidence interval

La valutazione pre-terapia di marcatori di esaurimento cellulare sui CD4 e CD8 può essere utile nel prevedere la risposta all'interruzione della ART

Gut, CMV: quali novità

Treatment with anti-α4β7 integrin antibody reduces virus-mediated gastrointestinal pathology by targeting distinct mucosal tissues

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Background: Our laboratory has recently demonstrated that in vivo administration of a monoclonal anti-α4β7 antibody (α4β7-mAb) during acute SIV infection following

1) intravenous,
2) intra-rectal or
3) repeated low-dose intra-vaginal SIV challenge lead to markedly lower gastro-intestinal tissue viral loads compared to rhesus macaques (RM) treated with a control mAb.

The purpose of the present study was to compare the tissues that served as primary targets of viral infection in the α4β7-mAb versus control mAb-treated RM, in order to identify mechanisms by which α4β7-mAb antibody reduces virus-mediated gastrointestinal pathology.

Gli anti-α4β7 riducono la diffusione intestinale del virus

RM trattati con α4β7:

Ridotto SIV DNA nelle biopsie intestinali di 5-25 volte rispetto ai controlli

Non differenze tra il SIV DNA nella milza e nei linfonodi

Immuno/PET a parità d VL, minor captazione intestinale negli animali trattati

Methods: Groups of 12-16 RM were administered a rhesus α4β7-mAb monoclonal antibody or an isotype-matched control rhesus IgG mAb (50 mg/kg) intravenously (i.v.) starting on day -1 and then every 3 weeks after infection. Each monkey was then repeatedly challenged with a low-dose SIVmac251 intra-vaginally or a single high-dose intrarectally.

Results: i.v. administration of α4β7-mAb blocked the detection of α4β7 on CD4+ T cells in the blood, cervicovaginal tissue, and GALT throughout the period of mAb administration. Viral DNA was reduced in GALT biopsies of the α4β7-mAb treated RMs compared to those treated with control mAb treated (median 3.5 vs. 12.8 copies/ng DNA respectively, p=0.006). Furthermore, in-depth analysis performed on a subset of animals (n=4/group) indicated that proviral DNA was 5 to 25 fold more abundant in jejunum, ileum, or colon of control-treated RMs compared to those treated with a4b7-mAb. In contrast, no difference in proviral loads in the spleen and lymph nodes from various sites was noted in the 2 groups.

Immuno-PET/CT assisted analysis revealed that for animals with comparable plasma viral loads, the α4β7-mAb treated monkeys showed a lower signal in the large intestine. In addition, only the control treated monkeys showed a clear PET/CT signal in lymph nodes surrounding the genital tract suggesting that treatment with α4β7-mAb prevents viral replication in this tissue, leading to different patterns of tissue localization of the virus between the two groups.

Conclusions: The α4β7-mAb either protects or delays intravaginal SIV transmission, reduces gastrointestinal pathology following infection, and results in both quantitative and qualitative differences in the level of viremia and tissue localization of virus.

SIV-induced translocation of bacterial products in the liver mobilizes myeloid dendritic and natural killer cells associated with liver damage

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Background: Disruption of the mucosal epithelium during immunodeficiency lentivirus infections permits translocation of microbial products into the circulation, causing systemic immune activation and driving disease progression. However, the specific effects of microbial products in liver, as a blood-filtering organ, are unclear.

Methods: In this study we investigated the effects of simian immunodeficiency virus (SIV) infection of rhesus macaques on microbial translocation in the liver by immunohistochemistry. We also compared liver infiltration by myeloid dendritic cells (mDCs), trafficking to the liver by lymphocytes, and liver-resident natural killer (NK) cell frequencies, phenotypes, and functions in naïve and chronically SIVmac239- or SIVmac251-infected rhesus macaques using flow cytometry.

Results: In livers of normal rhesus macaques very low levels of bacteria and LPS were detectable, but increased up to 20-fold in chronically SIV-infected animals. Increased microbial products in the liver of infected macaques was associated with production of the chemoattractant, CXCL16, by mDCs. Subsequently, lymphocytes expressing the CXCL16 receptor, CXCR6, were mobilized in blood and hypercytotoxic NK cells were recruited to the liver. Microbial accumulation, mDC activation and hepatic cytotoxic NK cell frequency were all significantly correlated with markers of liver damage.

Conclusions: Collectively, these data indicate that SIV-associated accumulation of microbial products in the liver initiates a cascade of innate immune activation resulting liver damage. These findings have implications for the liver pathology associated with HIV, especially in instances of coinfection with HCV.

Nel modello animale:

Nel fegato dei RM infetti una quantità di derivati microbici ed LPS 20volte superiore rispetto ai controlli sani

Aumentata espressione di chemochine (CXCL16) da parte dei mDC

Linfociti T CXCR6+ mobilizzati nel sangue

Aumento di NK residenti nel fegato

%MT, %mDC e % di NK si associano a markers di danno epatico

TULBPE04

Enhancement of microbiota in healthy macaques results in robust beneficial modulation of mucosal and systemic immune function

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[1]Drexel University, Philadelphia, United States, [2]University of Washington, Department of Pharmaceutics, Seattle, United States, [3]VGTI-Florida, Port Saint Lucie, United States, [4]National Cancer Institute, AIDS and Cancer Virus Program, Fredrick, United States, [5]University of Washington, Department of Microbiology, Seattle, United States, [6]Beth Israel Deaconess Medical Center, Boston, United States

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Background: With more than thirty million HIV-infected individuals worldwide, developing an effective vaccine to prevent new HIV infections remains a top priority in contemporary biomedical research. Given the critical role of mucosal surfaces in susceptibility of HIV infection, it is imperative that we induce effective mucosal responses.
However, current approaches to enhance mucosal immunity have not been successful in preventing HIV acquisition.

Methods: Modulating the microbiota in the GI tract is a safe and well-tolerated approach to enhance mucosal and overall health, and here we hypothesized that altering TLR signaling via microbiome enhancement may improve mucosal immunity. Thus we treated five macaques (SIV-) with the probiotic (PBio) VSL3 and sampled colon, rectum, blood and LN from prior to PBio treatment, and at days 28 and 80 post-treatment. We assessed cellular and humoral immunity and inflammation.

Results: rikingly, we found that PBio therapy resulted in significantly increased T follicular helper cells (Tfh; CD4+PD-1^{high}CXCR5^{high}, p=0.0085). In addition, immunohistochemistry confirmed that LNs had increased follicles after PBio treatment. Given the ability for Tfh to induce B cell responses, we measured surface IgA and IgG expression on B cells, and observed increased frequencies of B cells expressing IgA in the LN (p=0.0151) and colon (p=0.0072). To determine the method for increased Tfh, we measured IL-23 production by antigen presenting cells (APCs), and found significantly increased frequencies of IL-23+APCs in the colon (p=0.0173), which correlated with the frequency of LN Tfh (p=0.0358).

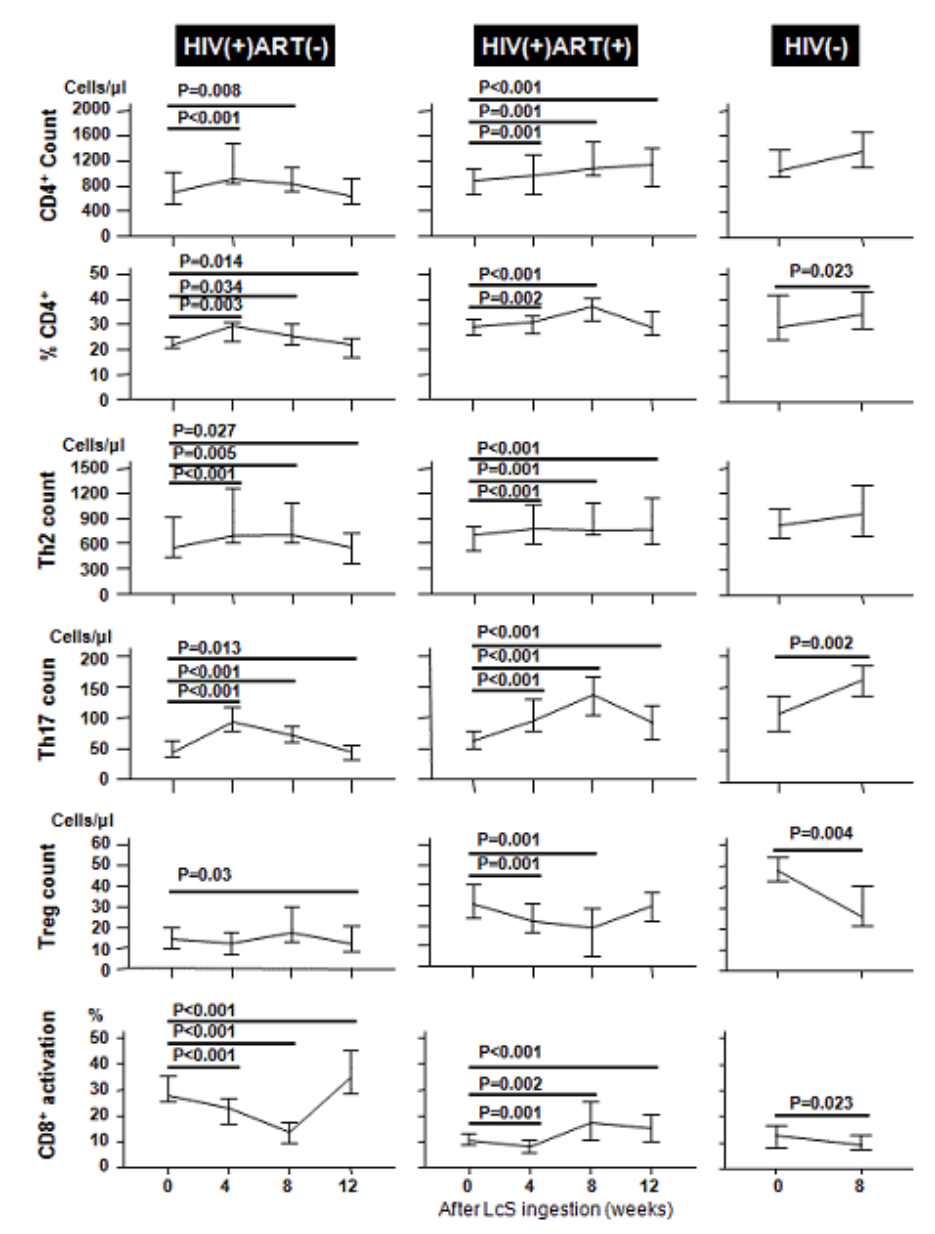
Conclusions: These data have potential implications for using PBio therapy to alter mucosal immunity in the context of vaccination or preventative approaches. In particular, the immunomodulatory properties of probiotic therapy in conjunction with HIV vaccination may provide an opportunity for enhanced mucosal HIV vaccine responses that could improve protection from infection by improving immune defenses at the mucosal portal of entry.

Aumento dei Tfh

Aumento del numero di follicoli all'interno dei linfonodi

Aumento di linfociti B esprimenti IgA nei linfonodi e nel colon

Aumento delle APC IL23+ nel colon che correla con la frequenza di Tfh nei linfonodi



Dopo 8 settimane di terapia con Lactobacillus casei Shirota:

Aumento dei CD4 negli HIV+

Aumento dei CD4/Th17+ in HIV+ e HIV-

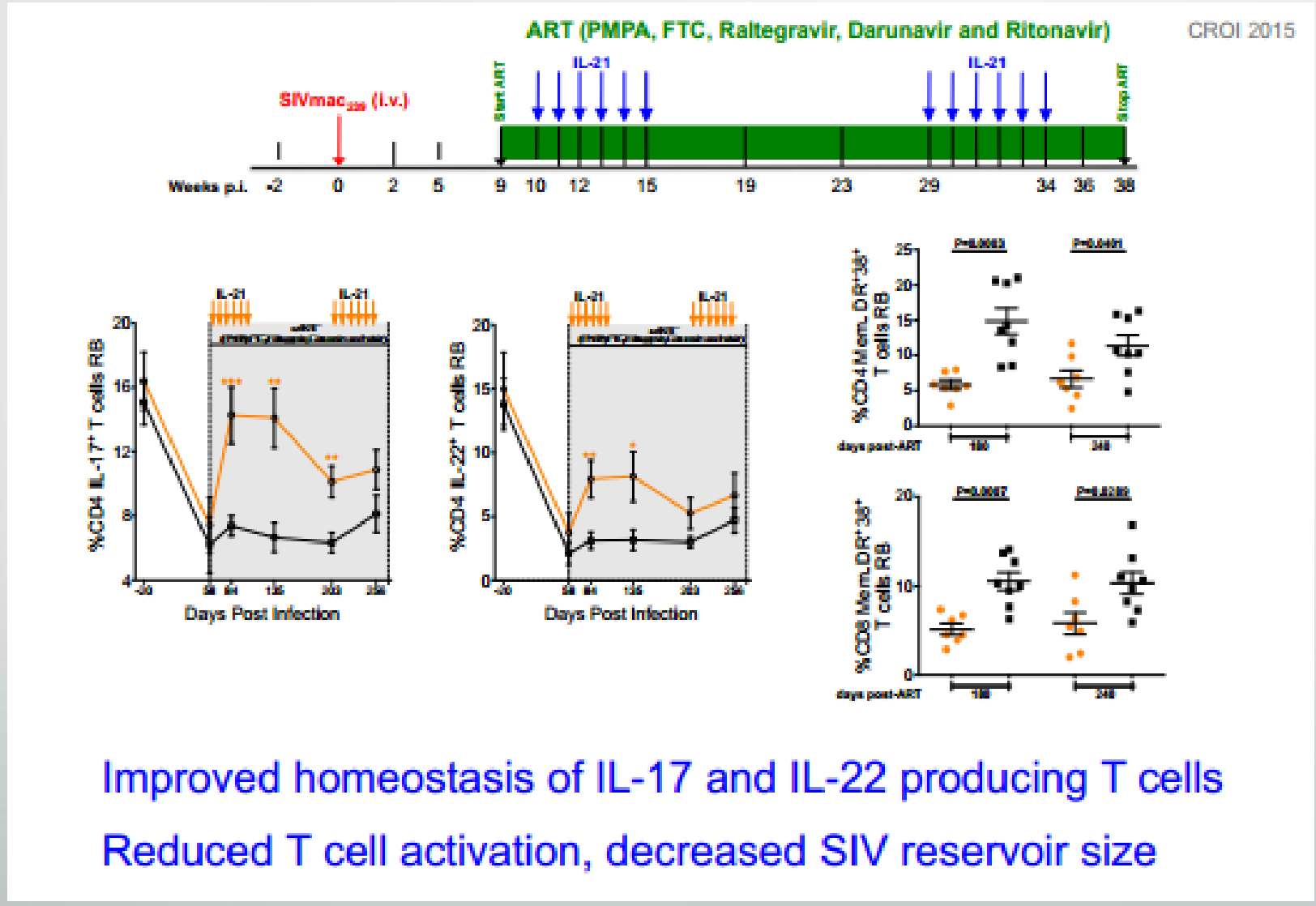
Netta riduzione dei CD8 attivati negli HIV ART- e lievemente anche negli HIV-

Riduzione significativa del VL negli HIV ART-

Non alterazioni del 16S rRNA in tutti i gruppi

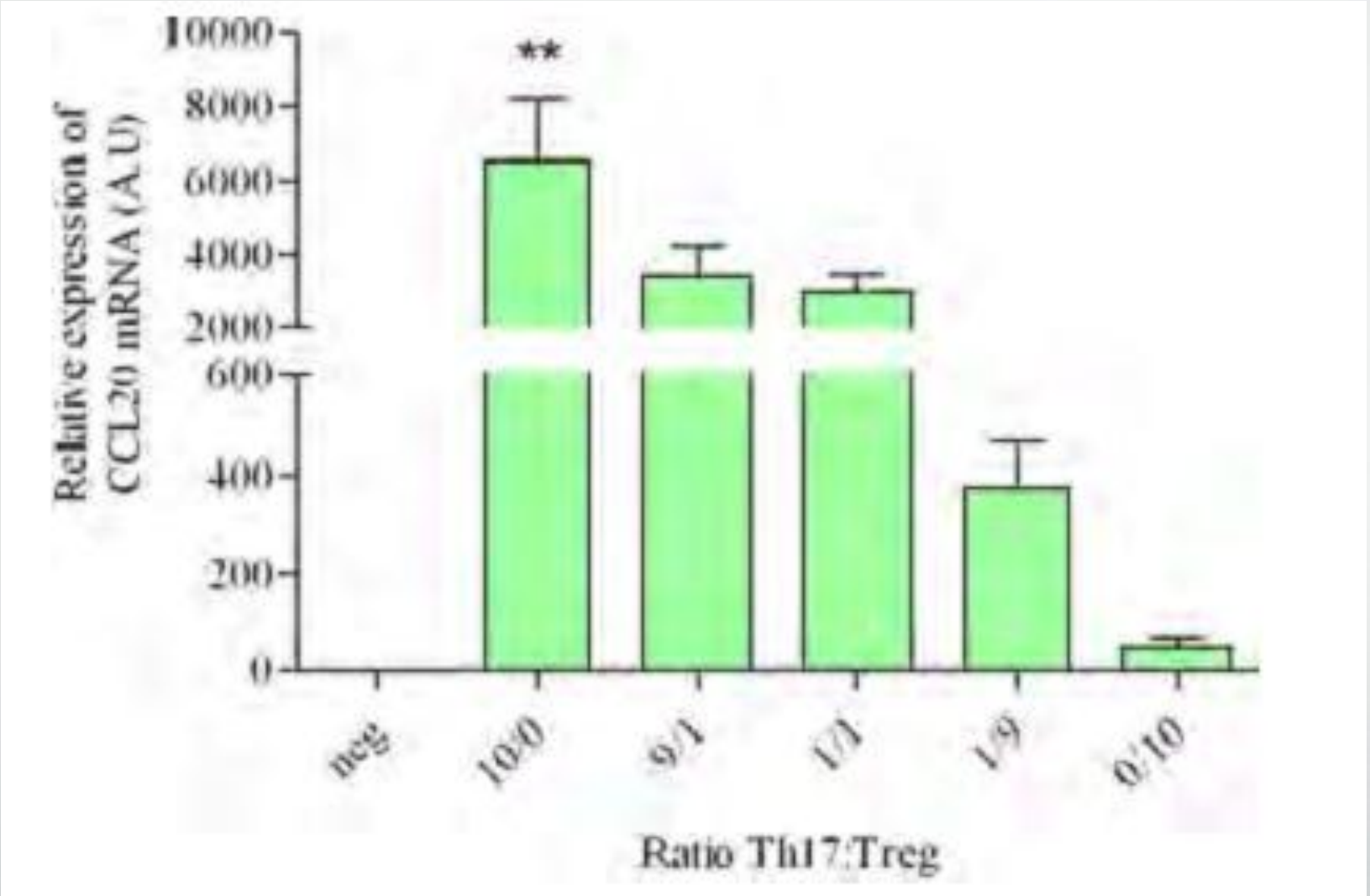
Aumento significativo dei Lactobacillus e Bifidobacterium nelle feci di entrambe i gruppi

IL-21 in RM

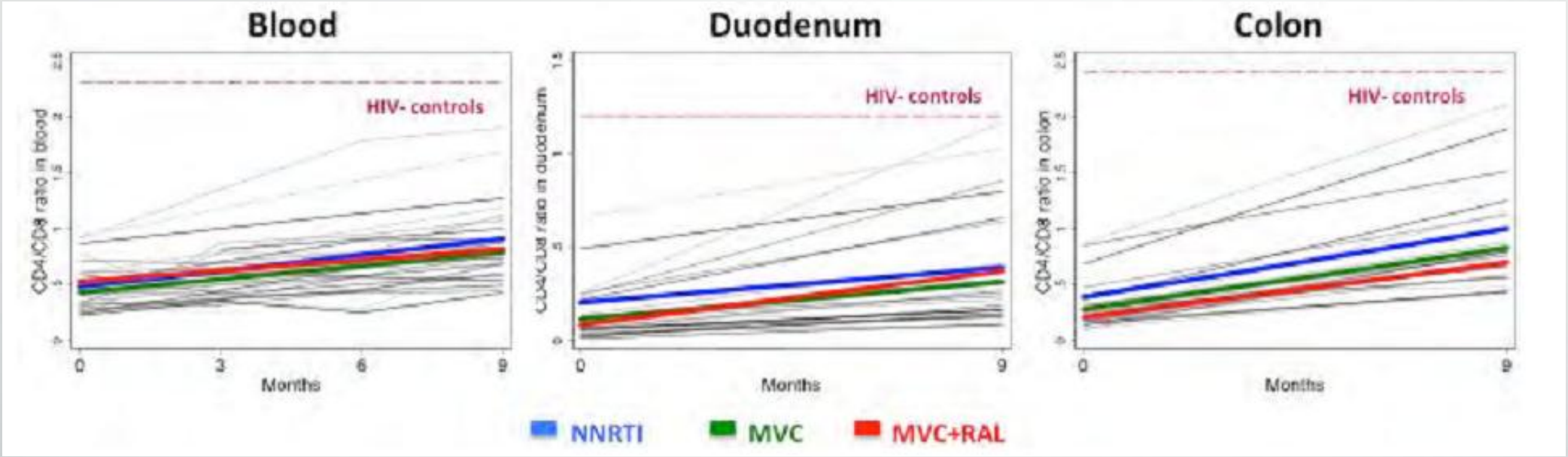


Improved homeostasis of IL-17 and IL-22 producing T cells

Reduced T cell activation, decreased SIV reservoir size

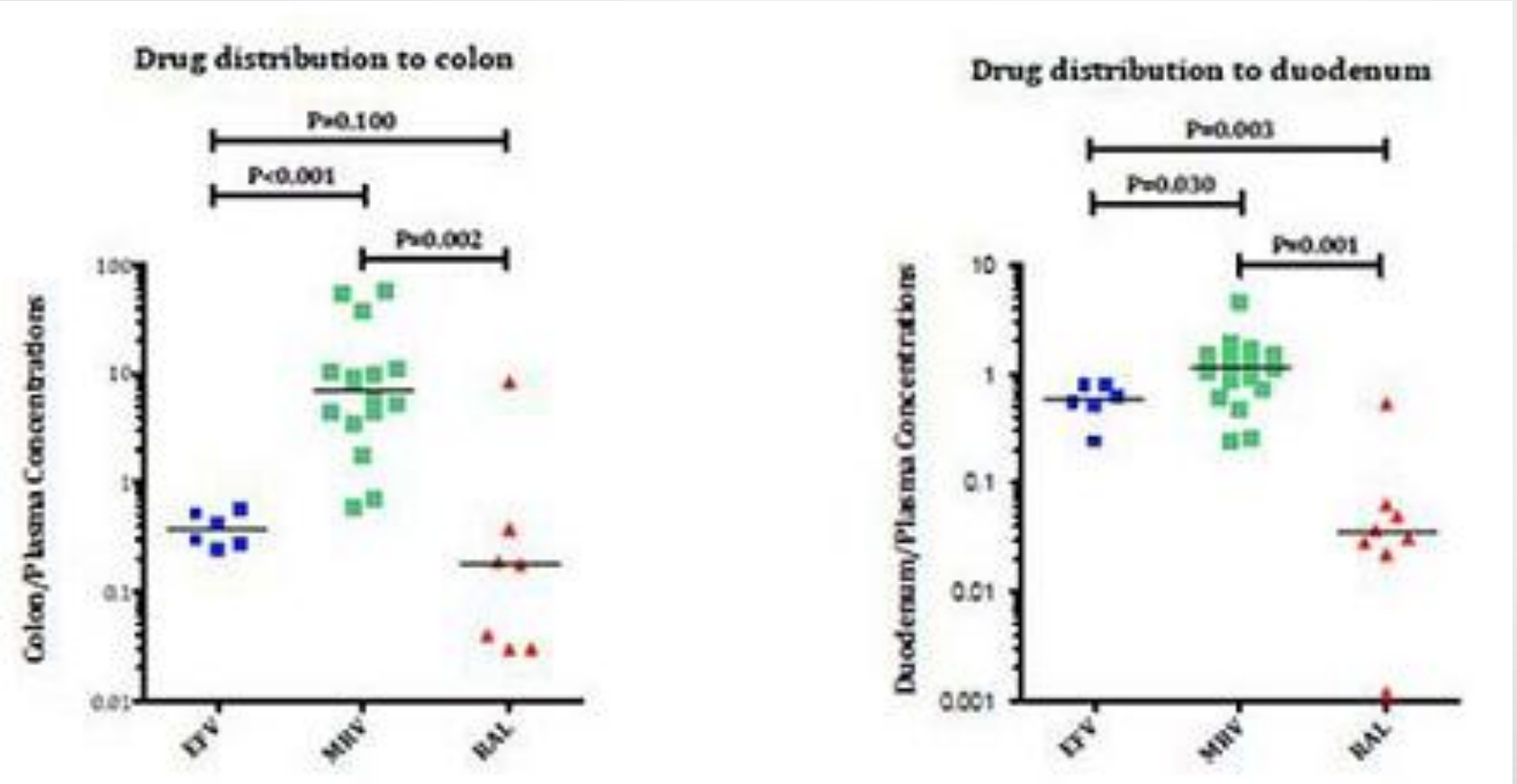


Il CCL20-CCR6 asse che guida l'homing dei Th17 nell'intestino risulta alterato negli HIV ART soppressi. La persistente alterazione del rapporto Th17/Treg nella lamina propria contribuisce ad una ridottta produzione di CCL20 da parte degli enterociti



Il recupero del rapporto CD4/CD8 durante la terapia antiretrovirale correla con riduzione della persistenza virale, dell'immunoattivazione e della maturazione delle cellule T nel sangue periferico e nel colon. Il duodeno presenta un comportamento differente

CROI 2015



Nel duodeno 4ART si associa a: minor concentrazione di CD8, maggior numero di CD4/CCR5+, CD8 naive/CD8 memory >1, ridotta concentrazione di sCD14 e HIV DNA

Il MRV è il farmaco con una miglior distribuzione nel gut e la sua concentrazione nel duodeno correla con %CD4, %CD8, CD4/CD8, %CD4 CD38 e %CD8 CD38

La concentrazione del MRV nel duodeno si associa ad una miglior maturazione dei CD8 tissutali

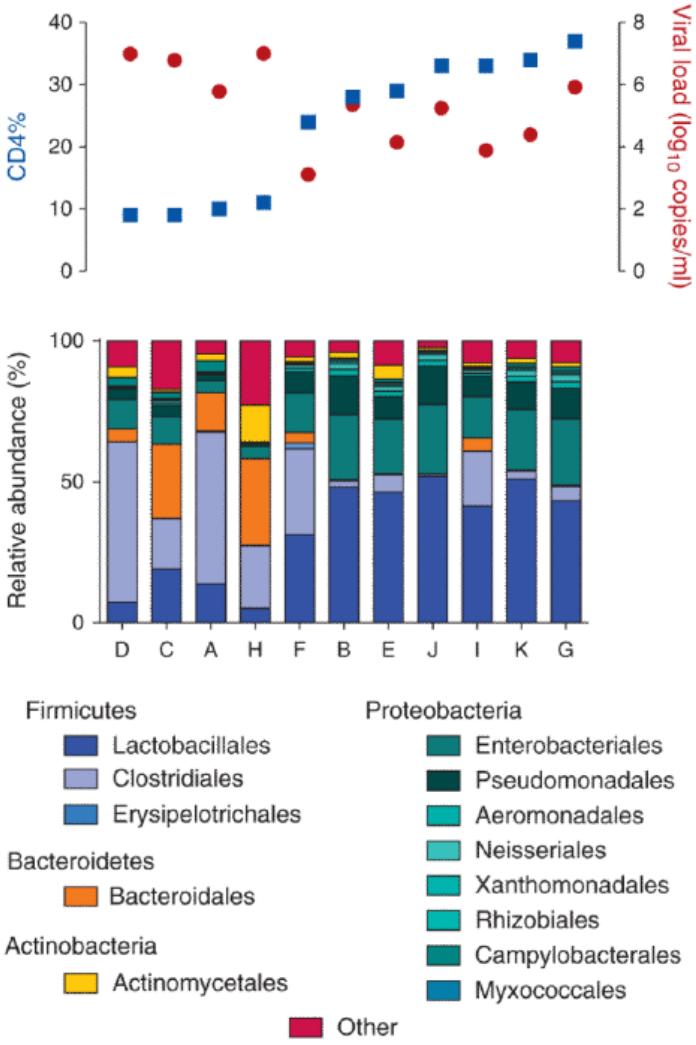


Fig. 2. Overview of participants' CD4%, viral load (VL) and gut bacterial profiles (GBP) at baseline

Participants' CD4% and VL are colored blue and red, respectively, and their corresponding GBP is in the bottom. Participants with lower CD4% and higher VL exhibit lower proportions of *Lactobacillales*.

300 **Effect of CMV and HIV Replication on T-Cell Exhaustion and Senescence During ART**

Jennifer M. Dan[1]; Marta Massanella[1]; David M. Smith[1]; Eric S. Daar[2]; Michael P. Dube[3]; Richard Haubrich[1]; Sheldon Morris[1]; Sara Gianella Weibel[1]

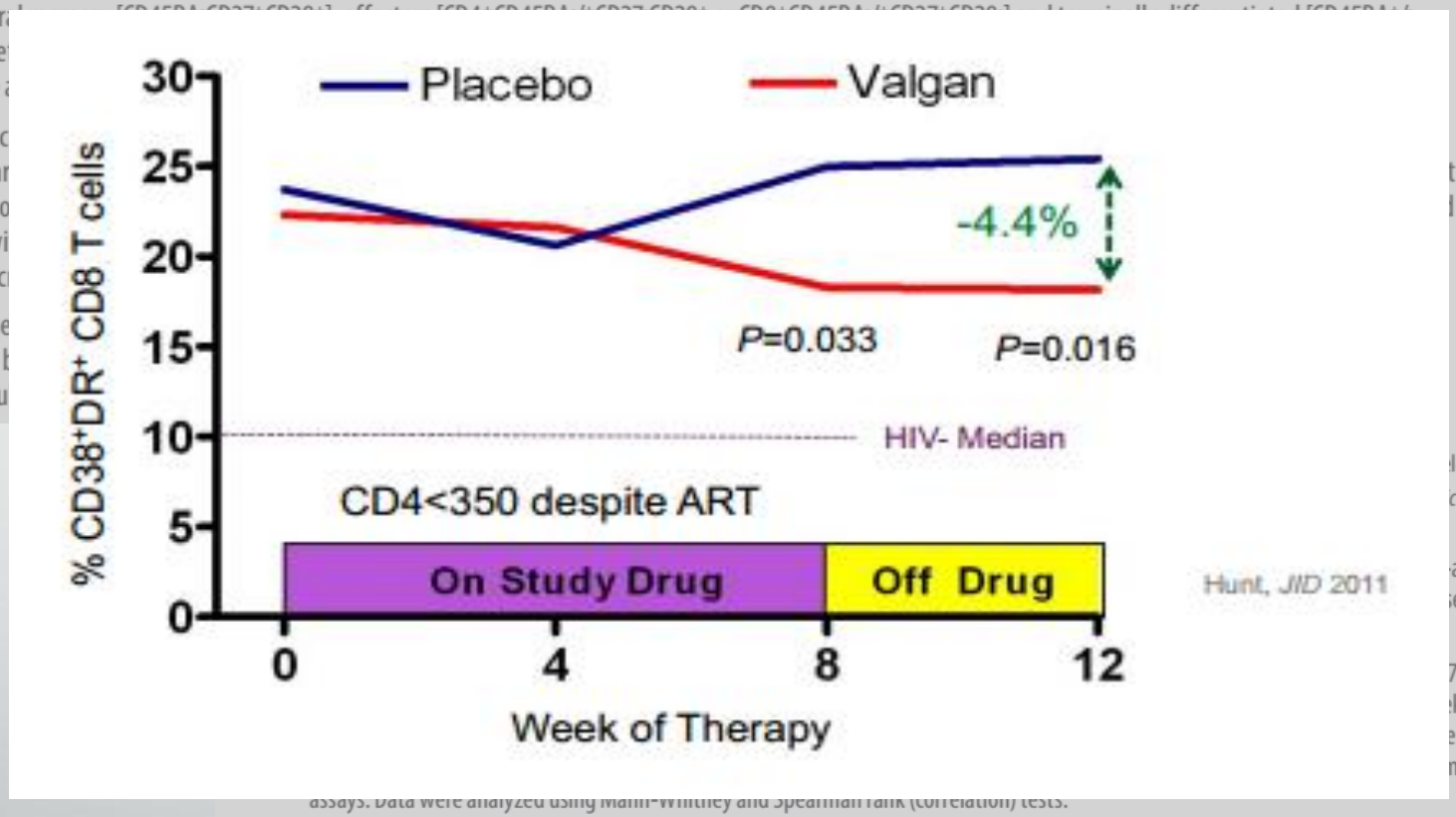
[1]*University of California San Diego, La Jolla, CA, US;* [2]*Harbor–University of California Los Angeles Medical Center, Torrance, CA, US;* [3]*University of Southern California Keck School of Medicine, Los Angeles, CA, US*

Background: HIV-infected men who have sex with men (MSM) are nearly universally infected with CMV, and both viruses are associated with T-cell dysfunction and inflammation-related morbidities. The effect of asymptomatic CMV replication and persistent HIV transcription during suppressive ART on markers of T cell exhaustion and senescence is poorly defined.

Methods: Paired seminal and blood samples from 45 asymptomatic chronically HIV-infected CMV-seropositive MSM on long term ART and with HIV RNA levels in blood plasma <50 copies/ml were studied. Levels of CMV DNA in semen and blood were measured by RT-PCR, and cell-associated HIV DNA and RNA transcripts (unspliced) were measured in PBMC by droplet digital PCR. Markers of T cell exhaustion (PD-1) and senescence (CD57) were measured in PBMC by flow cytometry for CD4 and CD8 T cells and subsets (naïve [CD45RA$^+$CD27$^+$CD28$^+$], centra
CD27$^-$CD28$^-$]). Associations be
determined using univariate a

Results: CMV DNA was detec
circulating CD4 T cells compar
HIV DNA) were positively asso
semen) or cellular HIV RNA wi
remained associated with inc

Conclusions: Our data sugge
increased PD-1 on T cells has b
infection, future studies shou



lo[4]; Irini Sereti[2]

ck, MD, US; [4]*National Institute of Dental and*

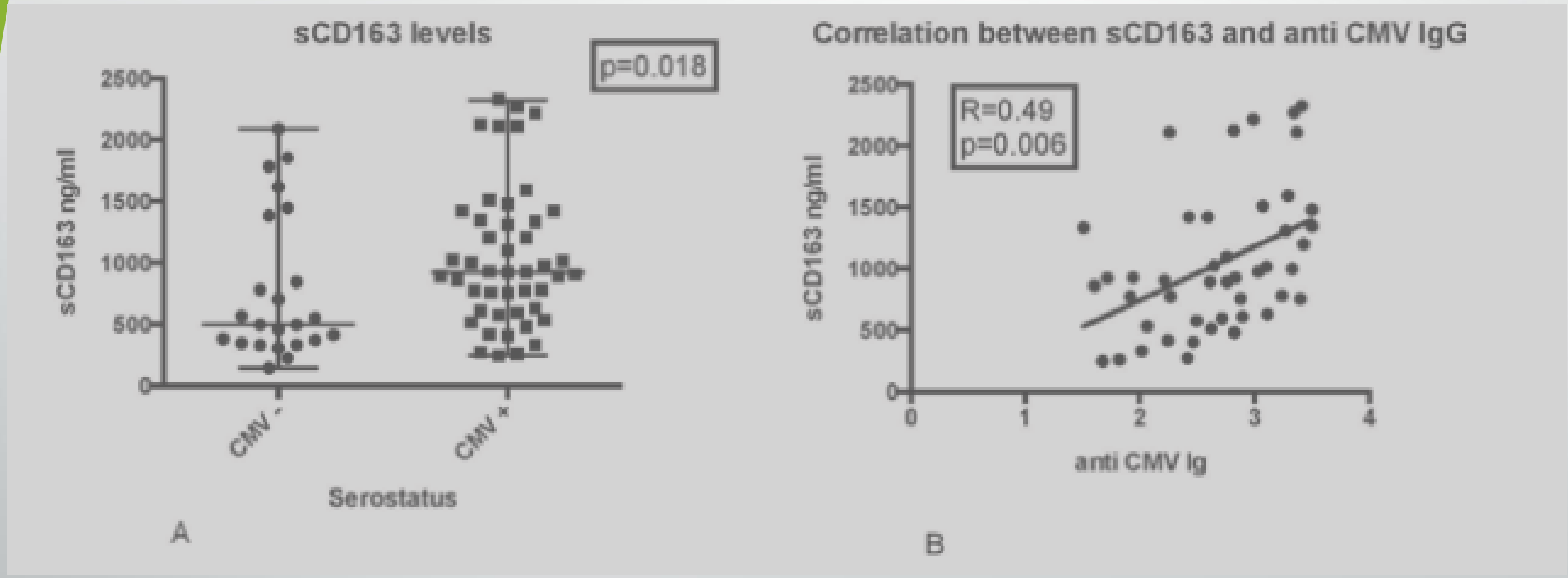
associated end organ disease (EOD). CMV
ease in chronic immune activation and non-

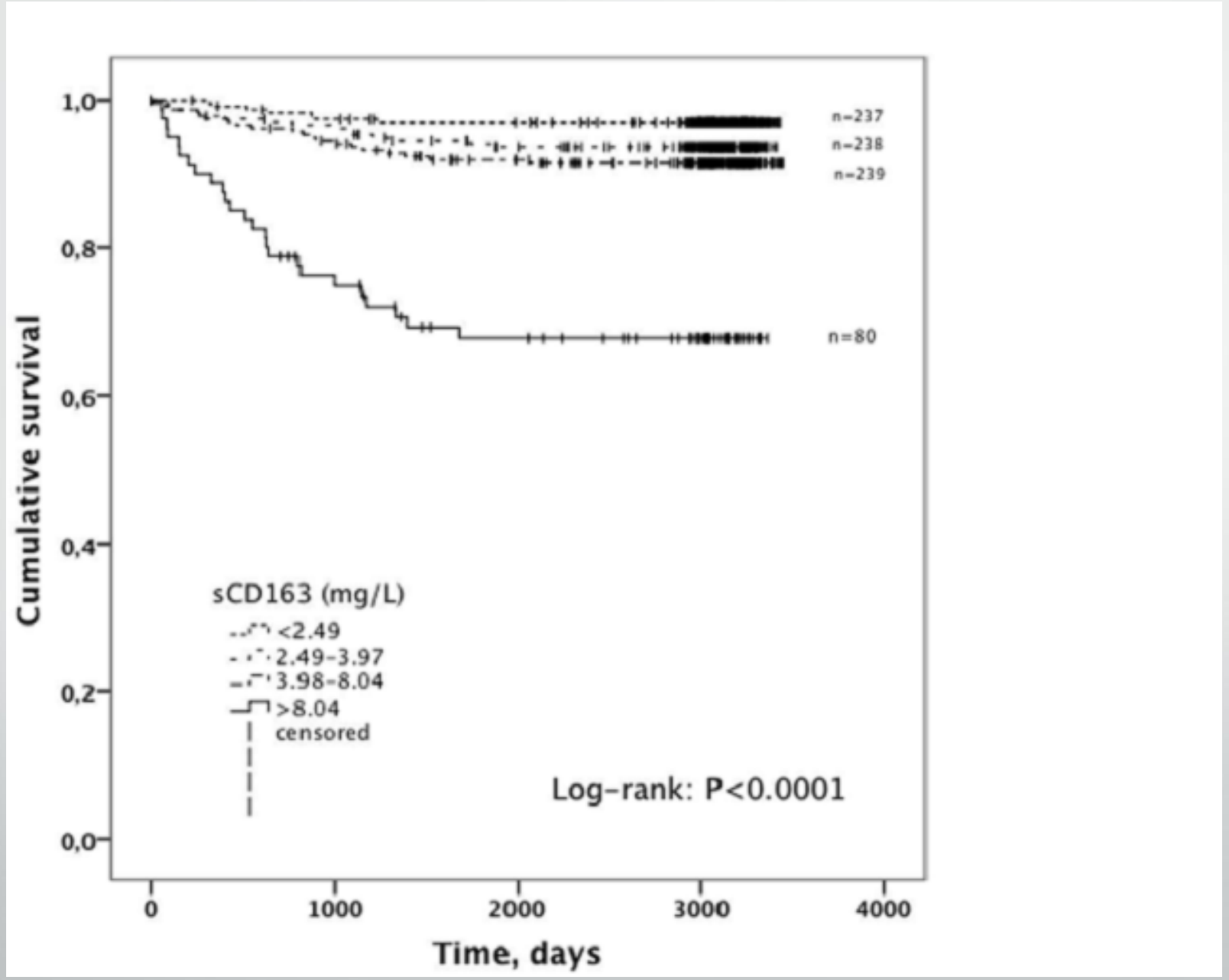
7) pre-ART were matched 1:1 by CD4 T-cell
k (W) 12 and 48 after ART initiation.
e activation and vascular injury (sICAM-3,
nogenic, TF Chromogenic) by ELISA-based

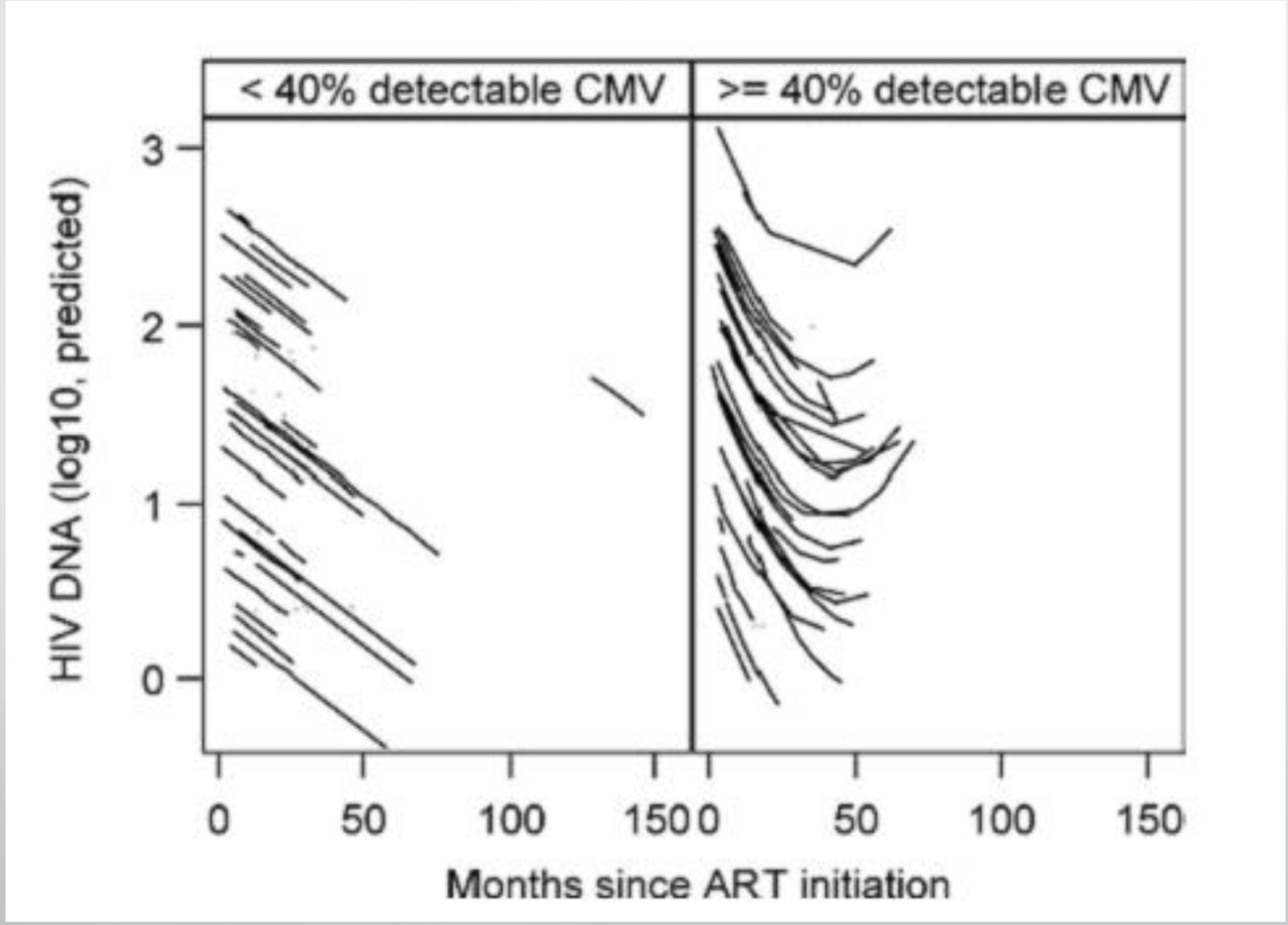
assays. Data were analyzed using Mann-Whitney and Spearman rank (correlation) tests.

Results: EOD cases were 66% male, with median age of 41 years, CD4 of 11 cells/μL, plasma HIV RNA of 5.12 $\log_{10}$ c/mL and CMV PCR 2.93 $\log_{10}$ c/mL units at W0. EOD cases had higher plasma levels of HIV RNA (p=0.01), CRP (p=0.03), IFN-γ (p=0.02), IL-10 (p=0.01), IL-8 (p=0.01), IL-6 (p=0.03), and SAA (p=0.001) compared to controls at W0. At W12, SAA (p=0.02), IFN-γ (p=0.0002), IL-10 (p=0.0003), IL-8 (p=0.01) and TNF-α (p=0.003) remained higher in EOD cases than in controls. At W48, IFN-γ (p=0.0.01), TNF-α (p=0.02) and IL-12 p70 (p=0.04) were persistently higher in EOD cases than in controls. Baseline, CMV IgG levels inversely correlated with sCD14 (r=-0.3, p=0.04), Fractalkine (r=-0.3, p=0.02) and HIV viral load (r=-0.33, p=0.01) but not with presence of EOD.

Conclusions: CMV end organ disease and viremia in HIV+ persons is associated with increased plasma levels of inflammatory and vascular injury biomarkers pre-ART with some persisting after ART. Our data suggest a protracted inflammatory response to CMV that may contribute to HIV pathogenesis and non-infectious complications.


sCD163 levels
p=0.018
sCD163 ng/ml
2500
2000
1500
1000
500
0
CMV -
CMV +
Serostatus
A
Correlation between sCD163 and anti CMV IgG
R=0.49
p=0.006
sCD163 ng/ml
2500
2000
1500
1000
500
0
0
1
2
3
4
anti CMV Ig
B


1,0
0,8
0,6
0,4
0,2
0,0
Cumulative survival
n=237
n=238
n=239
n=80
sCD163 (mg/L)
<2.49
2.49-3.97
3.98-8.04
>8.04
censored
Log-rank: P<0.0001
0
1000
2000
3000
4000
Time, days


< 40% detectable CMV
>= 40% detectable CMV
HIV DNA (log10, predicted)
3
2
1
0
0
50
100
150
0
50
100
150
Months since ART initiation

CROI 2015

Latenza virale: quali novità

389LB 2B4+PD1+ Naïve and Memory CD4+ T Cells Are Associated With Residual Viremia on ART

Cynitha Klamar; Feiyu Hong; John Bui; Anthony R. Cillo; Arcadio Agudelo-Hernandez; Deborah A. McMahon; Charles R. Rinaldo; John W. Mellors; **Bernard J. Macatangay**

University of Pittsburgh, Pittsburgh, PA, US

Background: CD4+ T cell expression of inhibitory receptors (IRs) has been reported to be a marker of latently HIV-infected cells. We determined whether co-expression of various IRs on naïve (T_N) and memory (T_M) CD4+ T cells is associated with persistent HIV-1 expression in patients on effective ART.

Methods: Expression of PD1, CTLA4, TIM3, 2B4, CD160, and LAG3, in CD4+ T cells from patients on suppressive ART was determined using flow cytometry. Boolean gating using Flowjo was done to evaluate 57 different combinations of inhibitory receptors in naïve (CD45RA+ CCR7+), central memory ($T_{CM;}$ CD45RA-CCR7+), and effector memory ($T_{EM;}$ CD45RA-CCR7-), CD4+ T cells. PBMC and plasma were tested for cell-associated HIV DNA (CA-DNA) and RNA (CA-RNA) and residual viremia using qPCR targeting 3′ pol. Correlation between markers was assessed using Spearman's rho.

Results: PBMC were analyzed from 30 patients on suppressive ART (<50 copes/ml) for a median of 7.4 years. Of the T_N and T_M expressing a single IR, frequencies were highest for those expressing TIM3 alone (mean: T_N=42.6%, T_M=23.9%) followed by PD1 and 2B4. Single-expression of the other 3 IRs was seen in <0.5% of the cells. Correlations between the frequencies of single-expression of TIM3, PD1, or 2B4 on T_N, T_{CM}, and T_{EM} and residual viremia were modest (with R values<0.45; p=0.01-0.04). No correlations were seen with CA-DNA or RNA. Evaluation of multiple IR co-expression (i.e. 2-6 IRs expressed) revealed that the combinations PD1/TIM3/2B4+, PD1/2B4+, PD1/TIM3+, PD1/2B4+ had the highest frequencies (mean: T_N=3.9% T_{CM}=6.1% T_{EM}=7.9%), whereas the other 53 IR combinations had frequencies <0.1%. Although PD1/TIM3+ cells had the highest frequencies (T_N=10.7% T_{CM}=19.1% T_{EM}=20.1%), PD1/TIM3 expression only modestly correlated with residual viremia for T_N only (R=0.42; p=0.02), and with CA-DNA in T_{EM} (R=0.40; p=0.03). By contrast, highly significant correlations were observed between residual viremia and PD1/2B4+ T_N (R=0.59; p<0.001) and PD1/2B4+T_{CM} (R=0.57, p=0.001), but not with CA-RNA or -DNA.

Conclusions: In patients on suppressive ART, a substantial fraction of naïve and memory CD4+ T cells co-express different combinations of PD1, 2B4, and TIM3. The specific combination of PD1 and 2B4 co-expression on naïve and central memory is strongly associated with the level of residual viremia on ART, suggesting that targeting more than one IR could be needed to maximize effects of HIV-1 persistence.

L'espressione combinata di PD1 e 2B4 su cellule naive e memory è fortemente associata ai livelli di viremia residua in pazienti in terapia antiretrovirale.

Per un miglior effetto sulla persistenza virale bisognerebbe agire su più di un Co-IR contemporaneamente

WEPEA143

CTLA-4-expressing memory CD4+ T cells are critical contributors to SIV viral persistence

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Background: Understanding the immunophenotype and anatomic location of latently infected cells represents a critical challenge in designing a cure for HIV. Among memory CD4+ T-cells, those expressing co-inhibitory receptors (Co-IRs) are strong candidates for being enriched in latent HIV, given their negative regulatory function and upregulation on T-cells following HIV infection. However, little is known regarding the dynamics of T-cells expressing multiple Co-IRs following suppressive ART and their contribution to the HIV/SIV reservoir, particularly in tissues.

Methods: We investigated the relationship between the level of Co-IR expression on memory CD4+ T-cells and their level of latent virus in 10 ART-treated, SIV-infected rhesus macaques (RMs). RMs initiated a 5-drug ART regimen 6-8 weeks after SIVmac251 infection, which was maintained until plasma viremia was < 60 copies/mL for at least 3 months. Blood and tissue levels of memory CD4+ T-cells expressing multiple Co-IR (PD-1, CTLA-4, TIM-3, 2B4, TIGIT) were longitudinally analyzed by flow cytometry. Memory CD4+Co-IR+ subsets were sorted twice during viral suppression based on their expression of PD-1, CTLA-4, and TIM-3, to quantify levels of cell-associated SIV-DNA and RNA.

Results: The majority of memory CD4+ T-cells from the blood, GI tract, lymph node, and spleen expressed multiple Co-IRs, specifically PD-1 and CTLA-4, and their frequencies remained stable or increased during SIV infection, even with suppressive ART. Following 1 month of viral suppression, both memory CTLA-4+(PD-1-) and PD-1+(CTLA-4-) CD4+ T-cells harbored significantly higher levels of SIV-DNA in the LN. Yet, after 3 months of suppression, only CTLA-4+ CD4+ T-cells, in the absence of other Co-IRs, were significantly enriched in SIV-DNA in the PBMCs, compared to Co-IR(-) cells, and in the LN, demonstrating the specific persistence of this virally infected subset. Furthermore, this subset did not express high levels of SIV-RNA, which suggests that these CTLA-4+ cells likely harbor latent SIV.

Conclusions: Despite comprising a small frequency of memory CD4+ T-cells, CTLA-4+ T-cells represent a novel subset of virally enriched cells that may critically contribute to persistence in ART-suppressed individuals. These findings highlight the benefit of therapeutically blocking both CTLA-4 and PD-1 to target a large fraction of the HIV reservoir.

CD4/CTLA-4+ cells rappresentano un nuovo subset cellulare che contribuisce in modo significativo alla latenza virale in RMs SIV+ in terapia

Possibile effetto terapeutico sul recervoir virale bloccando entrambe i recettori co-inibitori (PD-1 e CTLA-4)

371 Blockade of PD-L1 Does Not Reverse HIV Latency in CD4+ T Cells Ex Vivo

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Background: Blockade of the PD-1/PD-L1 pathways using monoclonal antibodies (mAb) to PD-1 has been reported to activate HIV expression from latently infected CD4 T cells ex vivo. To further evaluate this approach, we tested the ability of the human anti-PD-L1 mAb BMS-936559 to activate virion production from mononuclear cells obtained from patients on suppressive ART.

Methods: PBMC, total (t) CD4, and resting (r) CD4 cells were purified by negative selection of large-volume blood draws from HIV-infected donors. Freshly isolated PBMCs were cryopreserved and immunophenotyped for PD-1/PD-L1 expression. The remaining cells were incubated (1 million cells/well in triplicate) for 1 week with 20, 5, or 1.25μg/ml anti-PD-L1 mAb, with 20μg/ml isotype control [Zymogen DT-1D12g-4P (hIgG4)], or with anti-CD3/CD28-coated microbeads plus either anti-PD-L1 mAb or isotype control. On Day 8, cells were assessed for viability and supernatants tested for HIV RNA using the Roche Taqman v.2.0. A virologic response was defined as a >3-fold increase in HIV RNA over isotype control. Donors whose cells responded initially to anti-PD-L1 mAb were redrawn and tested again.

Results: PBMC, tCD4, and rCD4 cells purified from ten long-term (mean 8 years) suppressed donors. Cell viability was not reduced by treatment with anti-PD-L1 mAb. 9 of 10 donors responded to anti-CD3/CD28 in all cell types (mean fold-increases of 742, 1353, and 272 for PBMC, tCD4 and rCD4, respectively). Anti-PD-L1 mAb did not enhance responses to anti-CD3/CD28. PBMC from 2 of 10 donors (donor 3: 61-fold; donor 4: 7-fold) showed an initial response to anti-PD-L1 mAB that was not reproduced upon repeat blood draw. tCD4 cells from 2 of 10 donors (donor 2: 583-fold; donor 6: 84-fold) initially responded to anti-PD-L1. However, cells from donor 2 did not respond after a repeat draw. Cells from donor 6 responded on the first repeat draw but not the second. rCD4 from 0 of 10 donors responded to anti-PD-L1. PD-1/PD-L1 expression on CD4 and CD8T cells was evident on the day of cell isolation in all donors, but expression levels did not differ between responders, non-responders, and a healthy control.

Conclusions: Despite detectable PD-1/PD-L1 expression, increased HIV production from PBMC, total CD4 T cells or resting CD4 T cells after treatment with anti-PD-L1 antibody was infrequent and not reproduced longitudinally. Alternate strategies will be needed to activate proviral expression from latently infected CD4 T cells.

108 Treatment With a TLR7 Agonist Induces Transient Viremia in SIV-Infected ART-Suppressed Monkeys

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Background: Latent reservoirs of replication competent HIV-1 persist in patients on antiretroviral therapy (ART) and represent the major obstacle to HIV eradication efforts. Considerable effort has been directed to identify pharmaceutical agents capable of safely reactivating latent HIV-1 in ART-suppressed patients.

Methods: A study was conducted in SIV-infected rhesus macaques (RM) on ART to determine if administration of an oral toll-like receptor 7 (TLR7) agonist would induce transient plasma viremia and reduce viral reservoirs. Ten RM were infected with SIVmac251 by rectal challenge. Plasma SIV RNA levels were measured by RT-PCR (limit of detection 50 copies/mL). The RM received ART at ~9 weeks post-infection (PI) and became virologically suppressed by 24 weeks PI; virologic suppression was maintained through week 45. At 45 weeks PI, 4 RM were administered 7 doses of the TLR7 agonist at twice monthly intervals, while on ART. The first 3 doses were 0.1, 0.2 and 0.3 mg/kg and the last 4 doses remained constant at 0.3 mg/kg. Total viral DNA was quantified in peripheral blood mononuclear cells (PBMC), colon and lymph node biopsies taken pre- and post-completion of TLR7 treatment. Two weeks after TLR7 dosing, ART was discontinued.

Results: The first 3 doses of TLR7 agonist administered to the SIV-infected ART-suppressed RM had limited effect on plasma viremia. However, doses 4 through 7 led to transient and consistent increases in plasma virus (500 - 1000 SIV RNA copies/mL) in all treated RMs with a return to < 50 copies/mL within 4-7 days of TLR7 dosing. After completion of the TLR7 regimen, SIV DNA levels were reduced by 56-75% in PBMC, colon and lymphoid tissues. Viral DNA levels remained unchanged in the placebo control RM. To determine if these transient plasma virus blips and decreases in viral DNA content also reduced the size of the viral reservoir, ART was discontinued. While the plasma virus rebound kinetics in animals dosed with the TLR7 agonist were comparable to the placebo group after discontinuation of ART, the TLR7 treated animals showed a ~0.5 $\log_{10}$ reduction in plasma virus setpoint as compared to the placebo group.

Conclusions: Multiple oral administrations of a TLR7 agonist in SIV-infected ART-suppressed RM was safe, induced transient plasma viremia, reduced viral DNA content in PBMCs, colon and lymphoid tissues and established lower viral setpoint after ART cessation. These novel findings support clinical investigation of a TLR7 agonist in HIV-1 infected patients on ART.

La somministrazione orale di multiple dosi di TLR7 agonisti in SIV infetti RM in ART risulta ben tollerato, induce transitorio aumento della viremia plasmatica, riduce il contenuto di DNA virale nei PBMC nel colon e nei linfonodi e riduce il viral set-point alla sospensione della ART

TUPEA075

TLR7 agonist GS-9620 is a potent inhibitor of acute HIV-1 infection in human PBMCs

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Background: GS-9620 is a potent and selective oral TLR7 agonist that activates plasmacytoid dendritic cells (pDCs) to produce various cytokines including interferon-α (IFN-α). GS-9620 induced prolonged suppression of hepatitis B virus (HBV) in animal models of chronic infection and is now being tested in patients with chronic HBV infection. GS-9620 has also been shown to activate HIV expression in peripheral blood mononuclear cells (PBMCs) from virally suppressed patients and is being evaluated clinically for HIV-1 latency reversal. To further support the clinical testing of GS-9620 we investigated its effect on acute HIV-1 infection *in vitro*.

Methods: Anti-HIV-1 activity was tested in PHA-activated PBMCs or purified total CD4+ T cells using multi-cycle and single-cycle infection assays. Specific immune cell subsets (pDC, NK, B or CD8+ T cell) were depleted from PBMCs by negative selection prior to antiviral testing. Conditioned supernatant from GS-9620-treated complete or pDC-depleted PBMCs were tested for antiviral effect on purified HIV-infected CD4+ T cells. Cytokines were quantified by ELISA or Luminex assay. Blocking antibodies against IFN-α or IFN-α/β receptor were used to assess the role of IFN-α signaling in anti-HIV-1 activity.

Results: GS-9620 potently inhibited HIV-1 in multi-cycle infection of PBMCs (mean EC_{50}=0.536 ± 0.830 µM, n=27 donors). In a single-cycle PBMC infection assay, 48-hour pre-treatment significantly improved the potency of GS-9620 (EC_{50}=0.027 ± 0.030 µM, n=21 donors), consistent with a block in virus entry or early-stage HIV-1 replication. Depletion of pDCs, but not other immune cell subsets, reduced GS-9620 activity (21 to 277-fold, n=4 donors). IFN-α was detected in GS-9620-treated total PBMC cultures, but not in pDC-depleted cultures. Although GS-9620 was inactive in purified CD4+ T cells, HIV-1 replication was potently inhibited by GS-9620-conditioned PBMC media or recombinant IFN-α. IFN-α blocking antibodies abolished GS-9620 antiviral activity.

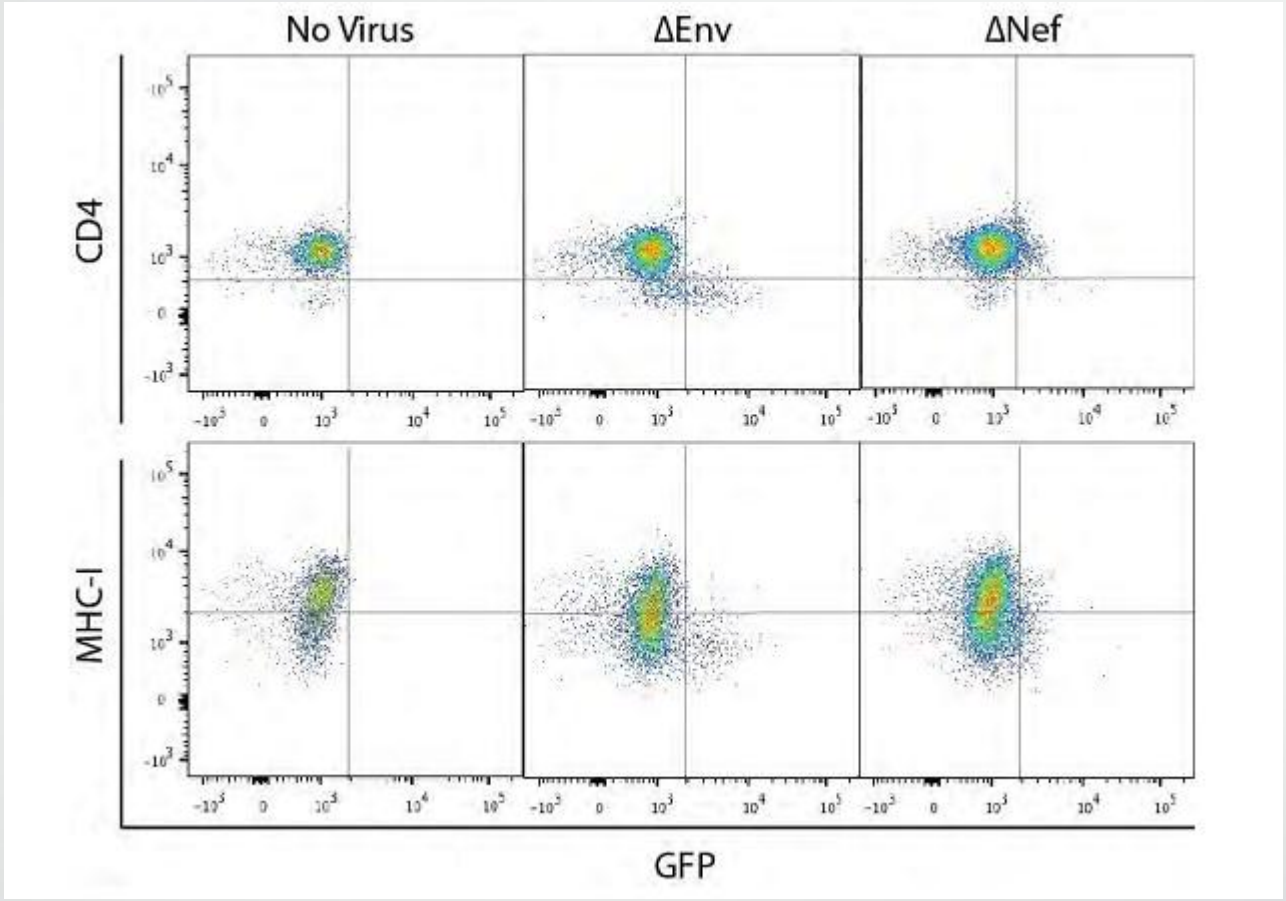
Conclusions: GS-9620 is a potent inhibitor of HIV-1 replication in primary PBMCs. Its antiviral activity is dependent on interferon-α produced by activated pDCs. Immune modulatory effects of GS-9620 leading to simultaneous activation of HIV-1 expression and inhibition of acute HIV-1 infection are important considerations for its clinical evaluation since the antiviral effect may help restrict potential local spread of virus upon *in vivo* latency reversal.

Effetto positivo dell'agonista del TLR7 (GS-9629) sulla inibizione della replicazione di HIV in vitro (PBMC).

Effetto sul blocco dell'ingresso del virus nelle prime fasi della replicazione

L'effetto antivirale dipende dall'INFα prodotto dalle pDCs

Anticorpi anti-INFα bloccano gli effetti dell'agonista del TLR7



Abilità di CD8 GAG specifici di eliminare CD4 latentemente infetti indotti a esprimere proteine precoci (nef) o tardive (env o gag), nonostante la ridotta espressione di MHC I o CD4

E quindi possibile eliminare CD4 latentemente infetti, recentemente riattivati, senza ricorrere all'induzione di un ciclo virale completo

WEPEA142

Extracellular ATP induces the rapid release of HIV-1 from virus containing compartments of human macrophages

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Background: The human immunodeficiency virus type-1 (HIV-1) infects CD4+ T lymphocytes and myeloid cells, in particular tissue macrophages. In comparison to T cells, infected macrophages differ both in terms of decreased to absent cytopathicity and for actively accumulating new progeny HIV-1 virions in Virus Containing Compartments (VCC). For these reasons, infected macrophages are believed to act as "Trojan horses" carrying infectious particles to be released upon cell death or functional stimulation.

Methods: The U937-derived chronically HIV-1 infected promonocytic cell line U1 was differentiated into macrophage-like cells (D-U1 cells) by PMA in the presence of urokinase-type plasminogen activator to favor virion retention in intracellular vacuoles and then shortly exposed to extracellular (e) ATP to induce their release. Primary human monocyte-derived macrophages (MDM) of HIV-1 seronegative donors were infected either with an R5 HIV-1 strain or with a VSVg-pseudotyped vector expressing eGFP. Both D-U1 cells and MDM were stimulated with eATP to induce the release of virions from VCC. Live imaging analysis was used to study the morphological effects of eATP on HIV-1 infected macrophages.

Results: Short term (5-30 min) eATP stimulation induced massive membrane blebbing and a rapid release of mature HIV-1 infectious virions from primary human MDM infected *in vitro* in the absence of cell death. The same phenomenon was reproduced in chronically infected D-U1 cells. Virion release was associated with a depletion of intracellular virions, as measured by intracellular p24 Gag staining and by visual imaging. Pharmacological inhibition of the microvesicle release pathway and of the ATP receptor (R) P2X7 prevented eATP-induced virion release from both acutely infected MDM and D-U1 cells.

Conclusions: Short (min) eATP stimulation induces the release of HIV-1 virions in both primary MDM and in D-U1 cells, via interaction with P2X7R and in the absence of significant cytopathicity. Pharmacologic interference with the microvesicle release pathway and with the P2X7R prevented this effect suggesting that they could represent novel exploitable targets for interfering with the reservoir of HIV-1 virions of infected tissue macrophages.

La breve stimolazione con ATP induce il rilascio di virioni di HIV da macrofagi acutamente o cronicamente infetti senza effetto citopatico

Effetto mediato da P2X7R

323 **P2X Type Purinergic Antagonists Can Block HIV-1 Infection and Associated Inflammation**

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Background: HIV-1 causes a chronic, incurable infection that is associated with chronic inflammation. Even in the face of virologic suppression with antiretroviral therapy this inflammation can persist. The mechanism of this inflammation is not clearly understood. Purinergic receptors are known to be mediators of inflammatory responses and can contribute to pro-inflammatory cytokine production and lymphocyte cell death. Purinergic receptor signaling has been found to be important for HIV-1 infection and we have recently found that inhibition of the P2X subtype purinergic receptors potently blocks HIV-1 productive infection at the level of membrane fusion. This study examines whether virus-induced purinergic signaling is responsible for inflammatory cytokine release during HIV-1 infection.

Methods: Our laboratory has developed methods using fluorescent constructs of HIV-1 to evaluate productive infection by flow cytometry and confocal microscopy. Infected supernatants can be subjected to multiplex bead capture assays to test for an array of human cytokines including IL-12, IL-10, IL-8, IL-6, TNF, and IL-1β. We have tested the effect of HIV-1 infection on peripheral blood mononuclear cells and observed levels of pro-inflammatory cytokine production.

Results: We observe that HIV-1 productive infection in CD4 T lymphocytes is potently blocked by P2X selective inhibitors. We further observed that exposure of peripheral blood mononuclear cells to HIV-1 results in induction of pro-inflammatory cytokines including IL-1β and IL-6 and that these levels are reduced with P2X inhibition. This suggests that P2X inhibitors can block both HIV-1 productive infection and associated inflammation.

Conclusions: Our findings distinguish P2X receptors as key signaling mediators of HIV-1 infection and inflammation. Studies in progress will examine the mechanism of P2X signaling in HIV-1 entry and will test whether purinergic antagonists can block HIV and reduce inflammatory sequelae of HIV-infection in humanized mouse models. We are exploring whether these drugs could be used as adjunctive antiretroviral therapy that could serve to reduce the morbidity and mortality associated with HIV-1 chronic inflammation.

Il trattamento dei CD4 con antagonisti di P2X blocca il rilascio di virioni di HIV e la produzione di citochine infiammatorie

CROI 2015

Biomarker	IRIS	No IRIS	Unadjusted p-value	Adjusted p-value
IFNγ (pg/mL)	5.65 (2.25, 10.6)	2.72 (1.44, 5.17)	<0.001	0.01*
CRP (mg/L)	4.54 (1.84, 13.7)	2.47 (1.27, 8.99)	0.03	0.28
IP-10 (pg/mL)	3.43 (3.19, 3.59)	3.31 (3.10, 3.49)	0.005	0.16
TNFa (pg/mL)	19.9 (15.4, 28.1)	15.5 (12.1, 21.9)	0.042	0.23
sCD14 (µg/mL)	2400 (1988, 3235)	2014 (1645, 2471)	<0.001	0.01*
IL-6 (pg/mL)	2.48 (1.49, 4.02)	1.71 (1.23, 3.08)	0.048	0.48
IL-8 (pg/mL)	10.5 (6.61, 15.4)	7.62 (4.84, 13.8)	0.04	0.79
IL-10 (pg/mL)	14.0 (9.38, 20.3)	7.60, 15.7)	0.04	0.19
Vitamin D (ng/mL)	8.22 (4.99, 13.8)	9.65 (5.83, 17.9)	0.038	0.04*
D-Dimer (mg/L)	1.32 (0.84, 2.34)	0.85 (0.52, 1.52)	<0.001	0.008*

Time-point	Biomarker	Inter-Tertile Range (33rd-67thcentile) or %>detection limit	Effect Type	Unadjusted RR, 95% CI	Unadjusted P-value	Adjusted RR, 95% CI	Adjusted P-value
Baseline	IP-10 (pg/ml) (n=137)	1370-3795	per inter-tertile range	1.6 (1.2, 2.2)	0.005	1.7 (1.1,2.5)	0.014
	LPS (pg/ml) (n=141)	162-228	per inter-tertile range	1.7 (1.3, 2.3)	<0.001	1.8 (1.2,2.6)	0.006
	sCD14 (ng/ml) (n=141)	1580-2082	per inter-tertile range	2.1 (1.5, 3.0)	<0.001	2.1 (1.2,3.7)	0.008
	16s rDNA (copies/ul) (n=141)	32-58	per inter-tertile range	1.4 (1.1, 1.9)	0.017	1.4 (1.1,2.0)	0.018
	IFN alpha 2 (pg/ml) (n=141)	46%	≤10.9 (detection limit) >10.9	1.0 (ref) 2.8 (1.4, 5.6)	0.003	1.0 (ref) 3.4 (1.3, 9.0)	0.011
	MCP-1 (pg/ml) (n=141)	218-342	lower tertile middle tertile upper tertile	1.0 (ref) 0.5 (0.2, 1.3) 2.1 (1.0, 4.5)	0.005	1.0 (ref) 0.4 (0.1, 1.1) 1.4 (0.5, 3.8)	0.042
Early (~Week 4)	IP-10 (pg/ml) (n=123)	699-1745	per inter-tertile range	1.6 (1.2, 2.2)	0.004	1.6 (1.1,2.2)	0.008
	LPS (pg/ml) (n=138)	174-244	per inter-tertile range	2.2 (1.5, 3.3)	<0.001	2.3 (1.5,3.6)	<0.001
Week 48	IP-10 (pg/ml) (n=131)	402-849	per inter-tertile range	1.4 (1.1, 1.8)	0.004	1.4 (1.1,1.9)	0.010
	LPS (pg/ml) (n=136)	148-204	per inter-tertile range	1.4 (1.0, 1.9)	0.095	1.2 (0.8,1.8)	0.34

Table Footer: IP-10, LPS and soluble CD14 were modeled on the $\log_{10}$ scale, RR = risk ratio (hazard ratio at baseline and risk ratio at early and week 48 time-points), CI = confidence interval, Unadjusted = adjusted for pre-enrollment CD4 ≤200 cells/mm^3 by design, Adjusted = additionally adjusted for baseline HIV-1 RNA, CD8 percent, history of AIDS, and randomized ACTG A5202 treatment

268 Monocyte/Macrophage Activation and Recruitment to Mucosal Sites in SIV Pathogenesis

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Background: Monocytes/macrophages (Mo/Ma) assume unique functions in HIV infection, being linked to microbial translocation, immune activation and inflammation (IA/INFL). We compared the fate of Mo/Ma in pathogenic, nonpathogenic and controlled SIVsab infection of pigtailed macaque (PTM), African green monkey (AGM) and rhesus macaques (RM) to better define their role in SIV pathogenesis.

Methods: We assessed Mo/Ma subsets (CD14, CD163, CD16), mobilization (CCR5, a4b7, CCR7, CXCR3), IA (CD80, 86, Ki-67, CCR5, TF), apoptosis (Cas-3), cytokine production (IL-6, IL-10, IL-12 and TNF-a), and phagocytic capacity of Mo/Ma in blood, LNs and intestine. We measured soluble Mo activation markers (sCD14, sCD163, sTF). Tissue infiltration of Ma was assessed by HAM56, CD68, CD163, CD16. Ma trafficking in tissues was assessed by administration of iron oxide microparticles.

Results: Increased CCR5 Mo expression correlated with their loss in circulation and increases in the gut in SV-infected PTMs. Increased CCR7 Mo expression was associated with Ma increase in the LNs in AGMs. No significant Mo/Ma variations were observed in RMs. Increased Mo/Ma proliferation occurred at all sites in PTMs, only in LNs in AGMs, and mainly in blood in RMs. Increased CCR5, CD80, CD86 and TF were observed on Mo/Ma from PTMs, while no changes occurred in AGMs. In RMs CD80 only increased in blood, while CD86 was decreased. Increased Mo/Ma apoptosis occurred early in PTMs and RMs, but was absent in AGMs. Phagocytic activity increased in PTMs but not in AGMs or RMs. Mo produced higher amounts of IL-6 and IL-12 in PTMs, IL-10 and 12 in RMs, while inflammatory cytokine did not increase in AGMs. In the gut AGM Ma produced IL-10, while PTM Ma produced IL-6 and TNF in addition to IL-10. RMs Ma did not show increases in cytokine production in the gut. Soluble markers of monocyte activation and sTF increased throughout infection in PTMs, but not AGMs. Massive infiltration with Ma was observed in LNs, gut, liver, lung and heart in chronically-infected PTMs but not in AGMs and RMs. Ma containing microparticles were found in liver, spleen, myocardium and pericardium in progressive hosts.

Conclusions: Progressive SIV infection is associated with increased activation, apoptosis and massive Mo recruitment to mucosal and nonmucosal tissues. These data suggest that Mo/Ma fuel immune activation and inflammation and development of comorbidities that distinguish progressive from nonprogressive SIV infection.

WEPEA121

Elevated levels of circulating nucleosomes in HIV-infected women: cause or consequence of chronic immune activation?

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Background: Circulating DNA is present in plasma/serum, mainly complexed with histones as nucleosomes. The detection of nucleosomes is representative of cell death from apoptosis or necrosis. Inflammation induced pyroptosis and activation induced apoptosis is associated with enhanced cell death in HIV infection. We hypothesize; higher circulating nucleosomes levels will be associated with cell death in HIV infected subjects contributing to inflammation/activation.

Methods: We studied 44 individuals from the Women's Interagency HIV Study (25 HIV+ antiretroviral (ARV) naïve with CD4 >350cell/mm^3; and 19 socio-demographically matched HIV negative controls). Immune activation (HLADR+CD38+) and apoptosis (intracellular Caspase-3) markers were assessed in PBMCs using multi-parametric flow-cytometry and inflammation markers [Tumor Necrotic Factor-Receptor- II (TNFR-II), & IL-6] were measured in paired plasma using ELISA. Circulating nucleosomes (c-Nucs) were quantified in plasma using Cell Death Detection Plus-ELISA to detect histone-associated DNA fragments (mono-nucleosomes and oligonucleosomes). Differences between groups were detected using t-test and associations between markers determined by Spearman's correlation coefficients.

Results: HIV+ve group had mean (SD) CD4 numbers 654 (269) and viral load with mean (SD) 17,036 (30,359) HIV RNA copies/mL. Significant differences were observed between HIV+ve compared to HIV-ve subjects in c-Nucs levels [Mean AU (SD) 48.24(27.81) vs. 28.37 (25.56) resp., p=0.02]; TNFR-II [Mean pg/mL (SD) 3392 (1796) vs.1630 (605) resp., p< 0.001]; CD8 T cell activation [% CD8 HLADR+CD38+ (SD) 9.75 (7.70) vs.2.48 (2.56) resp., p< 0.001] and CD8 T cell Caspase-3 [% (SD) 4.98 (3.74) vs.2.24 (1.02), p=0.003].
c-Nucs inversely correlated with CD4 Numbers (p=0.047, r= -0.300). Elevated c-Nucs levels were significantly associated with CD8 T cell activation (p=0.039, r=0.3117). Inflammation markers TNFR- II and IL-6 did not significantly correlate with c-Nucs. Importantly, c-Nuc levels significantly correlated with Caspase-3 expression in activated CD4 (p=0.014, r=0.367) and CD8 T cells (p=0.036, r= 0.316), programmed to undergo activation induced cell death by apoptosis.

Conclusions: Circulating nucleosomes correlated with activation induced cell death in HIV infected women. Circulating nucleosomes can stimulate the innate and adaptive immune system in HIV infection, keeping it chronically activated.

In HIV+naive (CD4 654 e VL 17036) vs HIV-

Significativo aumento di c-Nuc, TNFR-II, %CD8+DR+CD38+ e CD8Tcell Caspasi-3+

Correlazione inversa tra c-Nucs e CD4

Correlazione diretta tra c-Nucs e CD8 attivati

Correlazione diretta tra c-Nucs e CD4 e CD8+ attivati Caspasi-3+

Non correlazione tra c-Nucs e IL-6