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COMPETING INTEREST OF FINANCIAL VALUE ≥ £1,000:	
Speaker Name	**Statement**
Professor Mario Clerici:	Has received fees for attending advisory boards for GSK and ViiV (2011)
Date	**April 2012**

UNIVERSITÀ DEGLI STUDI DI MILANO
FACOLTÀ DI MEDICINA E CHIRURGIA

Sistema Sanitario Regione Lombardia

DISCORDANT RESPONDERS: WHAT HAVE WE LEARNT?

Mario (Mago) Clerici

Head, Department of Medical Sciences and Biotechnologies

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Fondazione Don Carlo Gnocchi Onlus

Birmingham, BHIVA, April 19, 2012

HIV INFECTION RESULTS IN QUANTITATIVE AND QUALITATIVE DEFECTS

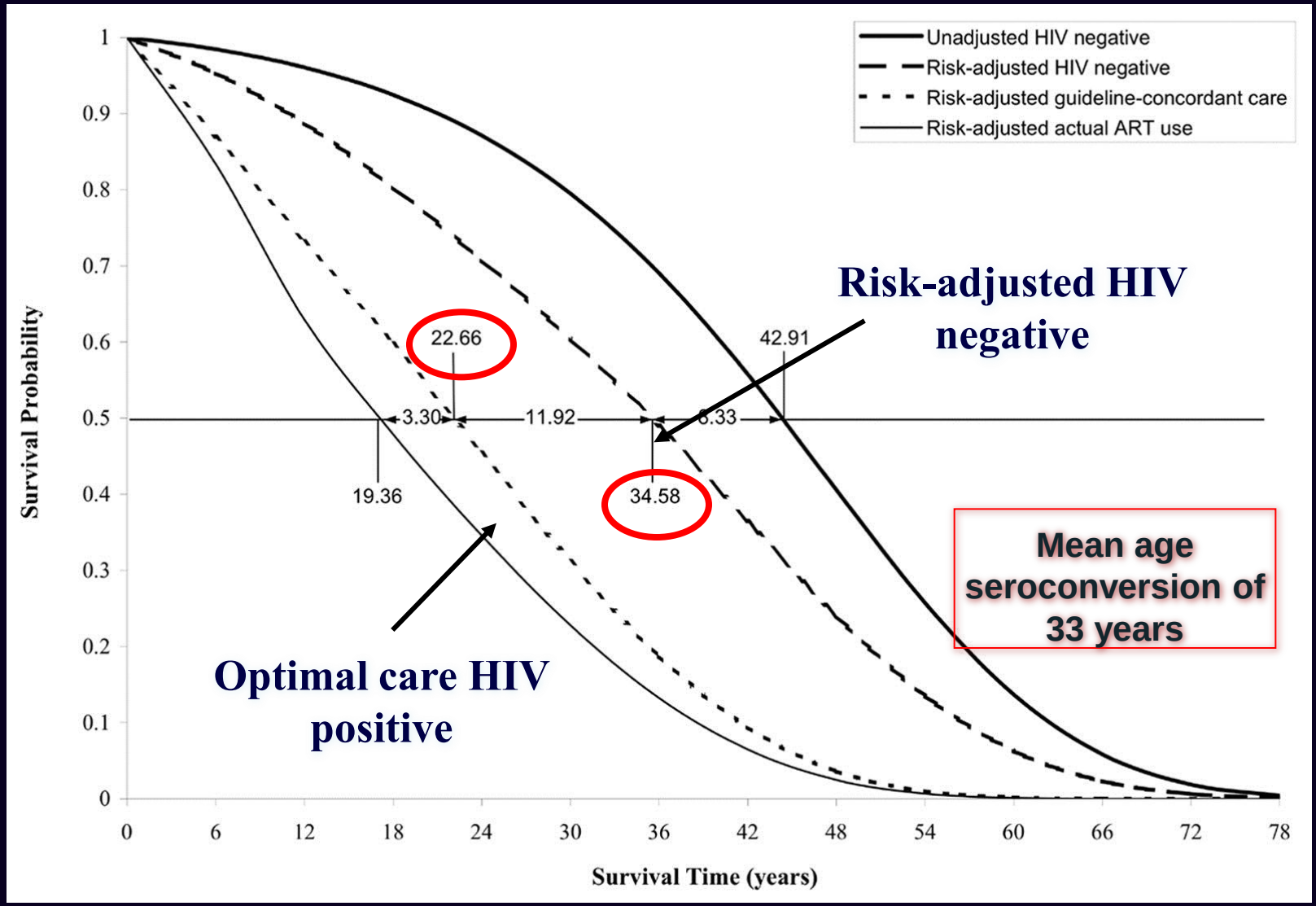
THAT AFFECT

BOTH CD4+ AND CD8+

T CELLS

THE ABILITY OF ANTIRETROVIRAL THERAPY (ART) TO RESTORE THE QUANTITATIVE AND QUALITATIVE DEFECTS OF CD4+ AND CD8+ T CELLS IS ONLY PARTIAL

Even with optimal care, well treated HIV disease does not restore full life expectancy

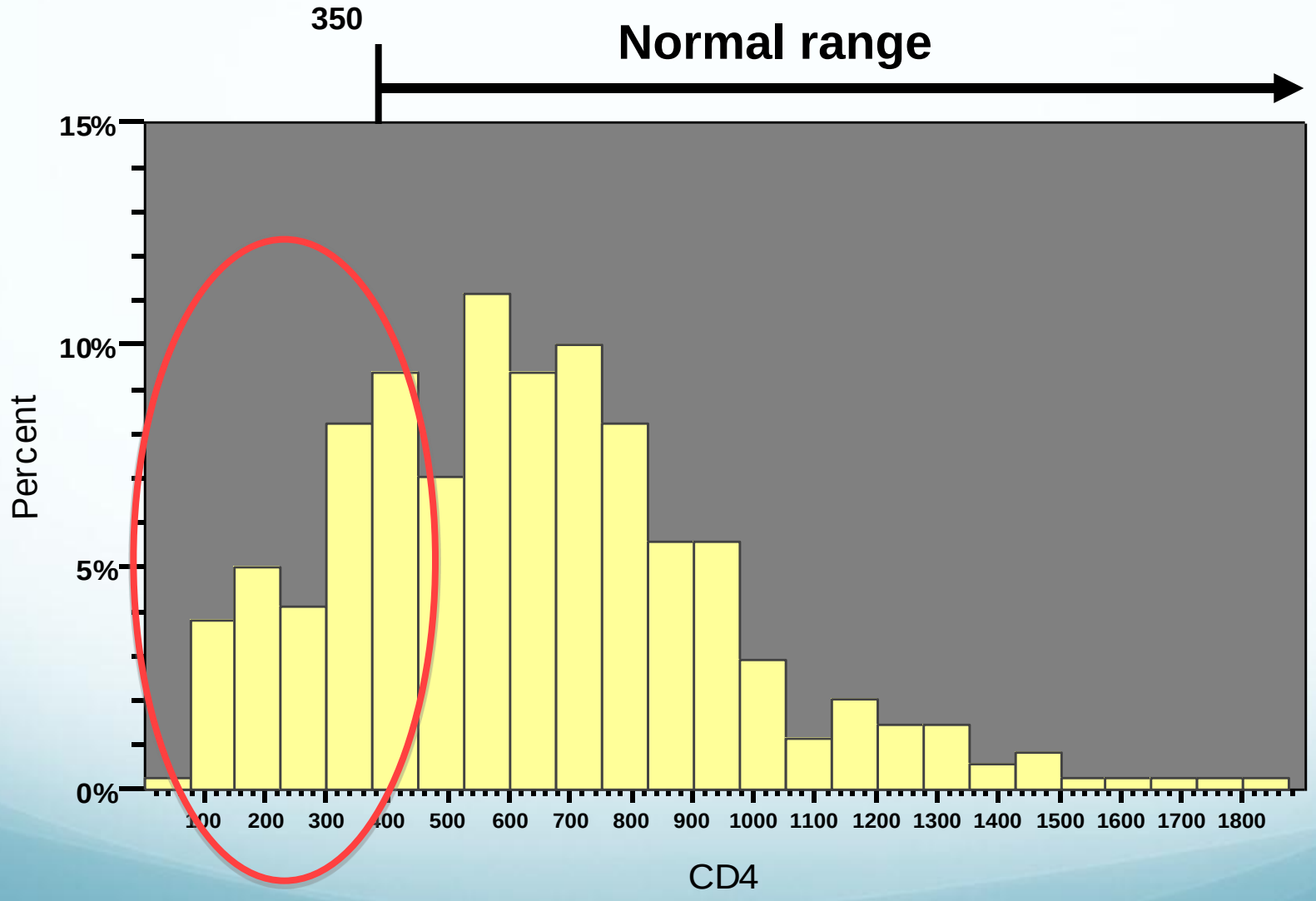


Losina et al CID 2009

On top of this…

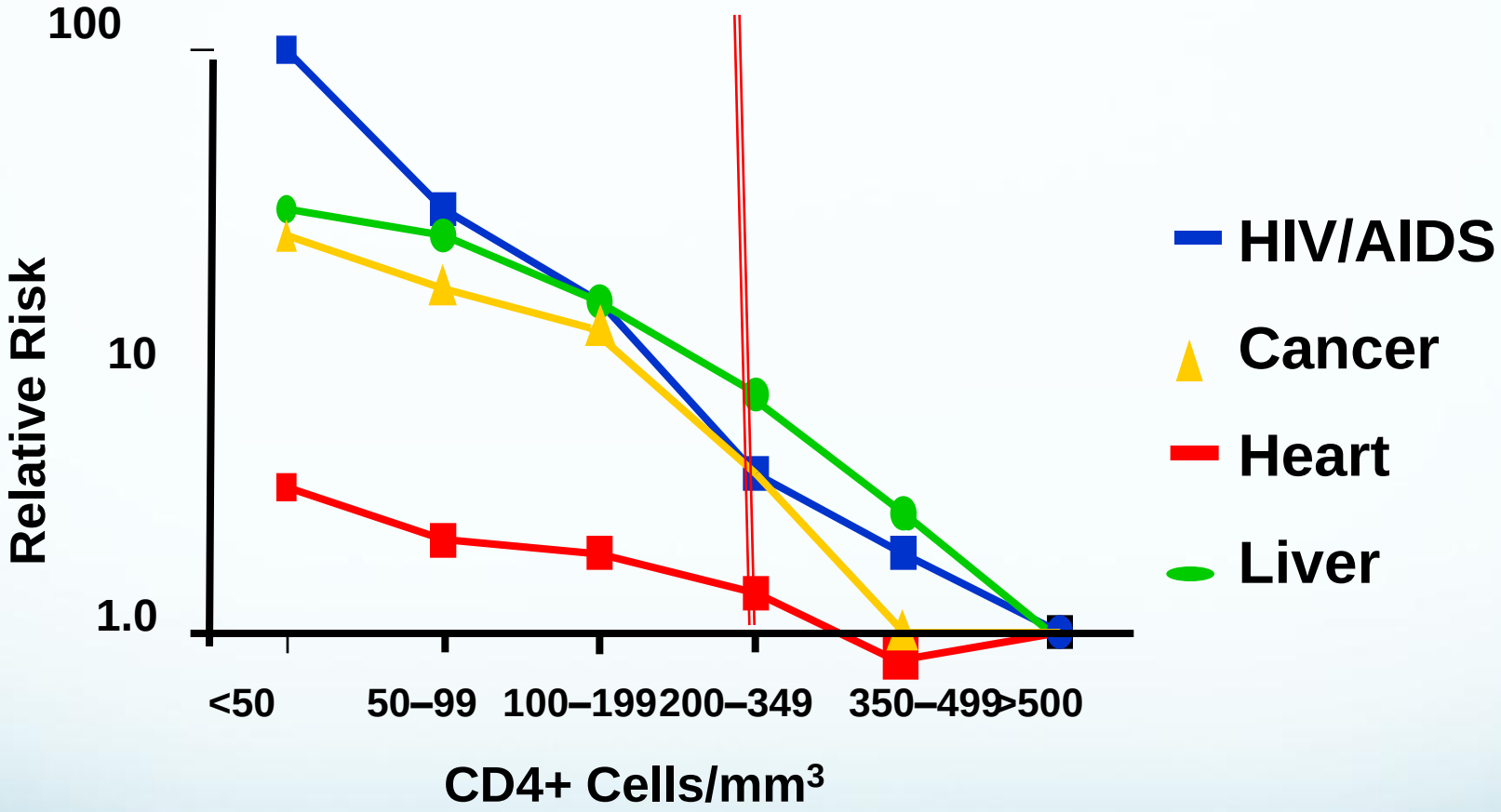
- **Immune restoration, i.e a satisfactory CD4 T cell increase in cART-treated patients, is incomplete in up to 30% of cases.**
- **Among persons with low CD4 cell counts, morbidities and mortality are increased.**

After > 5 yrs of HAART and VL < 400, up to 30% of patients have CD4 T cell counts below the normal range



Rodriguez, Myerson, 2011

Increased morbidity in HIV infection is related to CD4 T cell count on therapy (DAD)



Liver-related: Chronic viral hepatitis, liver failure (other); malignancy-related: malignancy, non-AIDS hepatitis; heart-related: MI, other CVD, other heart disease

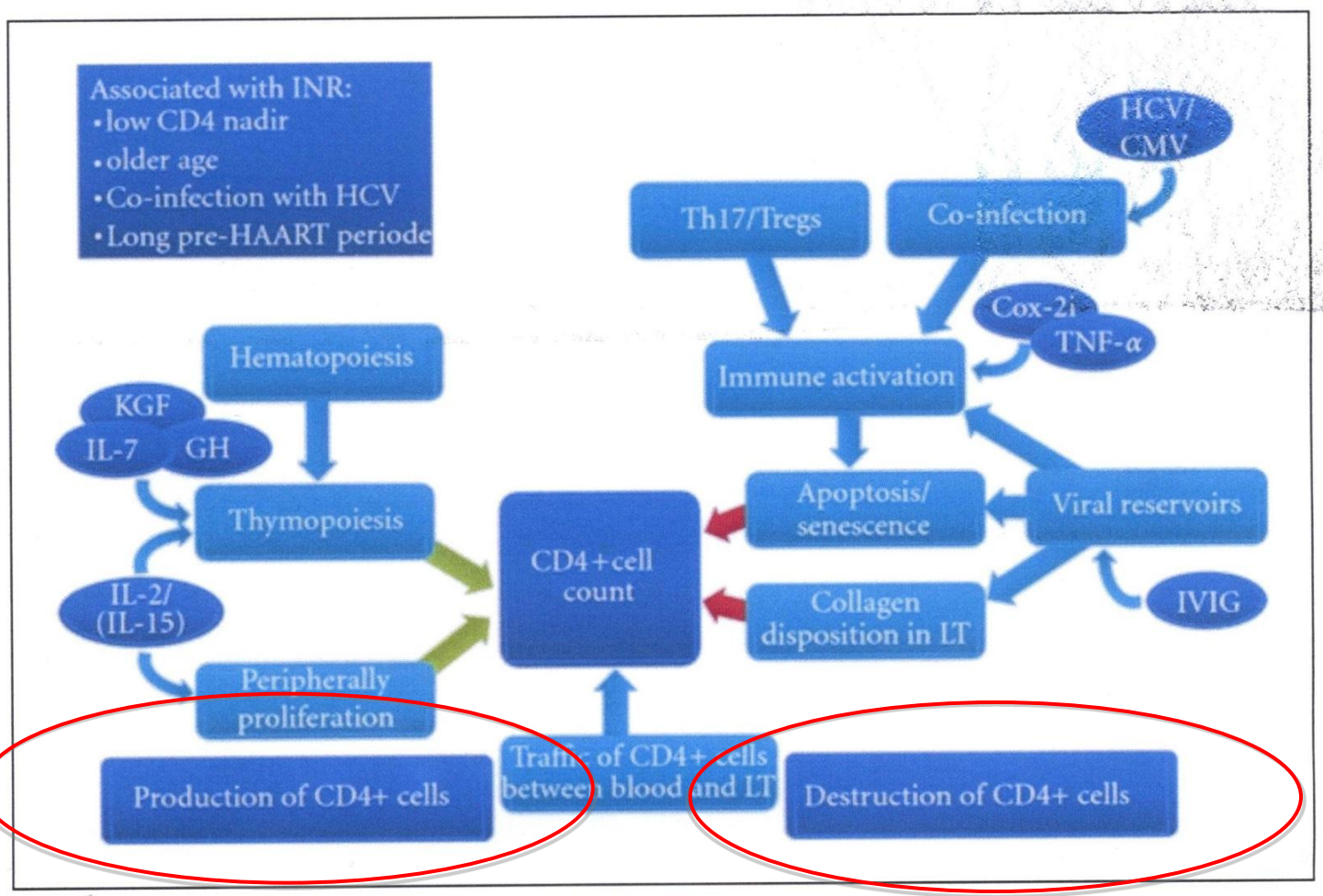
Why is this happening ?

Factors associated with lack of CD4+ T cell recovery:

- **Age**
- **Genetic background**
- **Concurrent infections (e.g. viral hepatitis)**
- **Adherence to antiretroviral therapy**
- **CD4+ T cell nadir**

From: Clin Dev Immunol. 2012; 2012: 670957.
Published online 2012 March 14. doi: 10.1155/2012/670957

JC Gaardbo, HJ Hartling, J Gerstoft, SD Nielsen



Background

Failure in de novo CD4+ T cell production:

- **persistent bone marrow impairment** *(Isfro A, Clin Infect Dis, 2008; Badolato R, Clin Infect Dis, 2008)*
- **smaller thymuses and lower thymopoietin levels** *(Marziali M, AIDS, 2006; Marchetti G, AIDS, 2006)*
- **normal plasmatic IL-7 levels** *(Marziali M, AIDS, 2006; Marchetti G, AIDS, 2006)*
- **defective IL-7R expression** *(Marziali M, AIDS, 2006; Marchetti G, AIDS, 2006)*

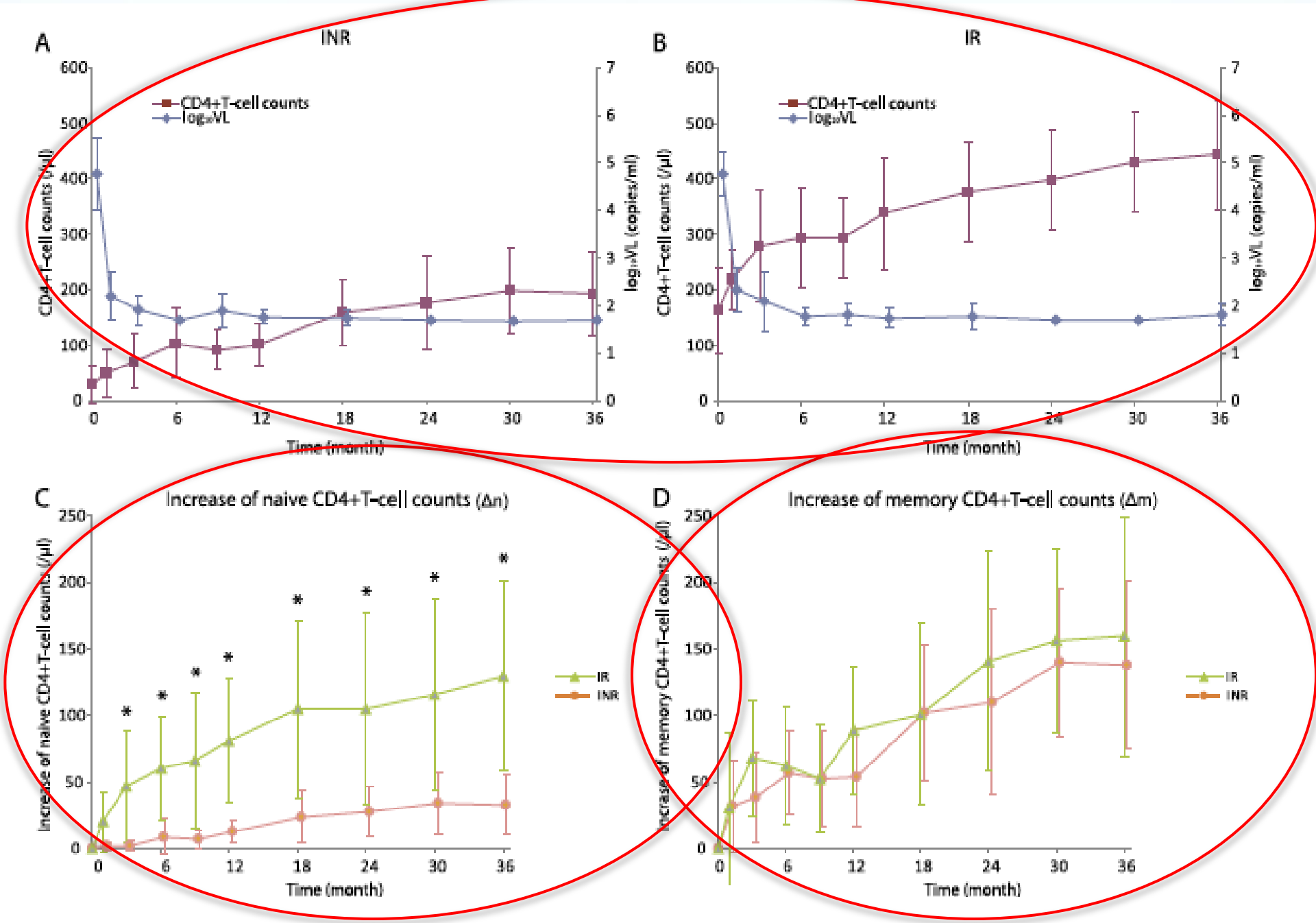
Excessive CD4+ T cell destruction:

- **ongoing viral replication** *(Ostrowski SR, JID, 2005; Chun TW, JID, 2002; Mavigner M PLOS One 2009)*
- **increased CD4+ T cell apoptosis** *(Negredo E CID, 2010)*
- **CD4+ T cell hyperactivation** *(Valdez H, AIDS, 2002; Hunt PW, JID, 2003; Pitrak DL, AIDS, 2001)*
- **persistent antigenic stimulation (LPS)** *(Brenchley JM, Nat Med, 2006)*
- **immunoregulatory mechaninsms (Treg)** *(Marziali M, AIDS, 2006)*

Reduced Thymic Output Is a Major Mechanism of Immune Reconstitution Failure in HIV-Infected Patients After Long-term Antiretroviral Therapy

Taisheng Li, Ning Wu, Yi Dai, Zhifeng Qiu, Yang Han, Jing Xie, Ting Zhu, and Yanling Li

Department of Infectious Disease, Peking Union Medical College Hospital, and Chinese Academy of Medical Sciences, Beijing


A
INR
CD4+T-cell counts
log10VL
CD4+T-cell counts (/μl)
log10VL (copies/ml)
Time (month)
B
IR
CD4+T-cell counts
log10VL
CD4+T-cell counts (/μl)
log10VL (copies/ml)
Time (month)
C
Increase of naive CD4+T-cell counts (Δn)
Increase of naive CD4+T-cell counts (/μl)
IR
INR
Time (month)
D
Increase of memory CD4+T-cell counts (Δm)
Increase of memory CD4+T-cell counts (/μl)
IR
INR
Time (month)

Background

Failure in de novo CD4+ T cell production: *(Benveniste O, JID, 2005)*

- **persistent bone marrow impairment** *(Isfro A, Clin Infect Dis, 2008; Badolato R, Clin Infect Dis, 2008)*
- **smaller thymuses and lower thymopoietin levels** *(Marziali M, AIDS, 2006; Marchetti G, AIDS, 2006)*
- **normal plasmatic IL-7 levels** *(Marziali M, AIDS, 2006; Marchetti G, AIDS, 2006)*
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- **persistent antigenic stimulation (LPS)** *(Brenchley JM, Nat Med, 2006, Piconi S, AIDS 2010)*
- **immunoregulatory mechanisms** *(Marziali M, AIDS, 2006, Piconi S, AIDS 2010)*

OPEN ACCESS Freely available online

PLoS one

HIV-1 Residual Viremia Correlates with Persistent T-Cell Activation in Poor Immunological Responders to Combination Antiretroviral Therapy

Maud Mavigner[1], Pierre Delobel[1,2,3], Michelle Cazabat[1,4], Martine Dubois[1,4], Fatima-Ezzahra L'Faqihi-Olive[1], Stéphanie Raymond[1,2,4], Christophe Pasquier[1,2,4], Bruno Marchou[2,3], Patrice Massip[2,3], Jacques Izopet[1,2,4]*

1 INSERM, U563, Toulouse, France, **2** Université Toulouse III Paul-Sabatier, Centre de Physiopathologie de Toulouse Purpan, Toulouse, France, **3** CHU Toulouse, Hôpital Purpan, Service des Maladies Infectieuses et Tropicales, Toulouse, France, **4** CHU Toulouse, Hôpital Purpan, Laboratoire de Virologie, Toulouse, France

Abstract

Background: The clinical significance and cellular sources of residual human immunodeficiency virus type 1 (HIV-1) production despite suppressive combination antiretroviral therapy (cART) remain unclear and the effect of low-level viremia on T-cell homeostasis is still debated.

Methodology/Principal Findings: We characterized the recently produced residual viruses in the plasma and short-lived blood monocytes of 23 patients with various immunological responses to sustained suppressive cART. We quantified the residual HIV-1 in the plasma below 50 copies/ml, and in the $CD14^{high}$ $CD16^{-}$ and $CD16^{+}$ monocyte subsets sorted by flow cytometry, and predicted coreceptor usage by genotyping V3 *env* sequences. We detected residual viremia in the plasma of 8 of 10 patients with poor $CD4^{+}$ T-cell reconstitution in response to cART and in only 5 of 13 patients with good $CD4^{+}$ T-cell reconstitution. CXCR4-using viruses were frequent among the recently produced viruses in the plasma and in the main $CD14^{high}$ $CD16^{-}$ monocyte subset. Finally, the residual viremia was correlated with persistent $CD4^{+}$ and $CD8^{+}$ T-cell activation in patients with poor immune reconstitution.

Conclusions: Low-level viremia could result from the release of archived viruses from cellular reservoirs and/or from ongoing virus replication in some patients. The compartmentalization of the viruses between the plasma and the blood monocytes suggests at least two origins of residual virus production during effective cART. CXCR4-using viruses might be produced preferentially in patients on cART. Our results also suggest that low-level HIV-1 production in some patients may contribute to persistent immune dysfunction despite cART.

Citation: Mavigner M, Delobel P, Cazabat M, Dubois M, L'Faqihi-Olive F-E, et al. (2009) HIV-1 Residual Viremia Correlates with Persistent T-Cell Activation in Poor Immunological Responders to Combination Antiretroviral Therapy. PLoS ONE 4(10): e7658. doi:10.1371/journal.pone.0007658

Editor: Douglas F. Nixon, University of California San Francisco, United States of America

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Competing Interests: The authors have declared that no competing interests exist.

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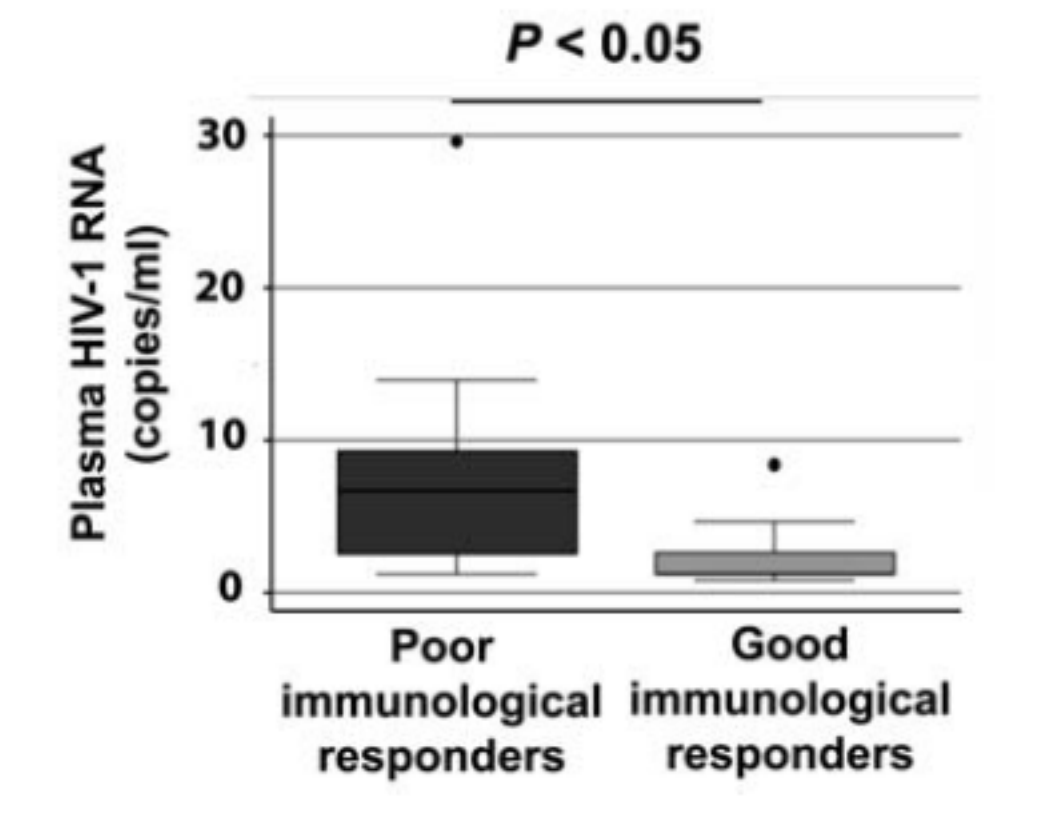


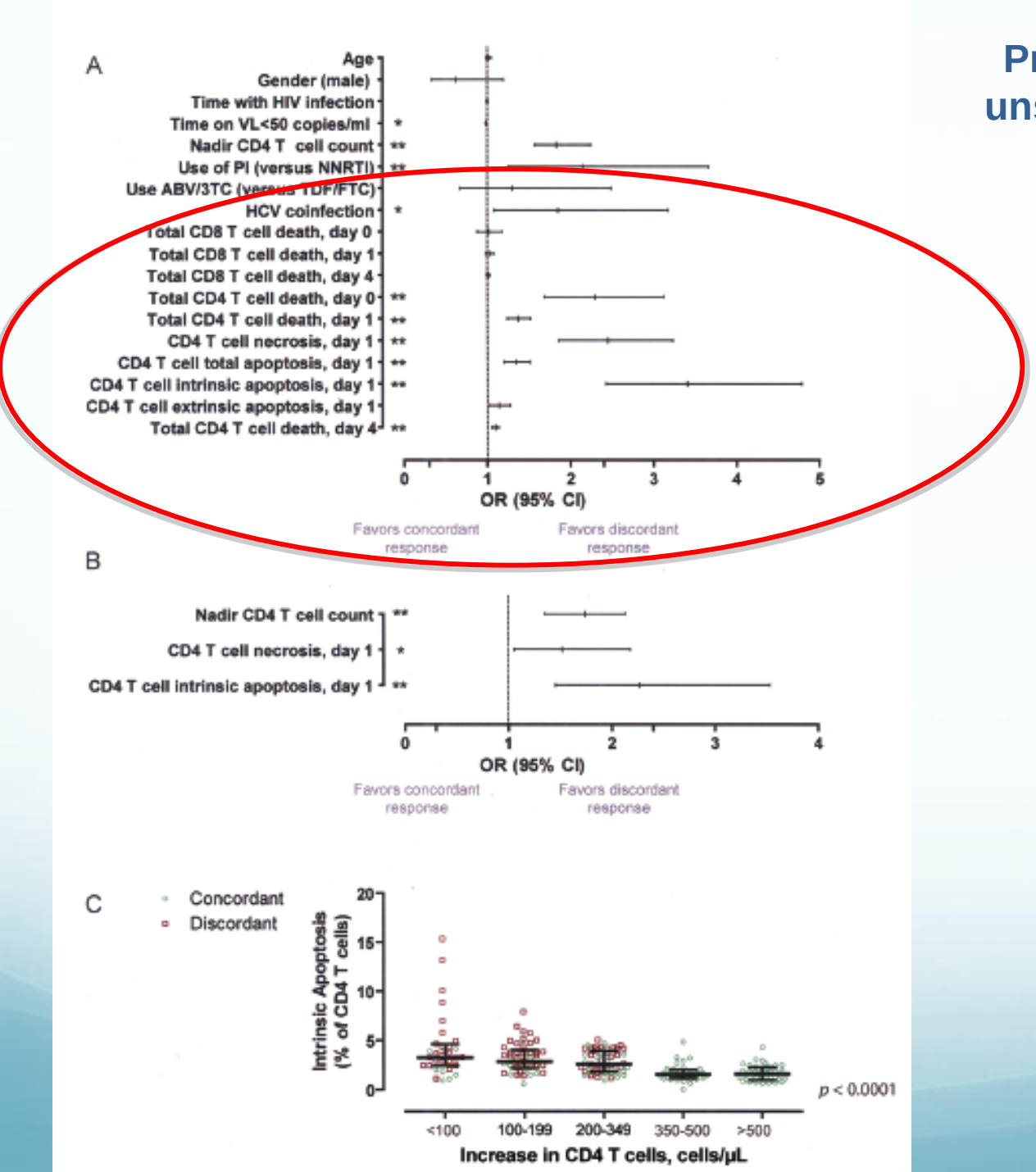
Figure 2. Residual HIV-1 RNA in the plasma. Comparison of the level of residual HIV-1 RNA in the plasma of the poor (n = 10) and good immunological responders to cART (n = 13).

Nadir CD4 T Cell Count as Predictor and High CD4 T Cell Intrinsic Apoptosis as Final Mechanism of Poor CD4 T Cell Recovery in Virologically Suppressed HIV-Infected Patients: Clinical Implications

Eugènia Negredo,[1,a] Marta Massanella,[2,a] Jordi Puig,[1] Núria Pérez-Álvarez,[1,3] José Miguel Gallego-Escuredo,[4,5] Joan Villarroya,[4,5] Francesc Villarroya,[4,5] José Moltó,[1] José Ramón Santos,[1] Bonaventura Clotet,[1,2] and Julià Blanco[2]

[1]Lluita contra la SIDA Foundation and [2]IrsiCaixa-HIVACAT Foundation, Institut de Recerca en Ciències de la Salut Germans Trias i Pujol (IGTP), Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, [3]Statistics and Operations Research Department, Technical University of Catalonia, [4]Biochemistry and Molecular Biology Department, University of Barcelona, Barcelona, and [5]Centro de Investigación Biomédica En Red Fisipatologia de la Obesidad y Nutrición, Spain.

Predictive factors for unsatisfactory immune recovery



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

Stefania Piconi[a,*], Daria Trabattoni[c,*], Andrea Gori[b], Serena Parisotto[c], Carlo Magni[a], Paola Meraviglia[a], Alessandra Bandera[b], Amedeo Capetti[a], Giuliano Rizzardini[a] and Mario Clerici[c,d]

AIDS 2010, 24:1991–2000

Materials and Methods

Inclusion criteria:

67 HIV-infected HAART-treated patients (> 7 years) divided in two groups :

CD4<500 cells/µl: 32 patients

CD4>500 cells/µl: 35 patients

} **HIV RNA< 50 copies/ml**

Conclusions

In patients with <500cell/µl after > 7 years of cART:

- **Treg cells as well as IL-10 and TGFβ-secreting CD4+ T cells are increased**
- **Viable CD4+ T cells are reduced whereas apoptotic and Caspase-expressing CD4+ T cells are increased**
- **Plasmatic LPS is increased and CD4+ T cells are hyperactivated**
- **TLR2 and TLR4 expression is higher**
- **IMMUNE ACTIVATION**

Can we increase circulating CD4 T cell counts in persons with HIV infection?

Will an increase in circulating CD4 T cell counts confer a clinical benefit?

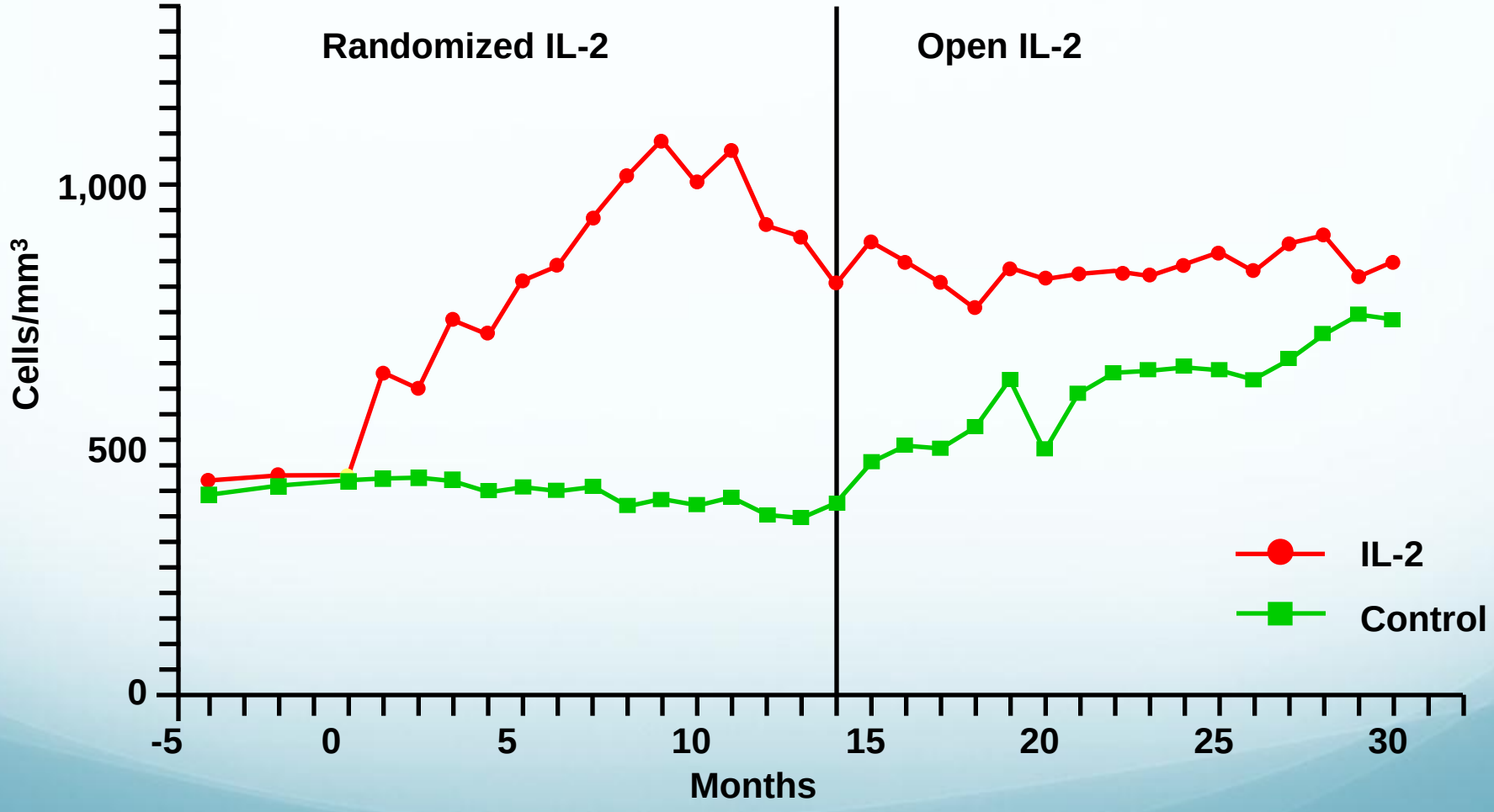
How to increase circulating CD4 T cell counts in HIV infection

- **Make more of them**
- **Redistribute them**
- **Disrupt the pathogenic mechanisms that drive their depletion (i.e. immune activation)**

How to make more T cells

- **Apply strategies that increase thymic production**
  - **Castration**
  - **Growth Hormone**
  - **Interleukin-7**
- **Apply strategies that promote extrathymic T cell expansion and/or survival**
  - **Interleukin-2**
  - **Interleukin-7**

Making more CD4 T cells: a controlled IL-2 trial



Kovacs et al. NEJM 1996

SILCAAT and ESPRIT: randomized controlled trials to evaluate clinical benefit of IL-2 in HIV infection

- **SILCAAT** (n=1.971)
  - CD4: 50-299
  - Avg CD4 = 202 at entry
  - FU = 7.6 yrs
  - Group CD4Δ =57
- **ESPRIT** (n = 4.117)
  - CD4 >300
  - Avg: CD4 – 454 at entry
  - FU = 6.9 yrs
  - Group CD4Δ = 153

Neither study could demonstrate a clinical benefit of IL-2 in terms of mortality or AIDS defining events

How to increase circulating CD4 T cell counts in HIV infection

- **Make more of them**
- **Redistribute them**
- **Disrupt the pathogenic mechanisms that drive their depletion (i.e. immune activation)**

1. **Reduce TLR-mediated immune activation**
2. **Work on the gut mucosa/flora (microbiota)**

blood

2011 118: 3263-3272
Prepublished online May 16, 2011;
doi:10.1182/blood-2011-01-329060

Hydroxychloroquine drastically reduces immune activation in HIV-infected, antiretroviral therapy –treated immunologic nonresponders

Stefania Piconi, Serena Parisotto, Giuliano Rizzardini, Simone Passerini, Roberta Terzi, Barbara Argenteri, Paola Meraviglia, Amedeo Capetti, Mara Biasin, Daria Trabattoni and Mario Clerici

Background

- **Defective CD4 recovery in ART is associated with TLR-mediated immune activation driven by alterations of gut permeability** ***Piconi et al, AIDS 24:1991-2000, 2010***

- **Hydroxychloroquine (HCQ) reduces (endosomal) TLR signalling.**

Materials and Methods

20 ART-treated HIV-infected patients:

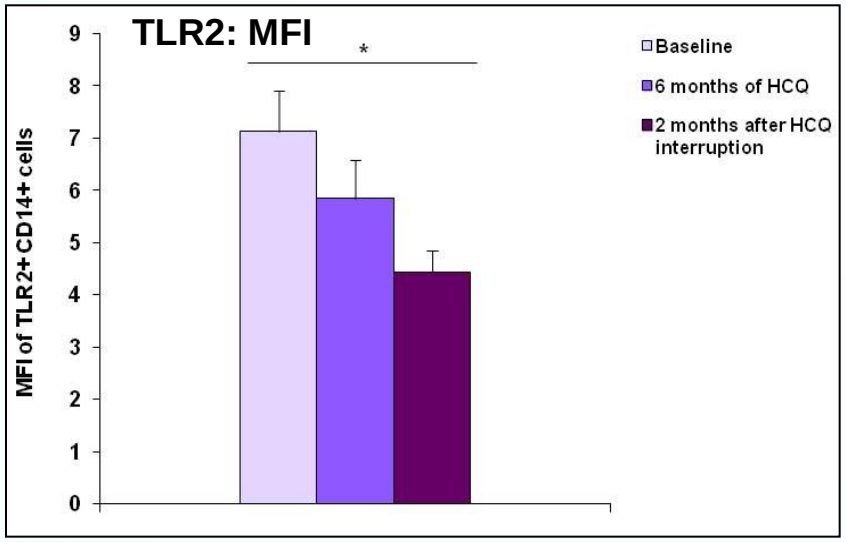
- **CD4+ count <200 cells/µl**
- **Undetectable viremia**

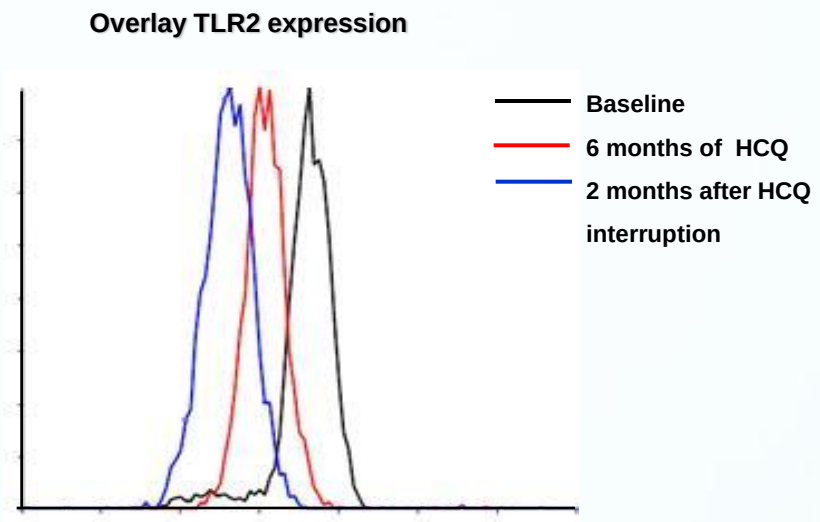
All patients received 400 mg/die of HCQ daily for 6 months.

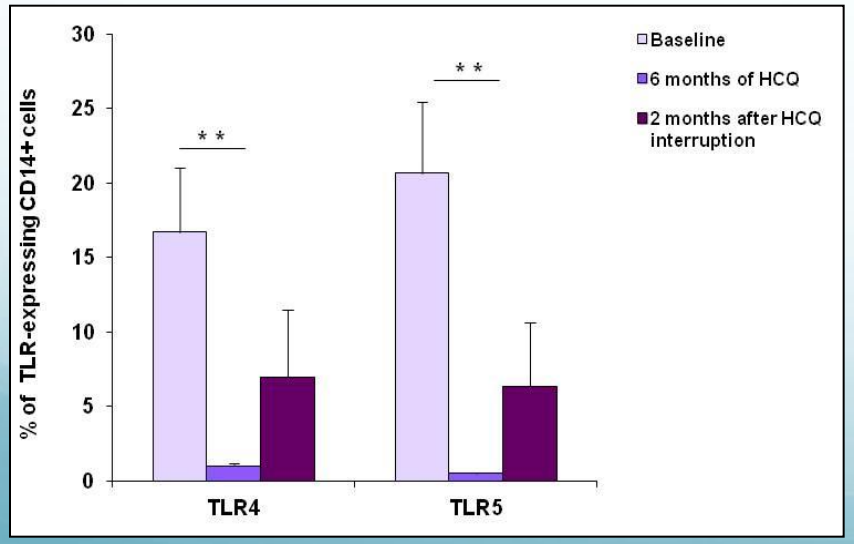
Immunologic parameters were evaluated at:

- **baseline**
- **after 6 months of HCQ treatment**
- **2 months after HCQ suspension**

Expression of TLRs on CD14+ cells

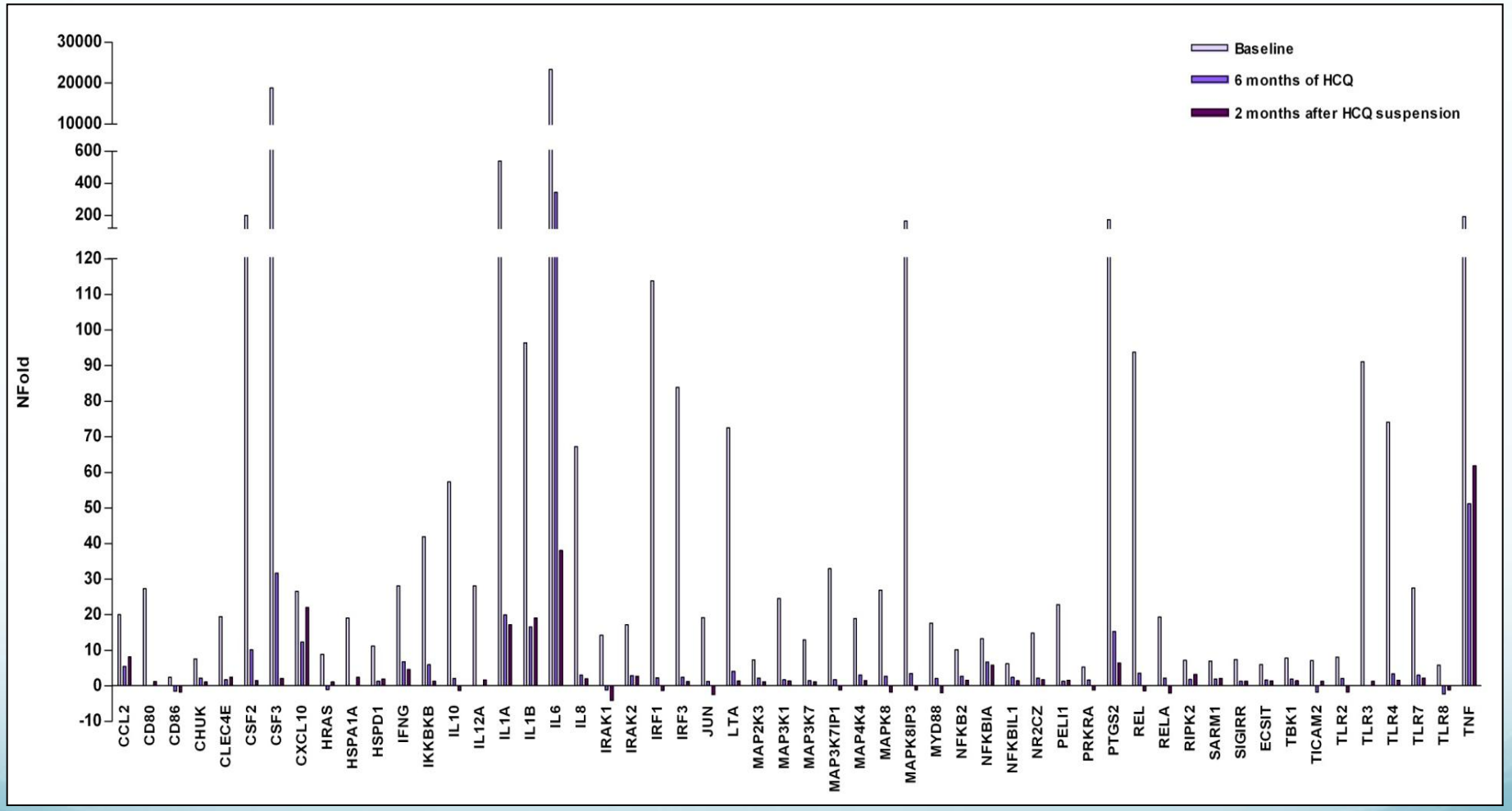




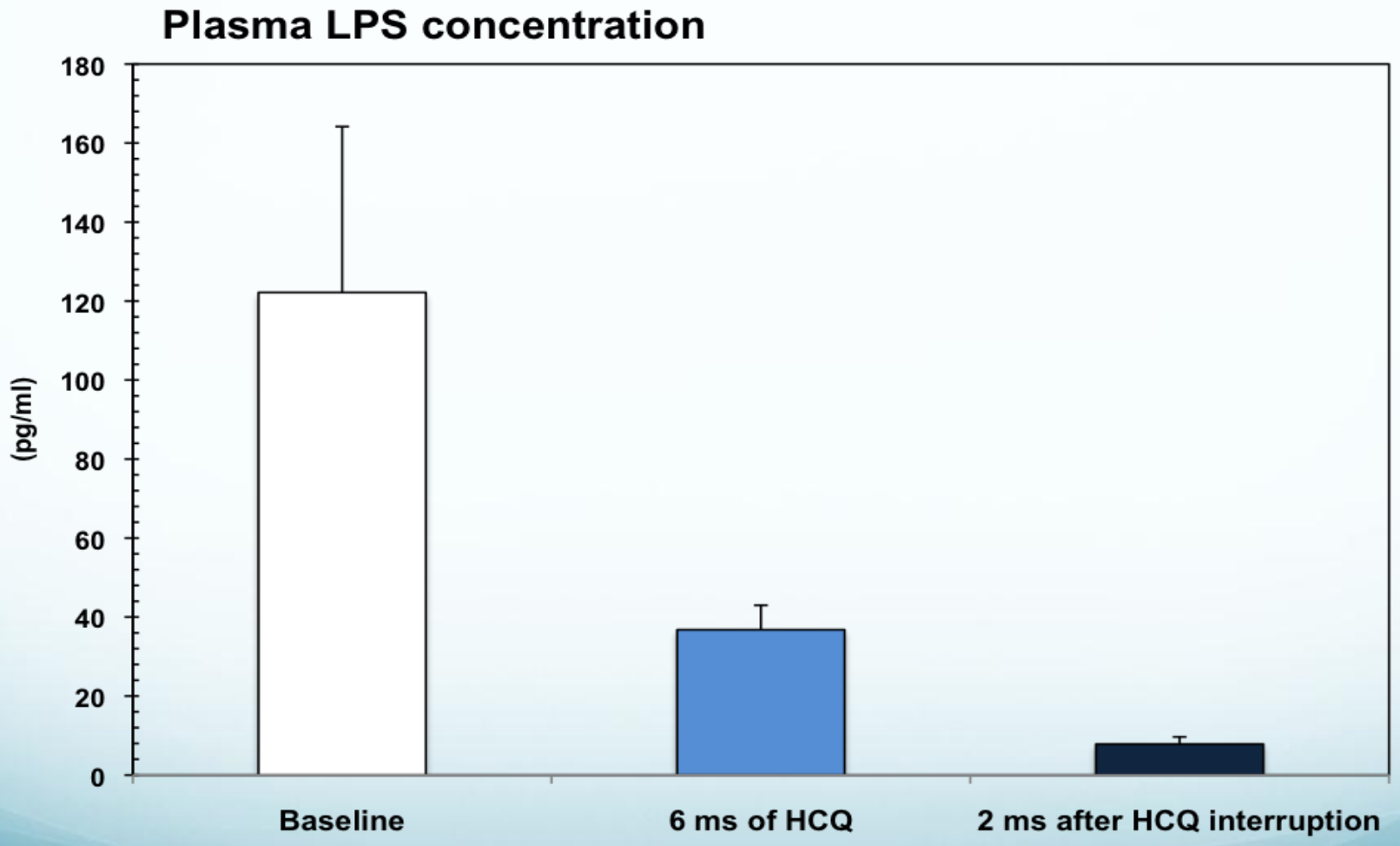


HCQ treatment reduced TLR expression on CD14+ cells

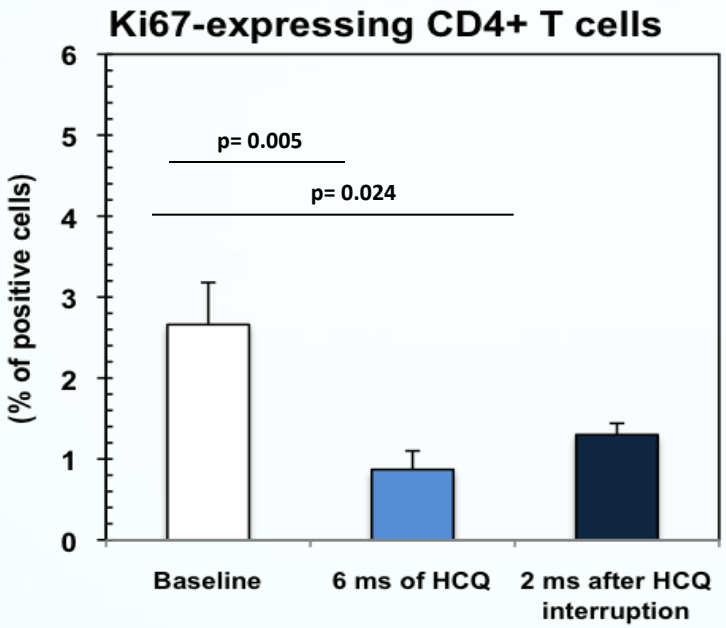
ssRNA-stimulated TLR signaling pathway

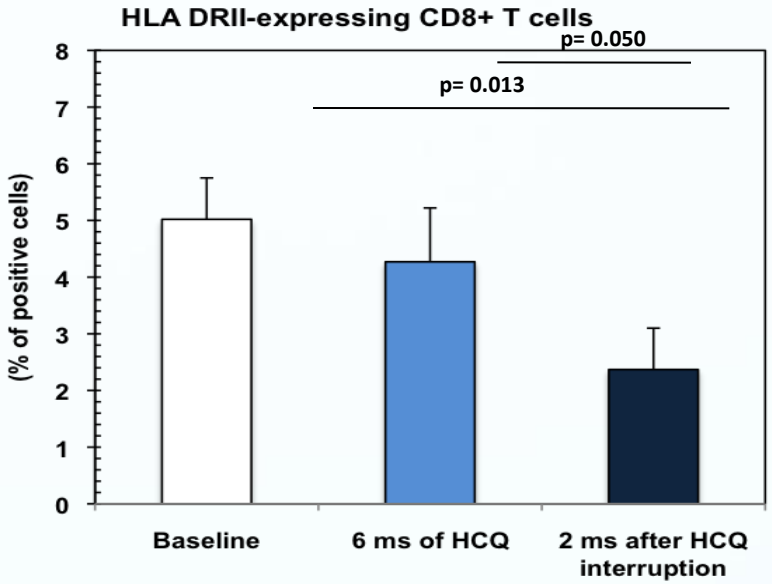


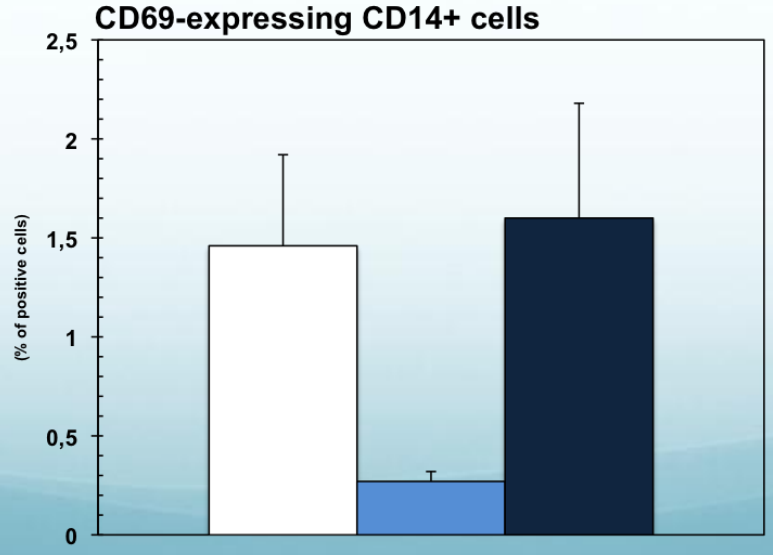
HCQ reduces microbial traslocation



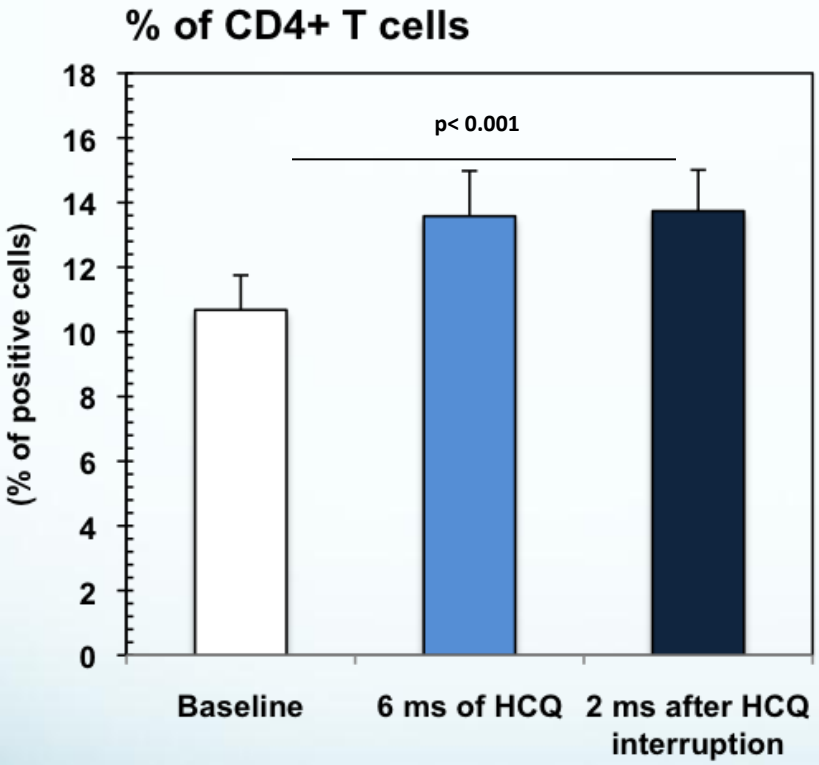
HCQ reduces CD4+, CD8+, and CD14+ activation

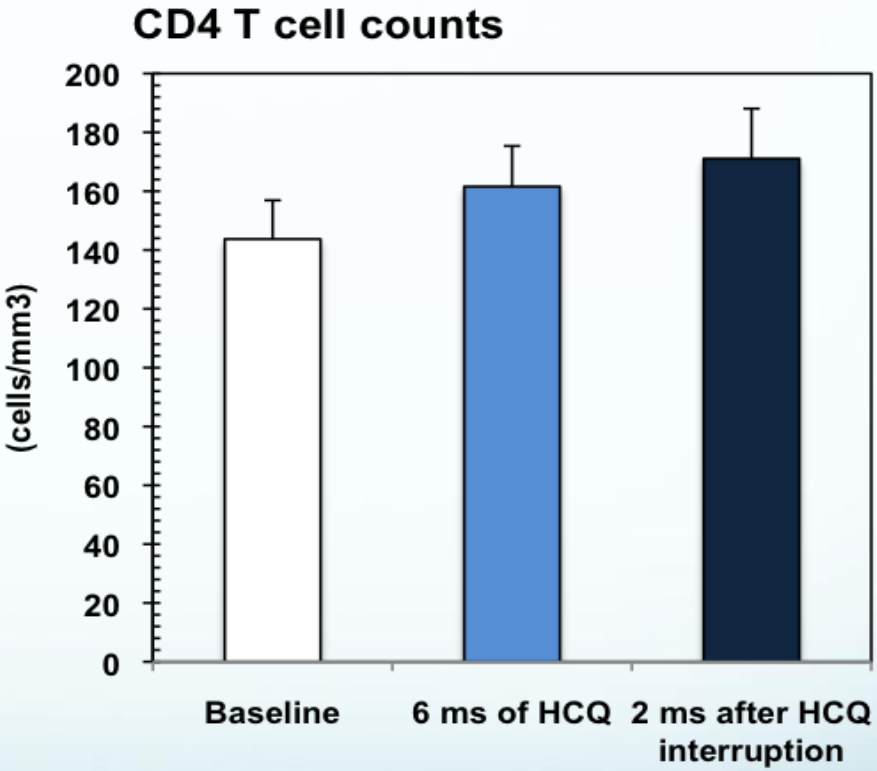






HCQ induces a (partial) increase in CD4+ T cells





1. Reduction in LPS and TLR-mediated immune activation
2. Diminished quantities of cells bearing activation-associated markers
3. Reduced production of proinflammatory cytokines

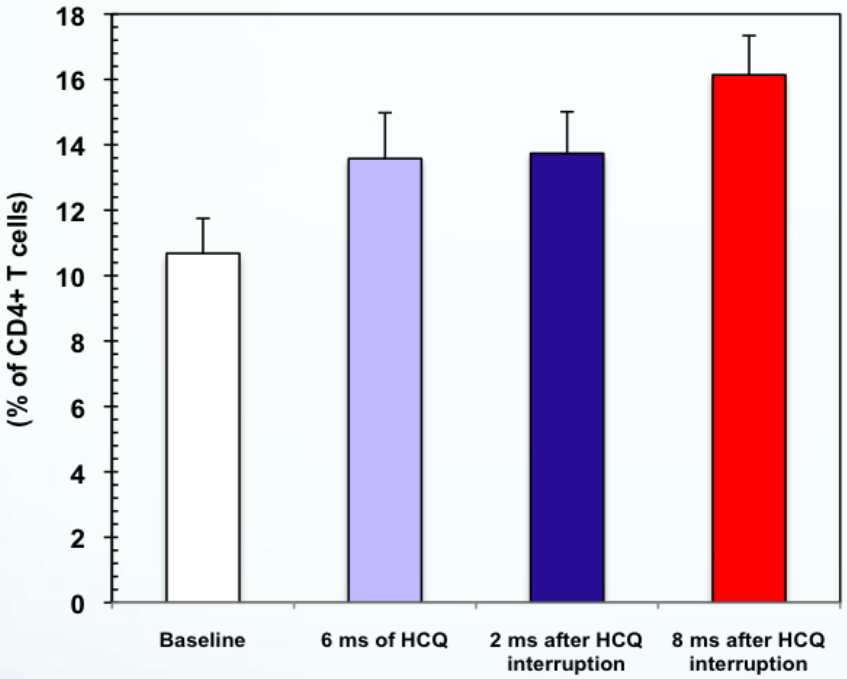
Despite all this, CD4 counts are not totally restored

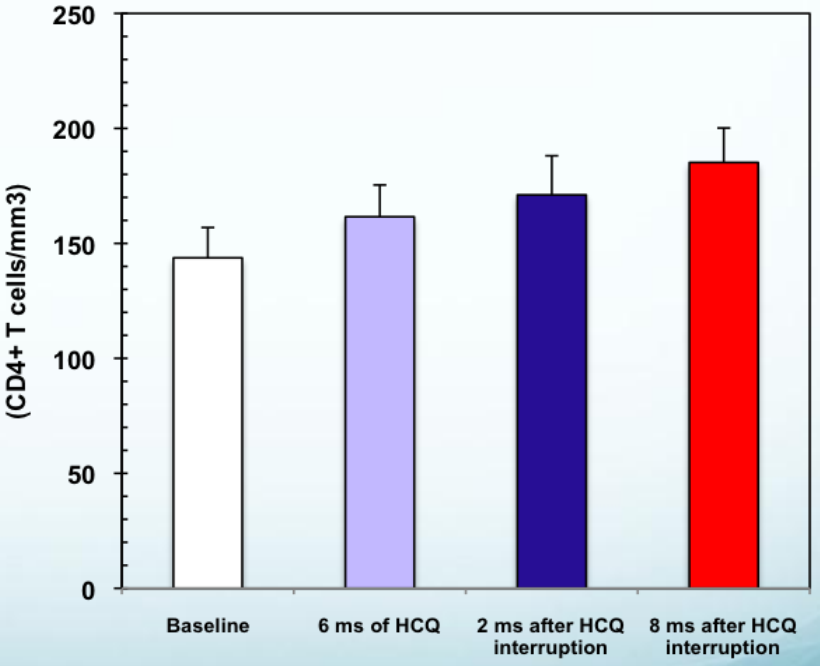
Not enough follow-up

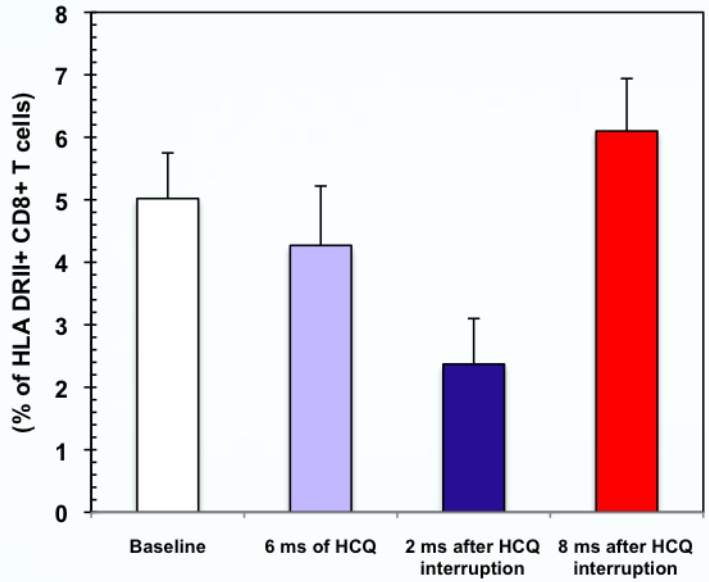
Immune activation is only partially responsible for CD4 counts

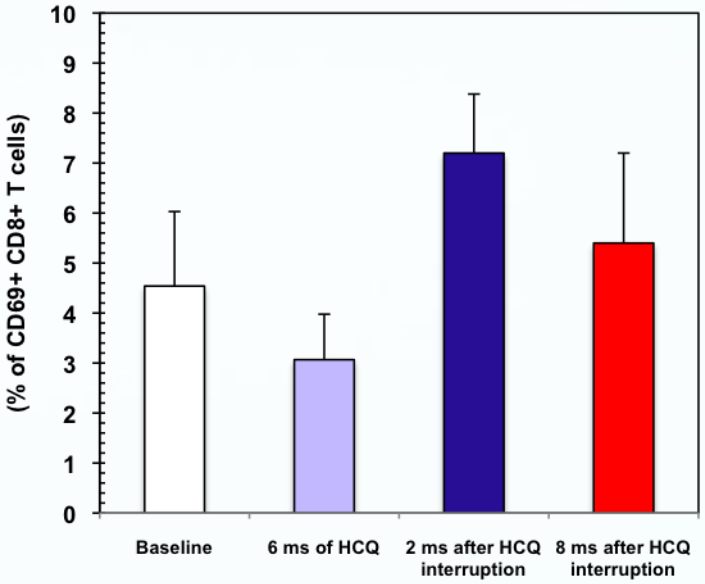
Other mechanisms (immune, hematologic) prevent optimal immune reconstitution in INR

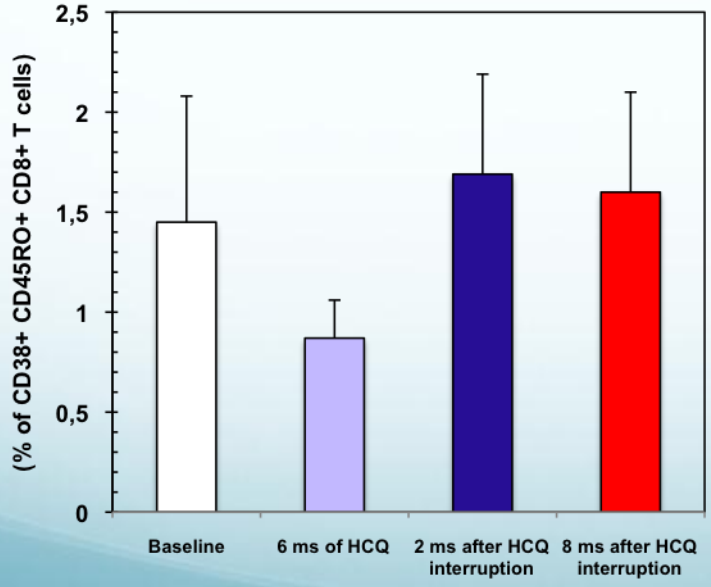
LONG TIME FOLLOW-UP: A STEADY INCREASE IN CD4+ T CELLS (8 mos. AFTER STOPPING HCQ)











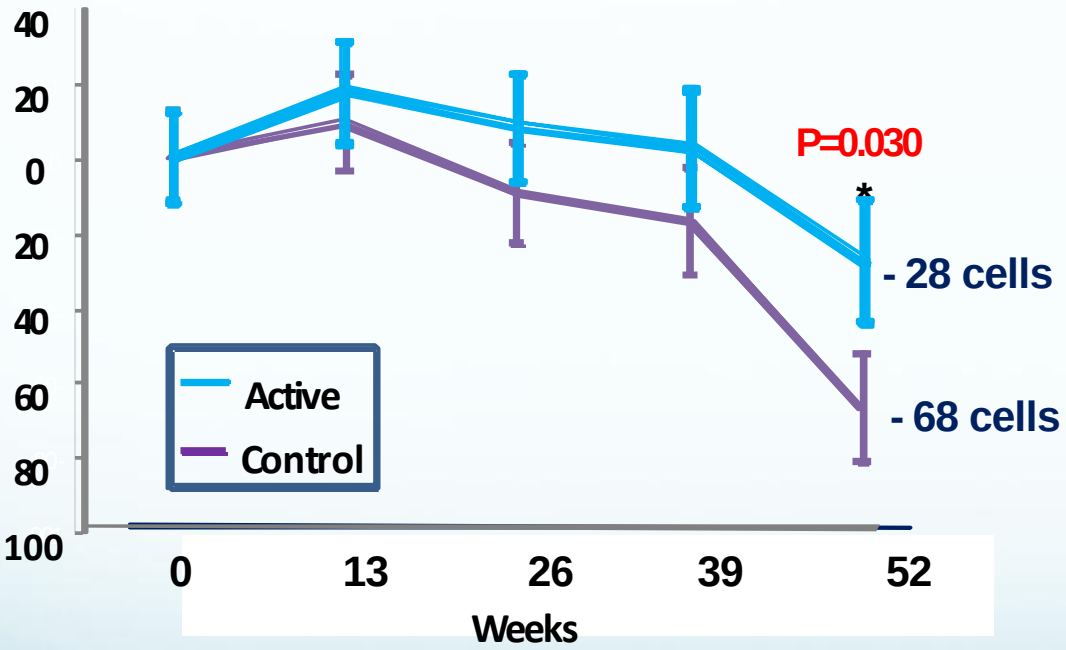
LONG TERM FOLLOW-UP: CONTROL OVER IMMUNE ACTIVATION IS LOST (8 mos AFTER STOPPING HCQ)

CD4 increase despite uncontrolled immune activation: is activation really responsible for incomplete CD4 reconstitution??

Act on the gut

WILL RESTORATION OF A PHYOLOGICAL GUT FLORA (MICROBIOTA) RESULT IN BENEFICIAL MODIFICATIONS OF IMMUNE PARAMETERS?

Prebiotics *reduces CD4+ decline and chronic immune activation but not viral load*



Prebiotics significantly slows the decline in CD4+ T-cell count in HIV-infected patients not on ART

Lange, et al. Submitted for publication

Nichole Klatt

Probiotic supplementation of ARV treatment during SIV infection of pigtail macaques results in enhanced GI tract CD4+ T cell frequency and immunological function

Laboratory of Jason Brenchley

Laboratory of Molecular Microbiology and

Program in Barrier Immunity and Repair

NIAID/NIH

March 7th, 2012

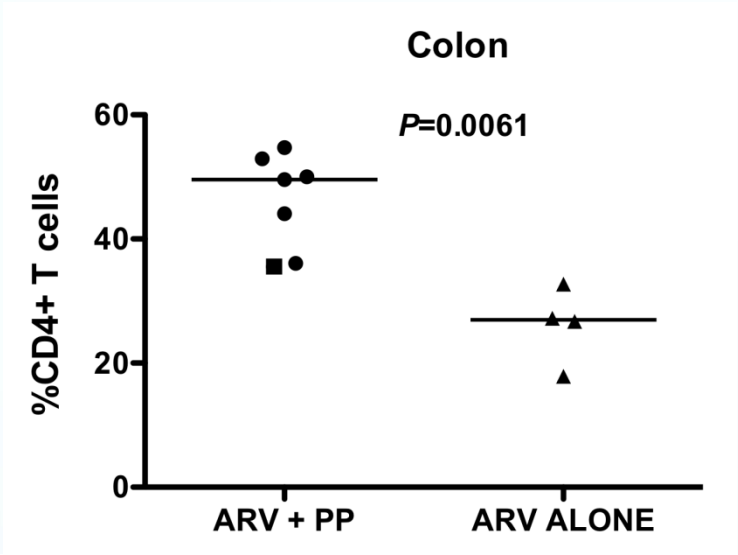
CROI

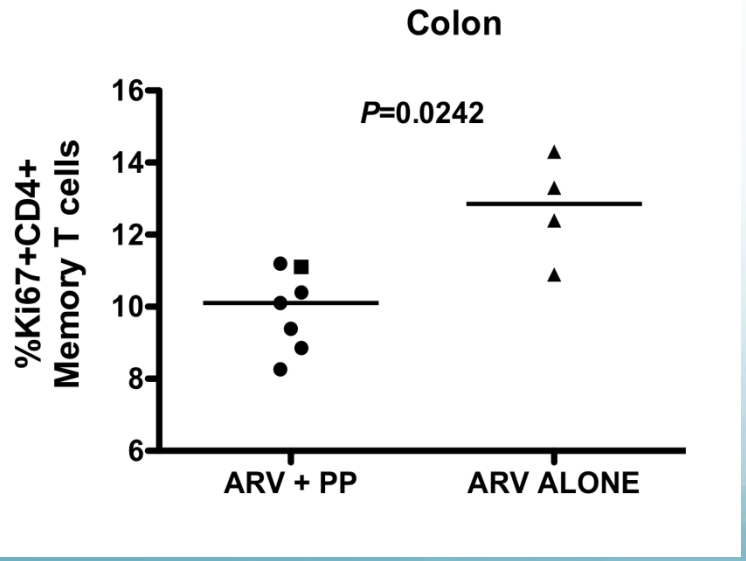
Seattle, WA

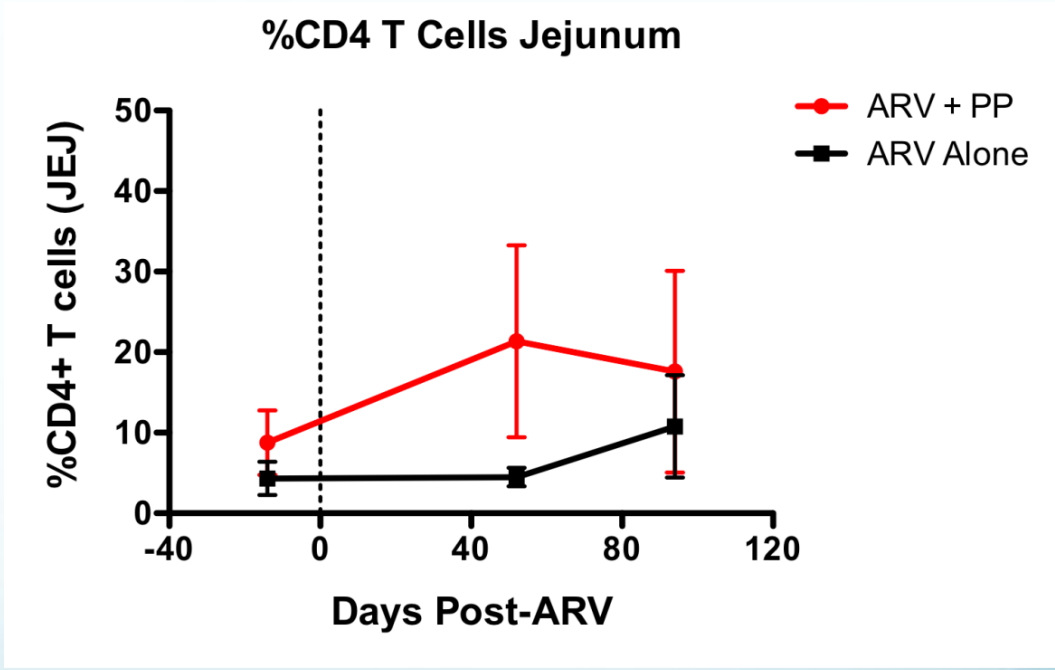
Abstract 95

Probiotic Supplementation of ARV Treatment during SIV Infection of Pigtail Macaques Results in Enhanced GI Tract CD4+ T Cell Frequency and Immunological Function

Nichole Klatt*[1], L Canary[1], X Sun[2], C Vinton[1], M Perkins[1], D Hazuda[3], J Lifson[4], E Haddad[2], J Estes[4], J Brenchley[1]







Conclusions

- **Even with optimal ART, life expectancy in HIV infection is shorter than normal; this is predicted by lower CD4 and higher inflammation**
- **Mechanisms of failed immune recovery are many and still partially unclear**
- **Approaches aimed at reducing TLR activity and/or targeting the microbiota could be beneficial in supplementing ART**

FULLY SATISFYING IMMUNE RECONSTUTION IS STILL AN UNREACHEABLE GOAL

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