CONVEGNO NAZIONALE

Let's stop HIV

Nuove prospettive
e popolazioni speciali

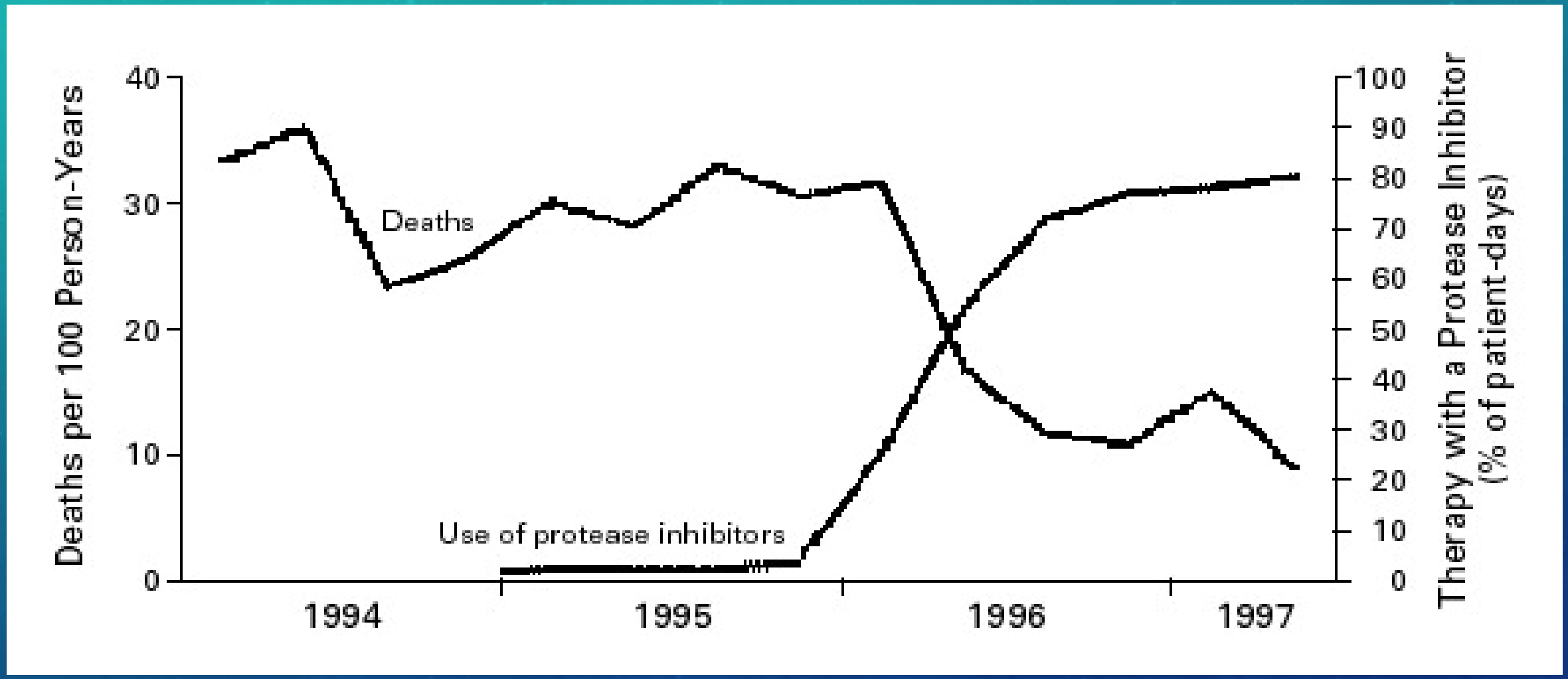
Quanto siamo vicini a una cura eradicante?

Giulia Carla Marchetti

QUANTO SIAMO VICINI AD UNA CURA (ERADICANTE)?

GIULIA MARCHETTI, MD, PHD

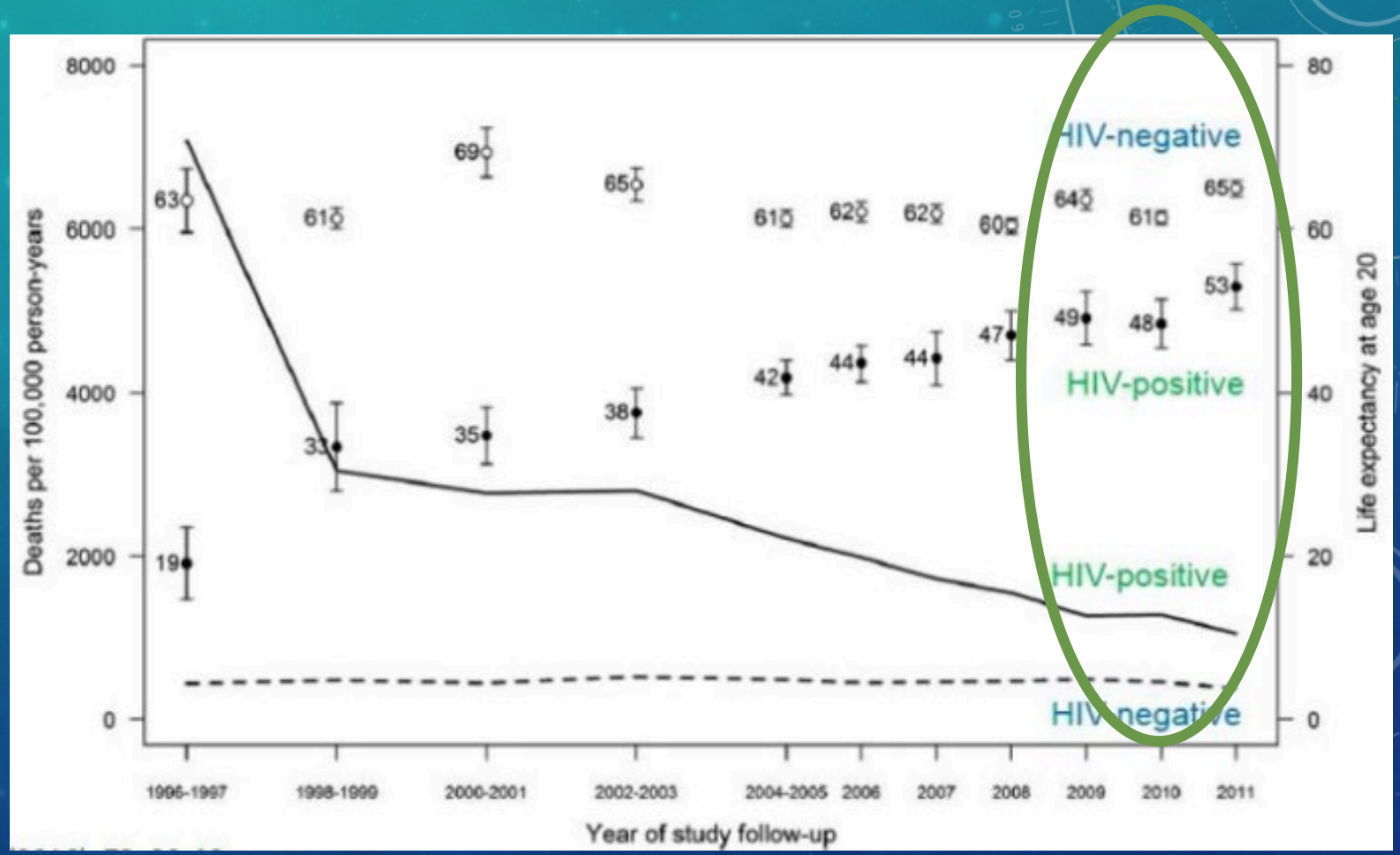
DEPT OF HEALTH SCIENCES, CLINIC OF INFECTIOUS DIS, UNIVERSITY OF MILAN, ASST SANTI PAOLO E CARLO



PALELLA FJ JR ET AL. N ENGL J MED 1998;338:853-860.

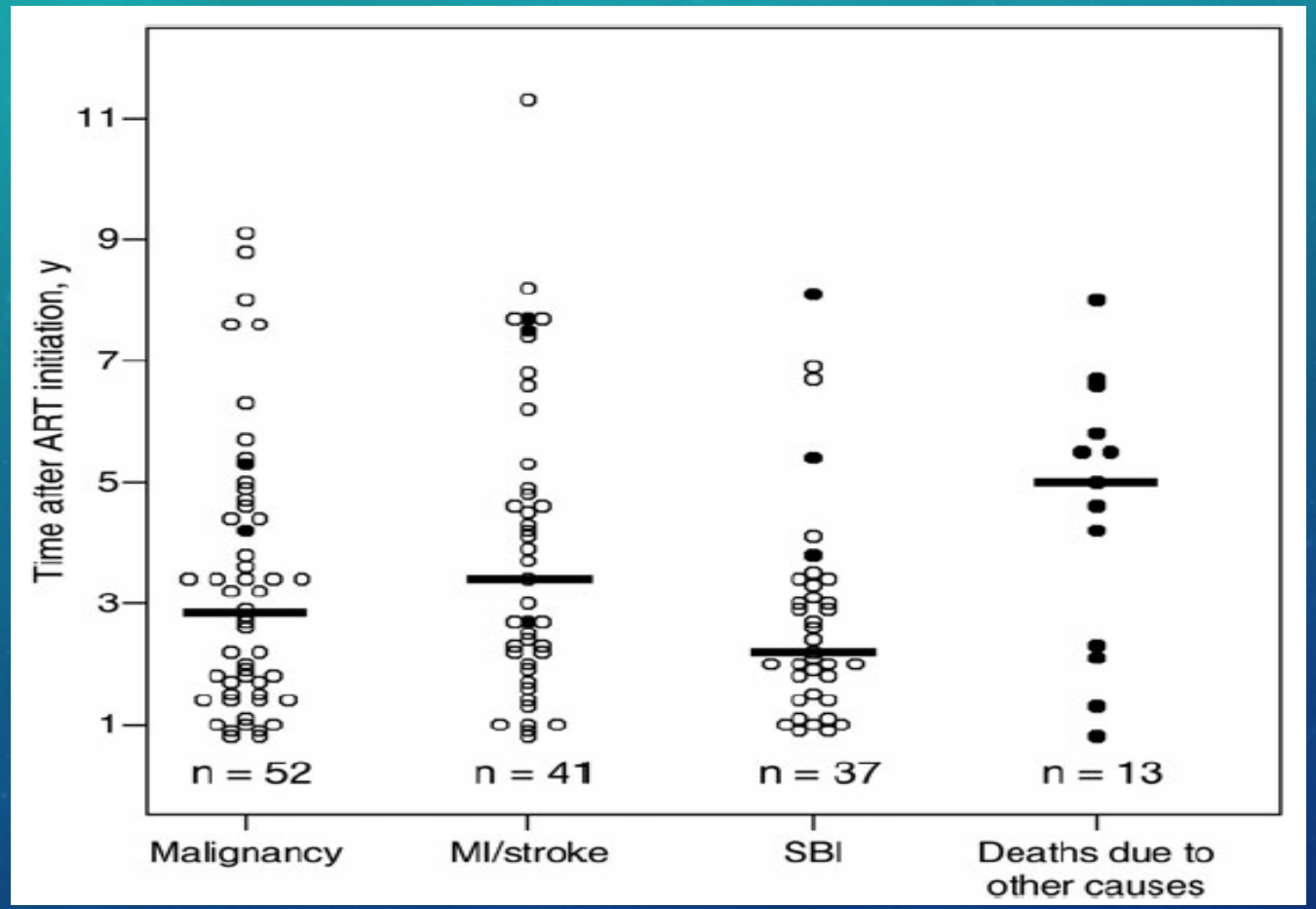
LIFE EXPECTATION AFTER AIDS - UPON VIRAL SUPPRESSION ON CART

Kaiser-Permanente, California



Marcus JL JAIDS 2016

RESIDUAL DISEASE AFTER AIDS - UPON VIRAL SUPPRESSION ON CART



Tenorio et al. JID 2014

How should we define success today?

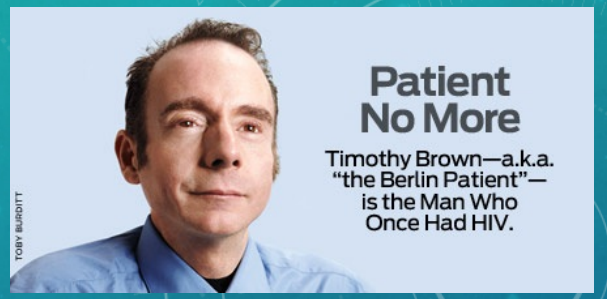
Eradication versus Remission

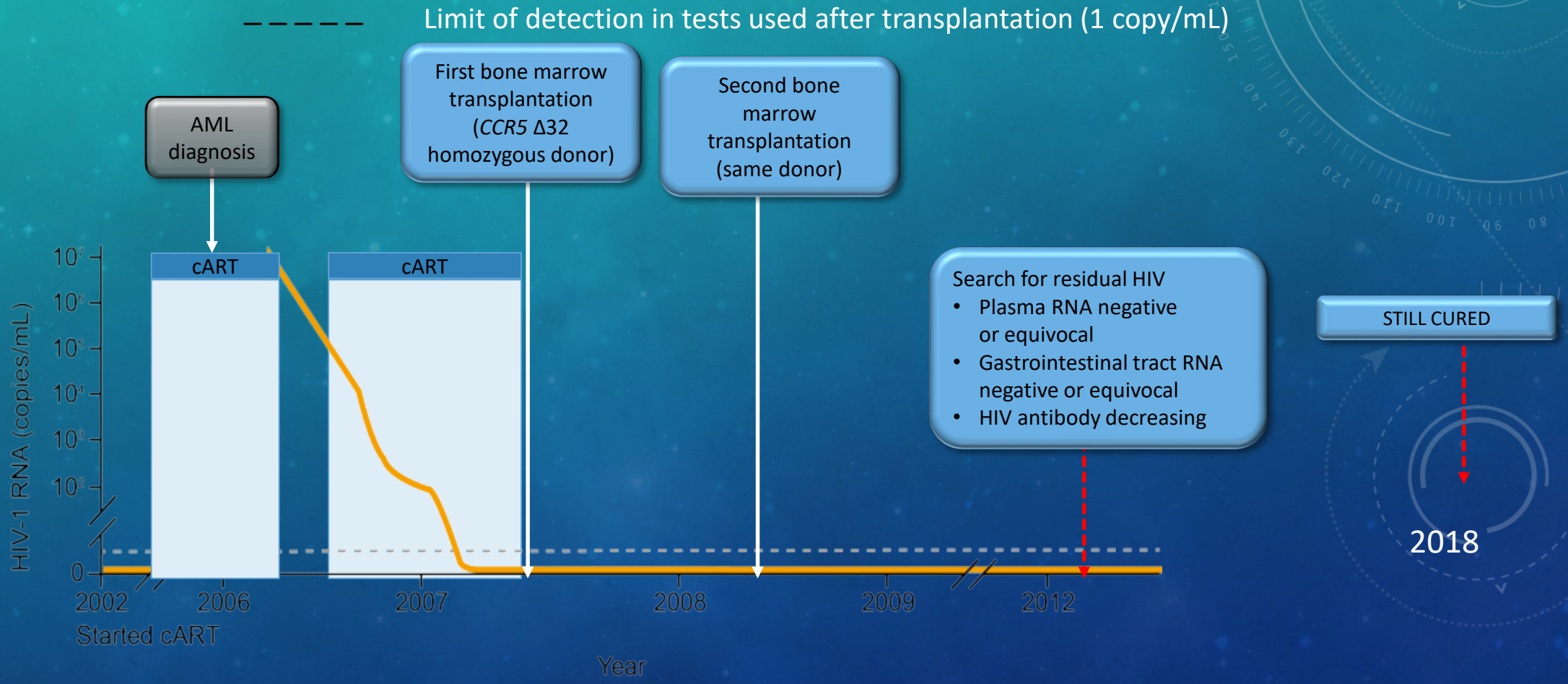
DEFINING SUCCESS
CURE VERSUS REMISSION

- Cure: complete removal of all replication-competent HIV cells
  - May have happended to Berlin (and London) patient, but impossible to prove

HIV CURE IS POSSIBLE

Timeline for the Berlin patient: the first and longest duration clinical cure case





AML, acute myeloid leukaemia; cART, combination antiretroviral therapy; CCR5, chemokine (C-C motif) receptor 5.
Kent SJ, et al. Lancet Infect Dis 2013;13:614–21

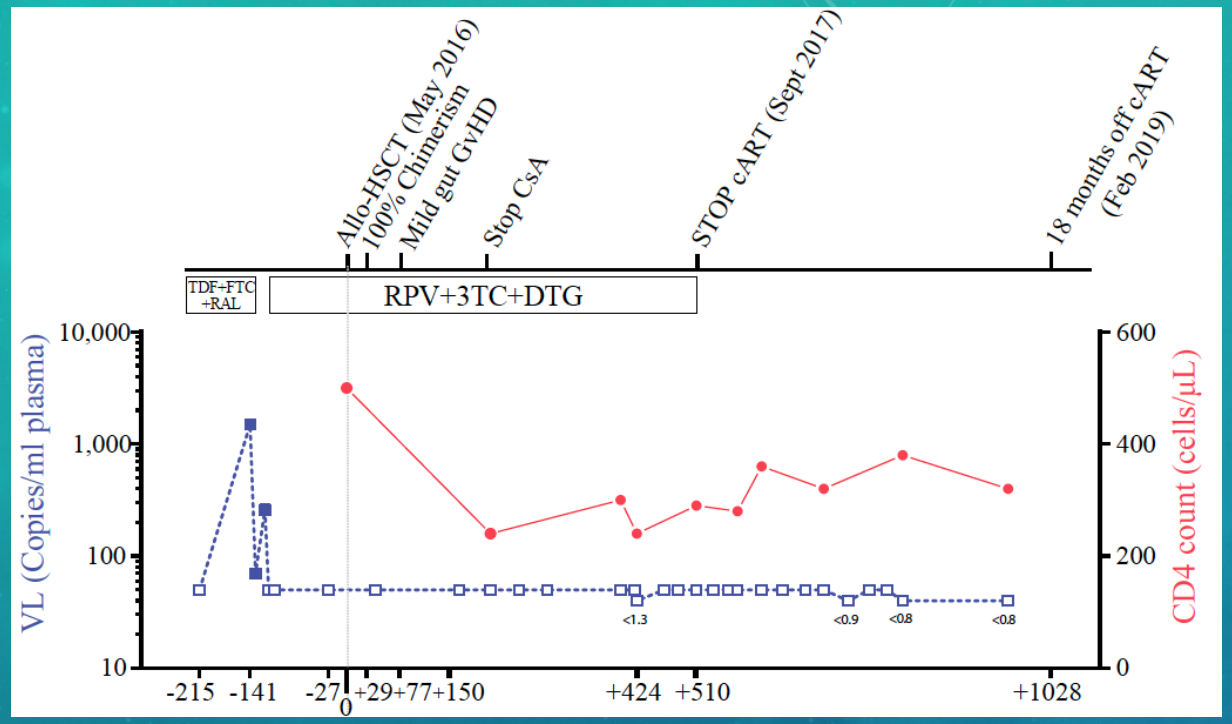
nature Accelerated Article Preview

Accelerated Article Preview Published online 5 March 2019.

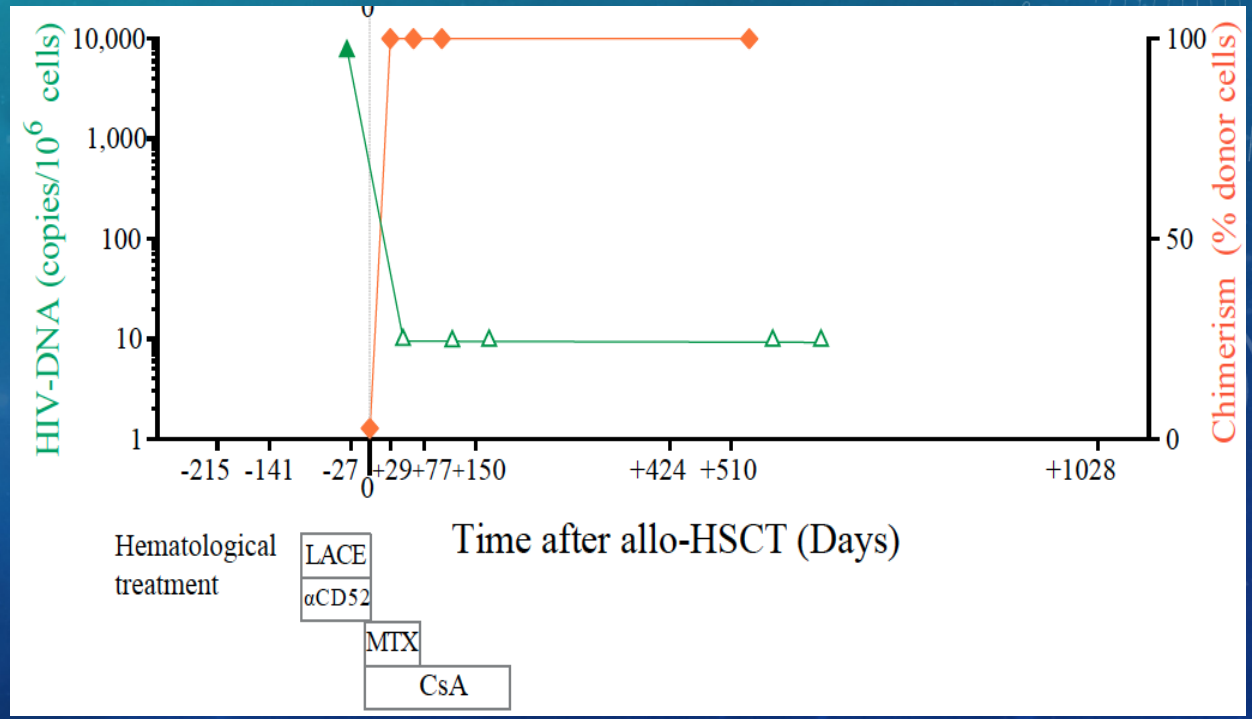
LETTER
doi:10.1038/s41586-019-1027-4

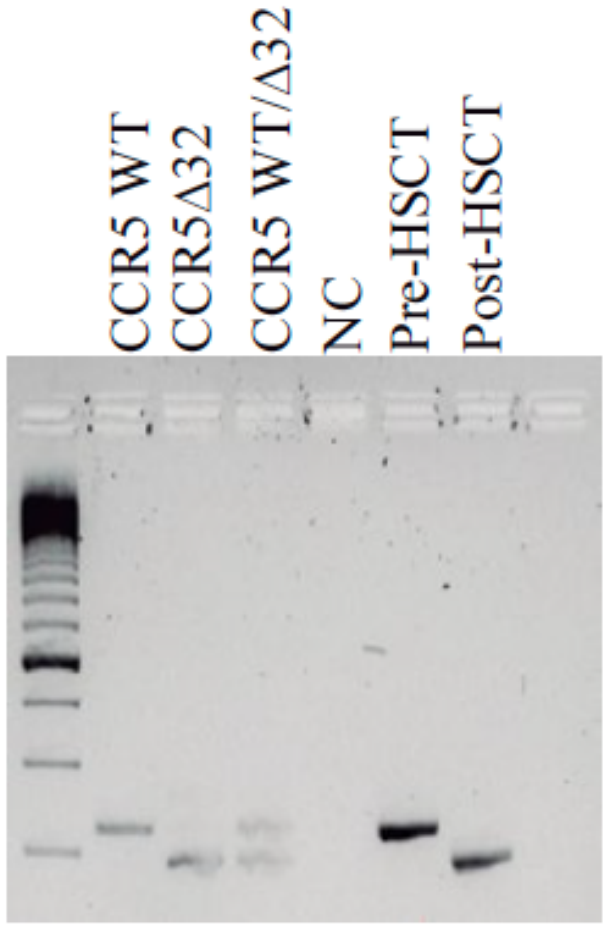
HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation

Ravindra K Gupta, Sultan Abdul-jawad, Laura E McCoy, Hoi Ping Mok, Dimitra Peppa, Maria Salgado, Javier Martinez-Picado, Monique Nijhuis, Annemarie M.J. Wensing, Helen Lee, Paul Grant, Eleni Nastouli, Jonathan Lambert, Matthew Pace, Fanny Salasc, Christopher Monit, Andrew Innes, Luke Muir, Laura Waters, John Frater, Andrew ML Lever, SG Edwards, Ian H Gabriel & Eduardo Olavarria

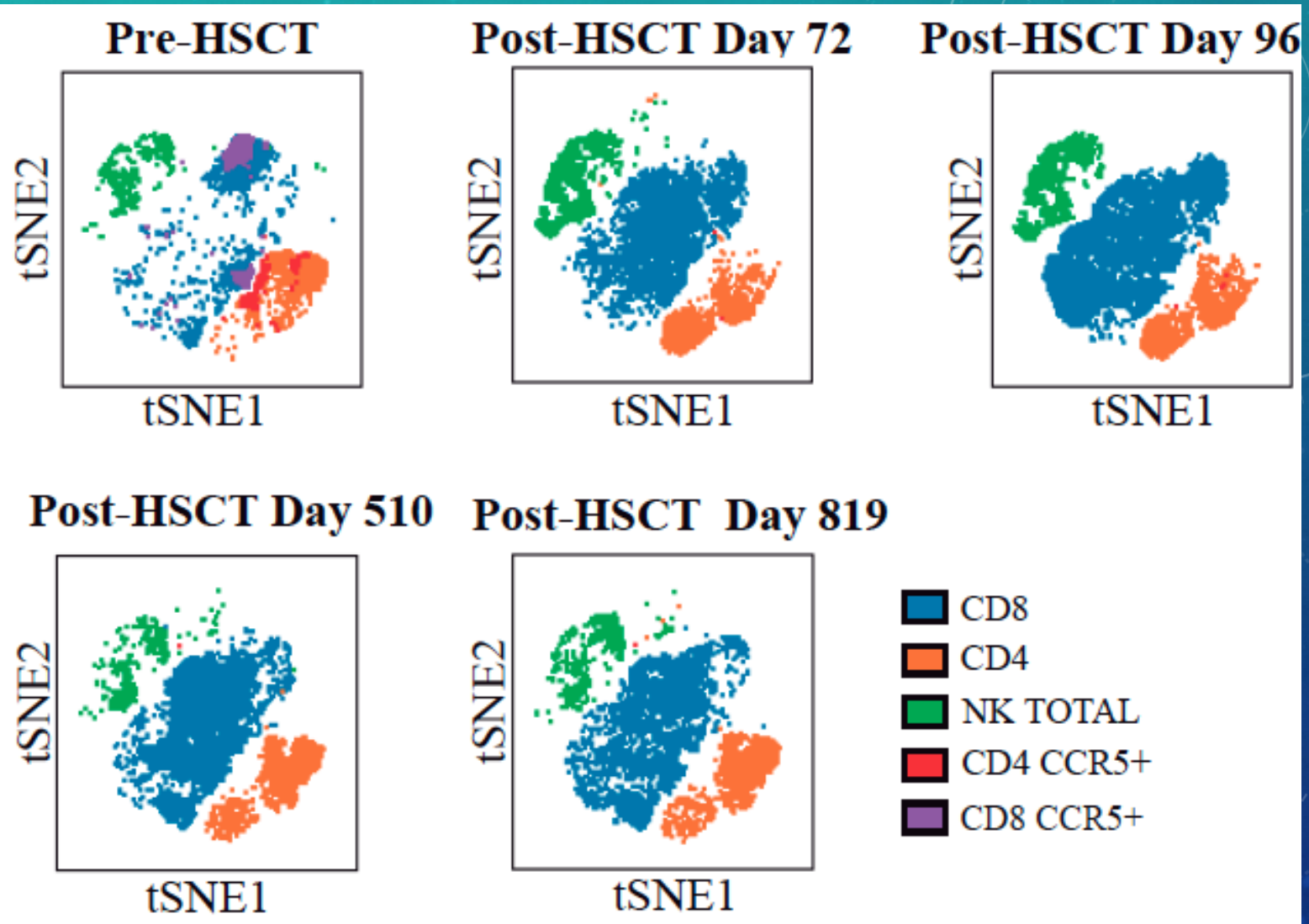


Stage IV non-Hodgkin lymphoma, homozygous CCR5 WT CCR5Δ32/Δ32 allo-HSCT transplant
Mild GVH

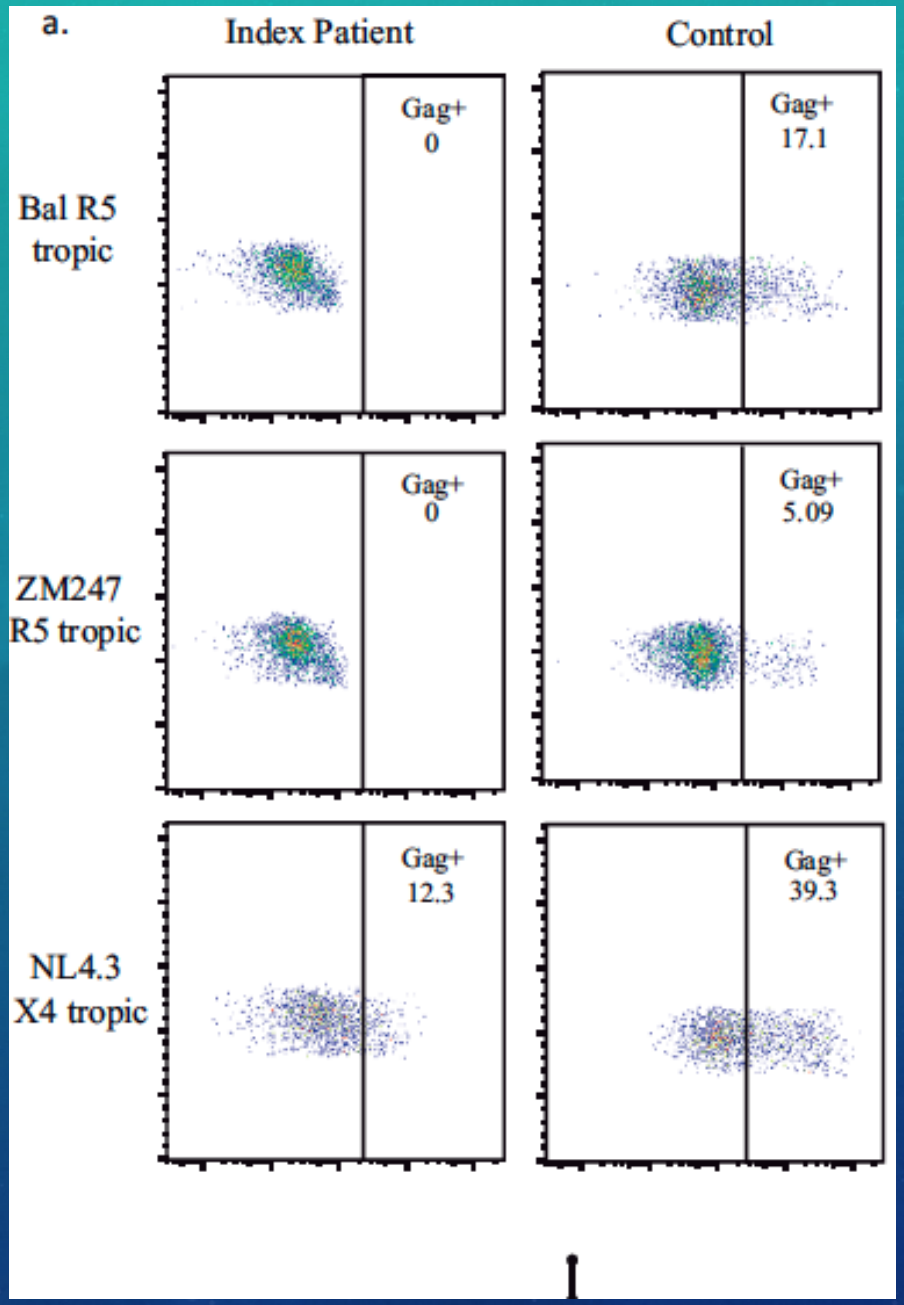


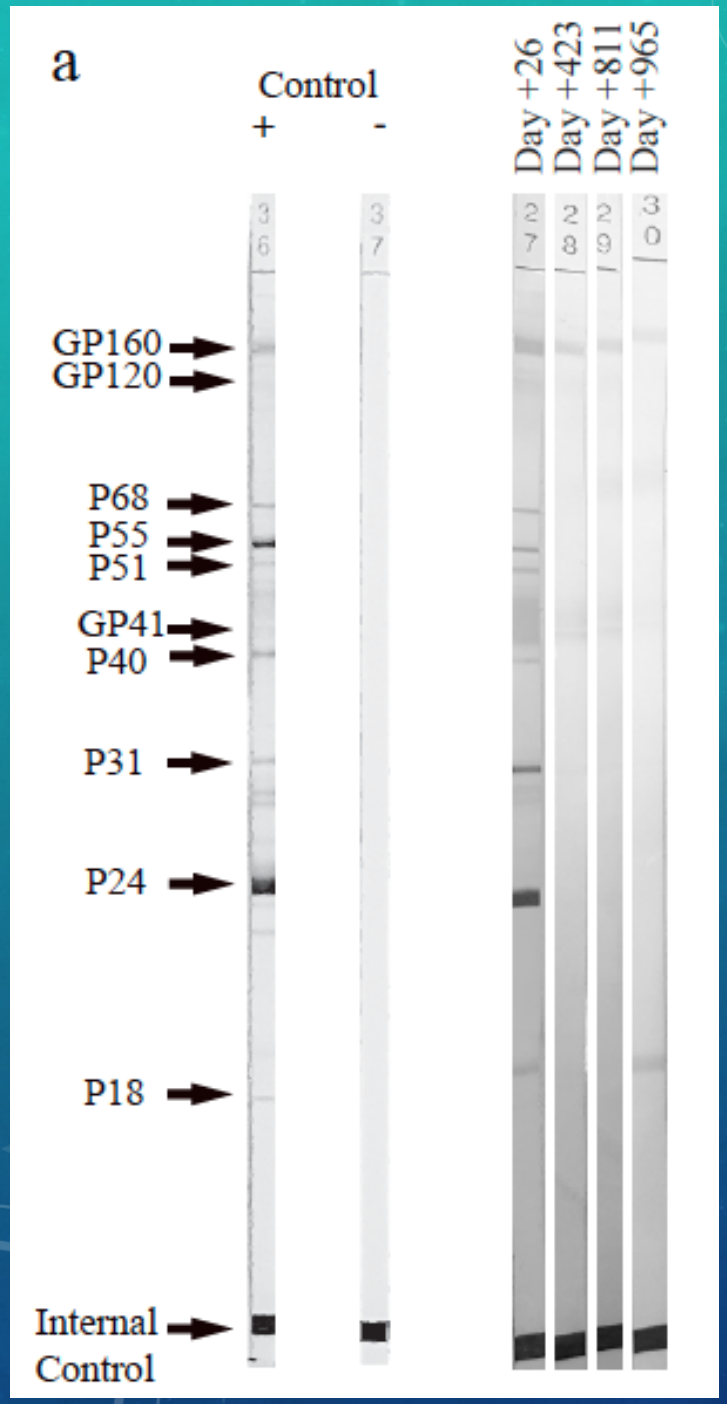

CCR5 WT
CCR5Δ32
CCR5 WT/Δ32
NC
Pre-HSCT
Post-HSCT

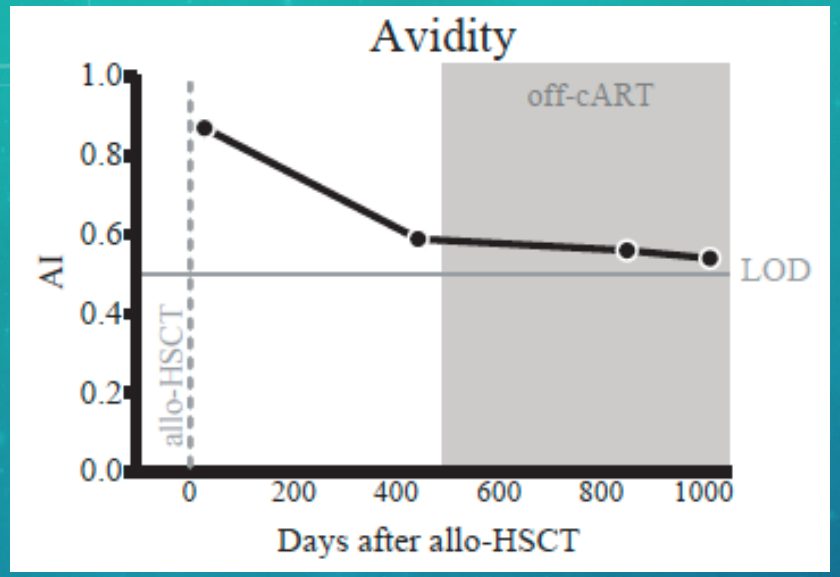
d

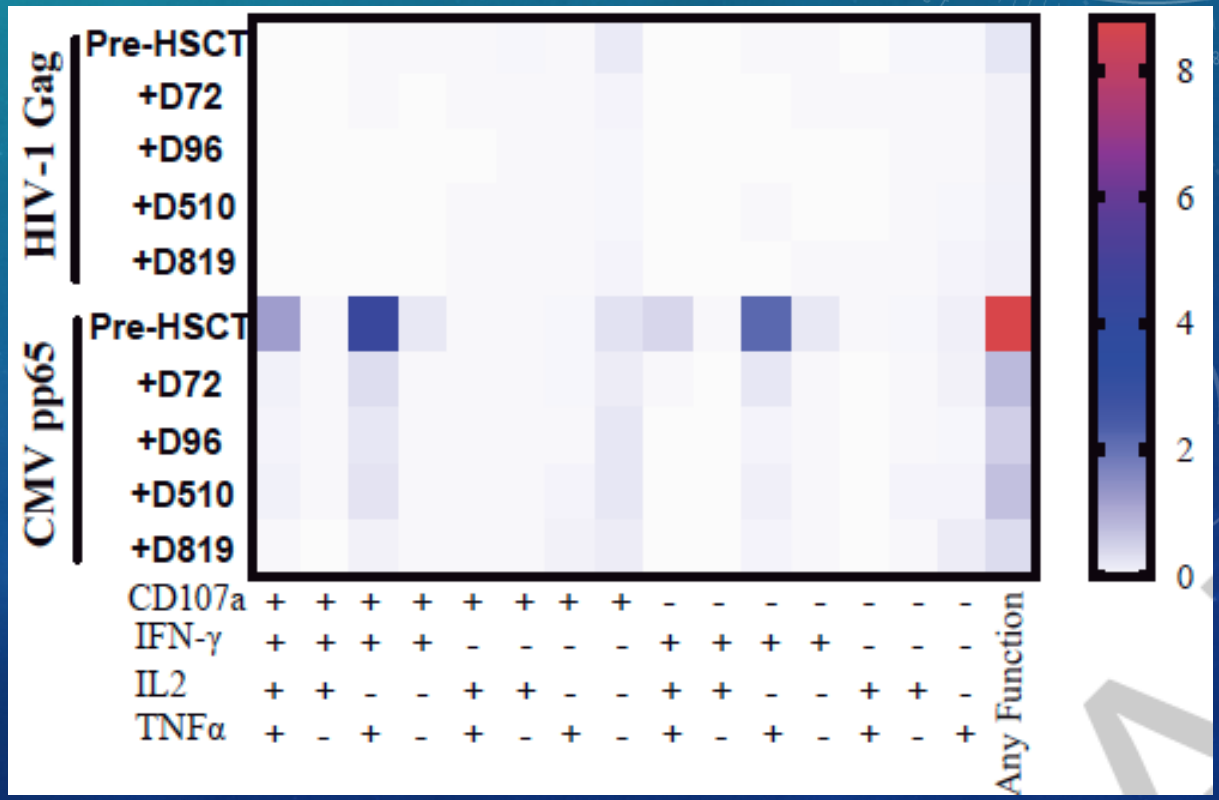

Pre-HSCT
Post-HSCT Day 72
Post-HSCT Day 96
Post-HSCT Day 510
Post-HSCT Day 819
tSNE1
tSNE2
CD8
CD4
NK TOTAL
CD4 CCR5+
CD8 CCR5+

INTRACELLULAR P24 GAG STAINING IN POST-TRANSPLANT CD4+ CELLS




a
Control
+
-
Day +26
Day +423
Day +811
Day +965
GP160
GP120
P68
P55
P51
GP41
P40
P31
P24
P18
Internal Control


Avidity
off-cART
LOD
allo-HSCT
AI
1.0
0.8
0.6
0.4
0.2
0.0
0
200
400
600
800
1000
Days after allo-HSCT


HIV-1 Gag
CMV pp65
Pre-HSCT
+D72
+D96
+D510
+D819
CD107a
IFN-γ
IL2
TNFα
Any Function
8
6
4
2
0

THE NEW YORKER

DAILY COMMENT

THE LONDON PATIENT AND A PLAN TO END THE H.I.V. EPIDEMIC IN THE UNITED STATES

Caveat to and lessons from the Berlin, London, Dusseldorf …patients:

- Extremely difficult to achieve
- Gene therapy approaches (from generation of HIV resistant CD4 + to the eradication of HIV infected cells by immune cell engineering)

DEFINING SUCCESS
CURE VERSUS REMISSION (FUNCTIONAL CURE)

- Remission: sustained virus (HIV) control or disease-free period in the absence of treatment
  - Replication-competent virus persists at levels that does not cause harm or that is not transmitted

FUNCTIONAL CURE – THE QUESTION(S)

Can we generate HIV-specific immune responses capable to fully contain viral replication even in tissue (sanctuaries) once cART is stopped?

FUNCTIONAL CURE – THE ASSUMPTION(S)

- LTNPs* and ECs**: high CD4 counts and/or HIV RNA control without therapy
- PTCs***: long-term virological remission following the interruption of cART started during early(est) infection

**LTNPs, long-term non progressors; **ECs, elite controllers; ***PTCs, post-treatment controllers*

Grabar et al. AIDS 2009; Saag & Deeks CID 2010; Saez-Cirion Plos Path 2013

FUNCTIONAL CURE – THE STRATEGY

First “debulk” the disease (cART) also reaching tissues, then eradicate or control the infection by enhancing immune function (immunotherapy)

FUNCTIONAL CURE – WHAT DO WE NEED? (AND WHY HAVE WE FAILED?)

- **Low viral burden**
- **Low inflammation**
- **Sustained host responses, that are primed, reside in tissues, target susceptible epitopes**

FUNCTIONAL CURE – WHAT DO WE NEED? (AND WHY HAVE WE FAILED?)

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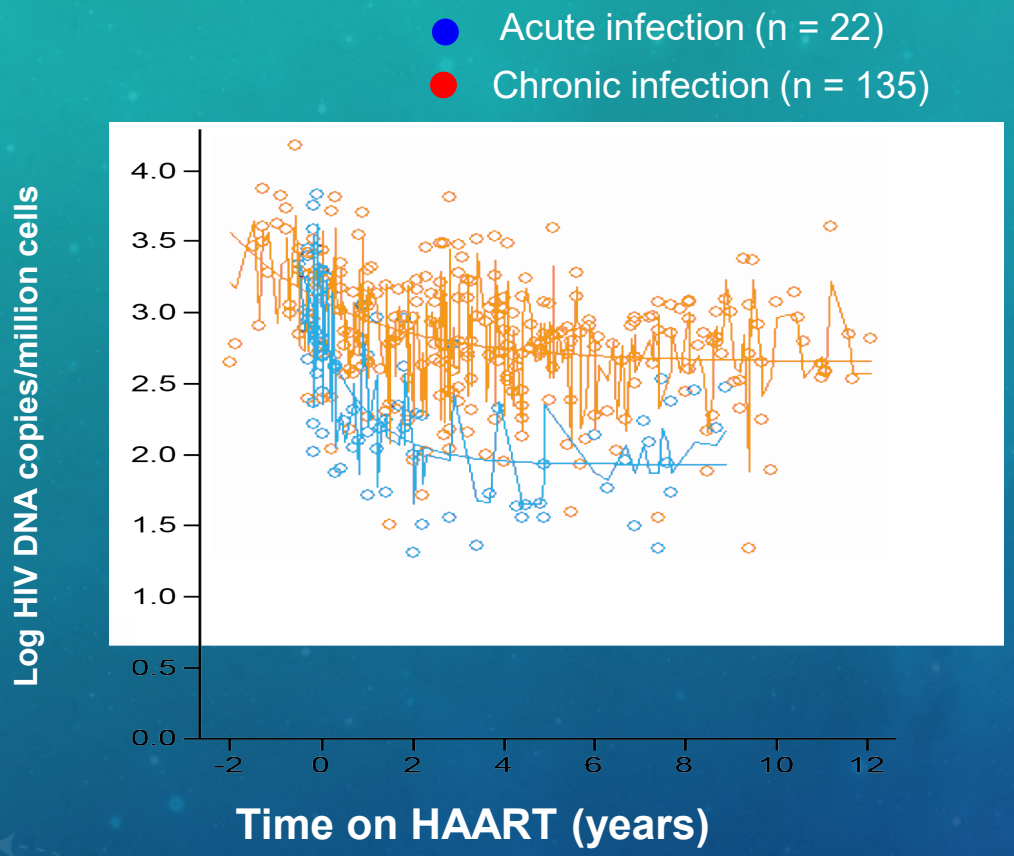
- **Sustained host responses, that are primed, reside in tissues, target susceptible epitopes**

1. DEBULKING THE VIRUS

Need to “push “ the viral reservoir below a threshold. But which?

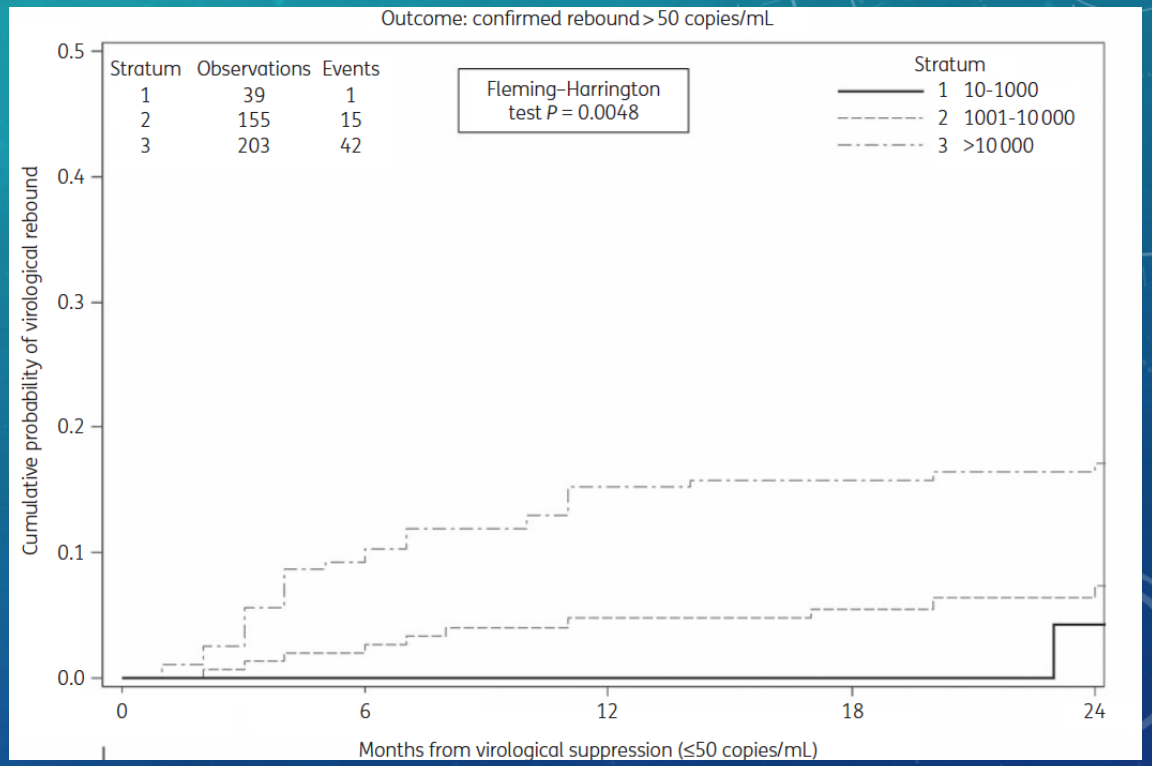
WHAT IS THE THRESHOLD BELOW WHICH ART COULD BE SAFELY STOPPED?



Hocqueloux L, et al. JAC 2013.

>400 patients starting first-line cART

Viral rebound by 24 months first-line cART



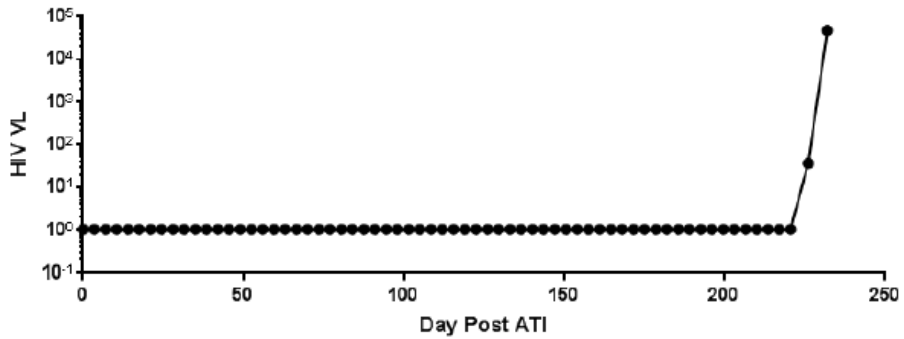
Ceccherini-Silberstein et al. JAC 2018

EARLIEST TREATMENT (ADULTS)

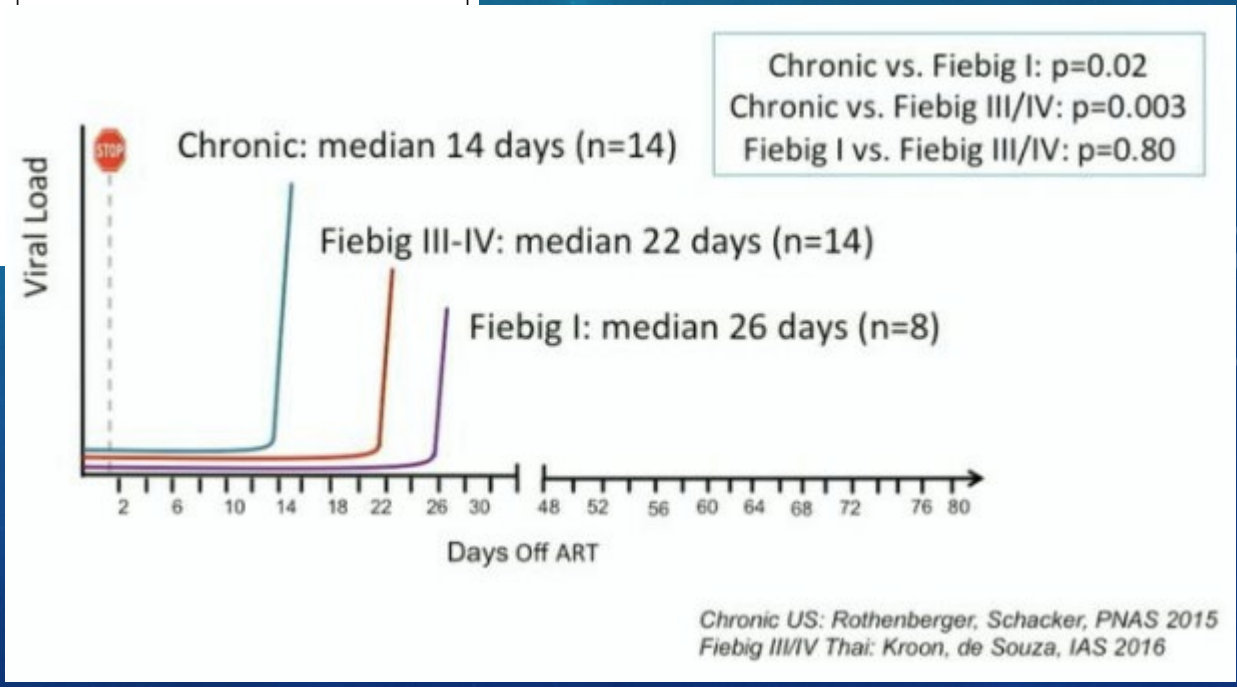
HIV-1 persistence following extremely early initiation of antiretroviral therapy (ART) during acute HIV-1 infection: An observational study

PLOS | MEDICINE

Timothy J. Henrich[1]*, Hiroyu Hatano[2], Oliver Bacon[2,3], Louise E. Hogan[1], Rachel Rutishauser[1,2], Alison Hill[4], Mary F. Kearney[5], Elizabeth M. Anderson[5], Susan P. Buchbinder[2,3], Stephanie E. Cohen[2,3], Mohamed Abdel-Mohsen[2,6], Christopher W. Pohlmeyer[7], Remi Fromentin[8], Rebecca Hoh[2], Albert Y. Liu[2,3], Joseph M. McCune[1], Jonathan Spindler[5], Kelly Metcalf-Pate[7], Kristen S. Hobbs[1], Cassandra Thanh[1], Erica A. Gibson[1], Daniel R. Kuritzkes[9,10], Robert F. Siliciano[11,12], Richard W. Price[13], Douglas D. Richman[14,15], Nicolas Chomont[8], Janet D. Siliciano[10], John W. Mellors[16], Steven A. Yuki[17,18], Joel N. Blankson[7], Teri Liegler[2], Steven G. Deeks[2]



PrEP during early (day 1) detectable infection followed by ART resulted in the lack of any detectable reservoir, using multiple highly sensitive methods



Chronic US: Rothenberger, Schacker, PNAS 2015
Fiebig III/IV Thai: Kroon, de Souza, IAS 2016

EARLIEST TREATMENT (NEWBORNS): THE CLOSEST THAT WE GET TO HIV CURE ? – VERY EARLY ART, NO MEMORY CELLS

Parameters	Mississippi [2]	Canadian [4]	Milan [5]
Time to viral rebound	27 months	<1 month	<1 month
ART onset	30 hours	<24 hours	12 hours
Pre-ART HIV RNA. copies/ml	19,812	808	152,560
Time to HIV RNA < 50 copies/ml on ART	1 month	6 months	3 months
Time on ART before interruption	18 months	3 years	3 years
Cell-associated HIV DNA	Undetected[a]	Undetected	Undetected
Replication-competent virus	Negative viral outgrowth assay[a]	Negative viral outgrowth assay	Negative viral culture
HIV antibody	Non-reactive[a]	Non-reactive	Non-reactive
HIV-specific T cells	Undetected[a]	Not reported	Detected
Others	Normal frequencies of activated T cells[a]	Detected cell-associated HIV RNA	High frequencies of activated T cells

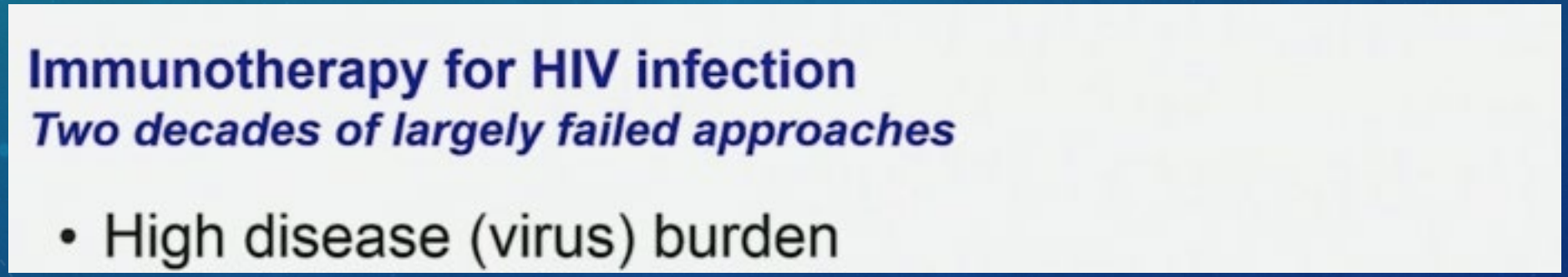
[a]The reservoir and immunity testing in the Mississippi baby were performed after ART was interrupted. The testing on the Canadian and Milan babies was performed during ART. ART: antiretroviral therapy.

Ananworanich & Robb JIAS 2014
4- Brophy J et al. 20th IAS Conference, Melbourne 2014;
5- Giacomet V et al. Lancet 2014

+ Frange et al. cART until 5yo; 11 years off cART HIV RNA<4 cp/ml, HIV DNA <2,2Logcp/10^6PBMC - IAS 2015 , Vancouver- Canada

1. DEBULKING THE VIRUS

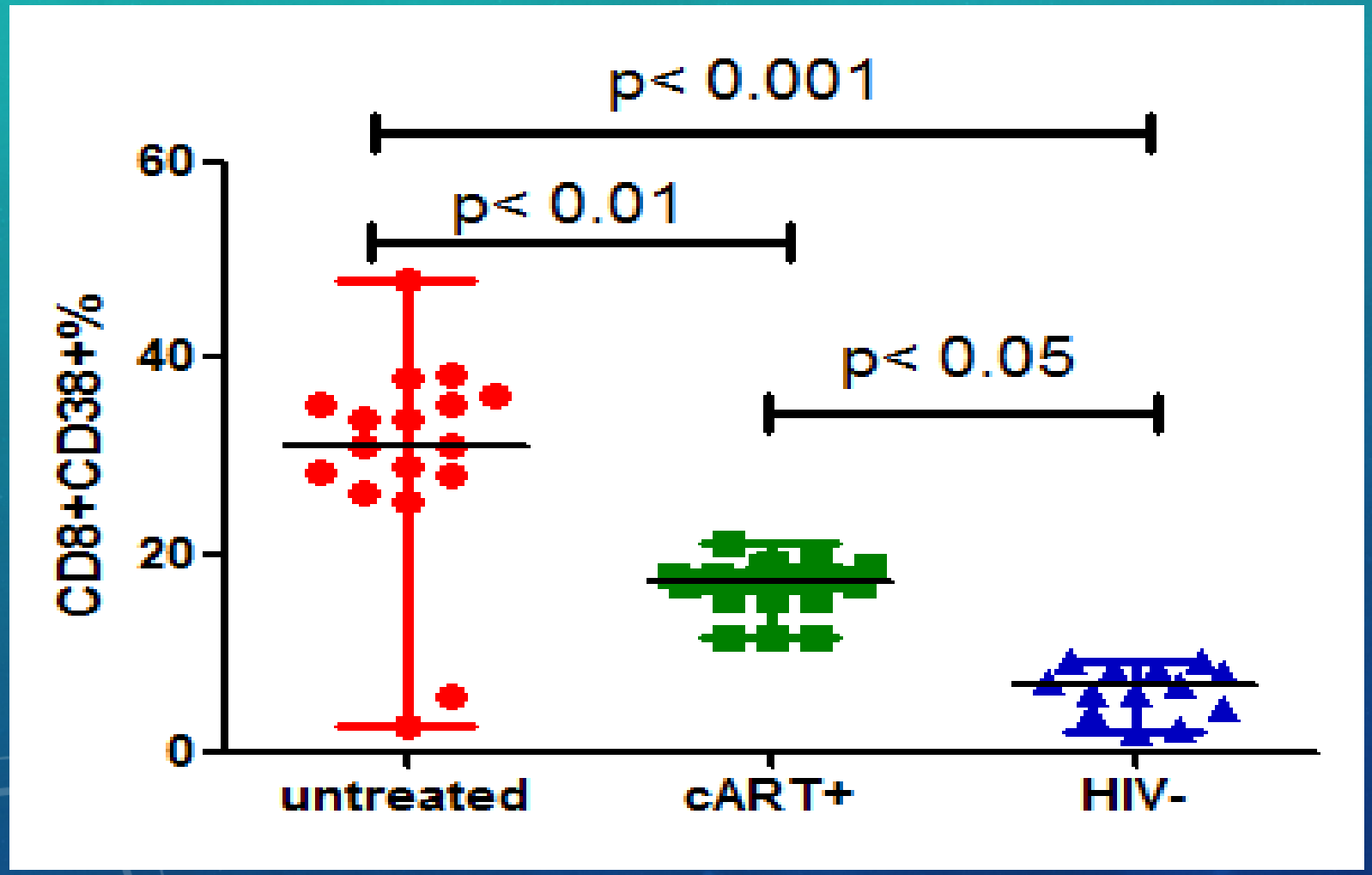
Need to “push “ the viral reservoir below a threshold. But which?



FUNCTIONAL CURE – WHAT DO WE NEED? (AND WHY HAVE WE FAILED?)

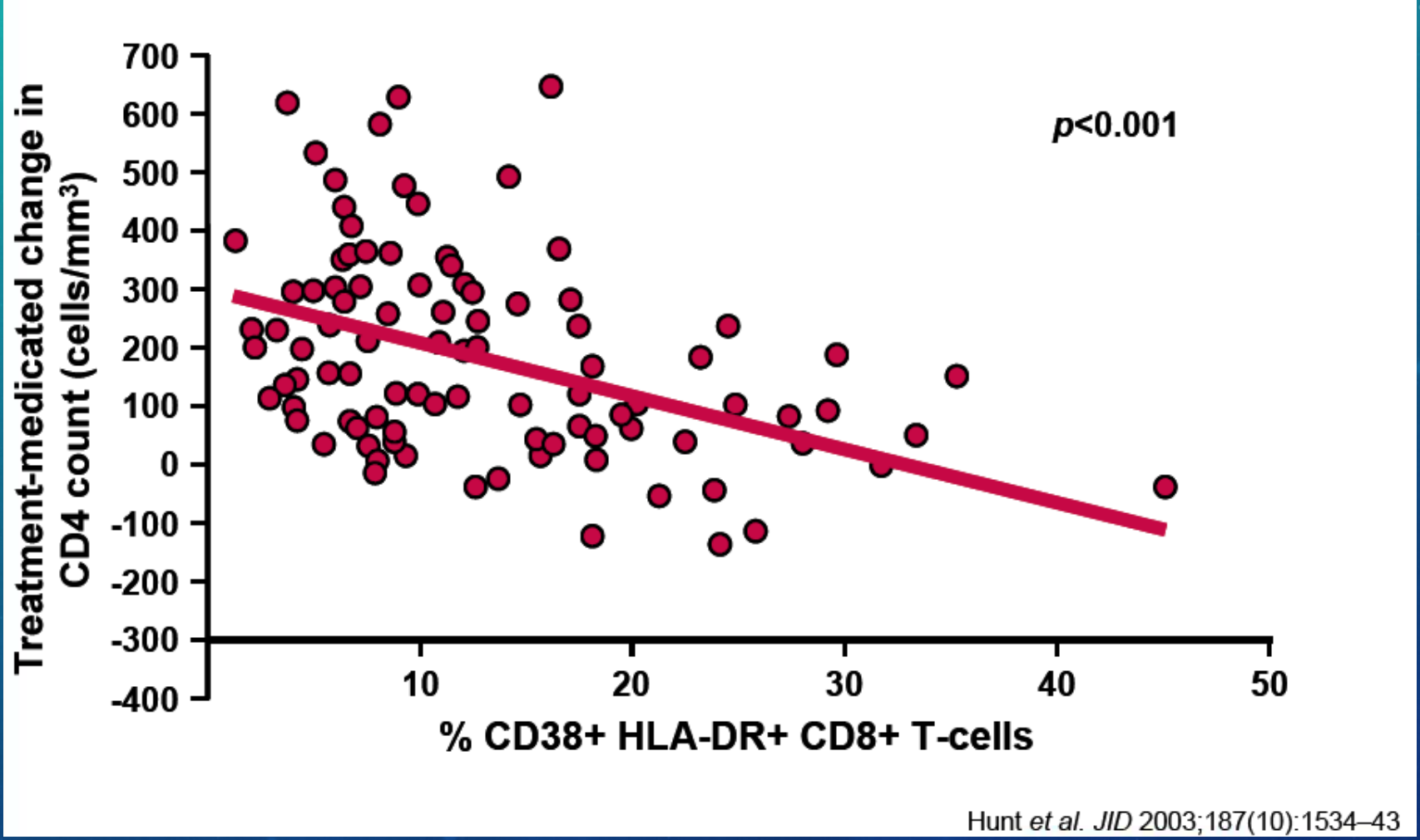
- Low viral burden
- **Low inflammation**
- Sustained host responses, that are primed, reside in tissues, target susceptible epitopes

2. REDUCING INFLAMMATION



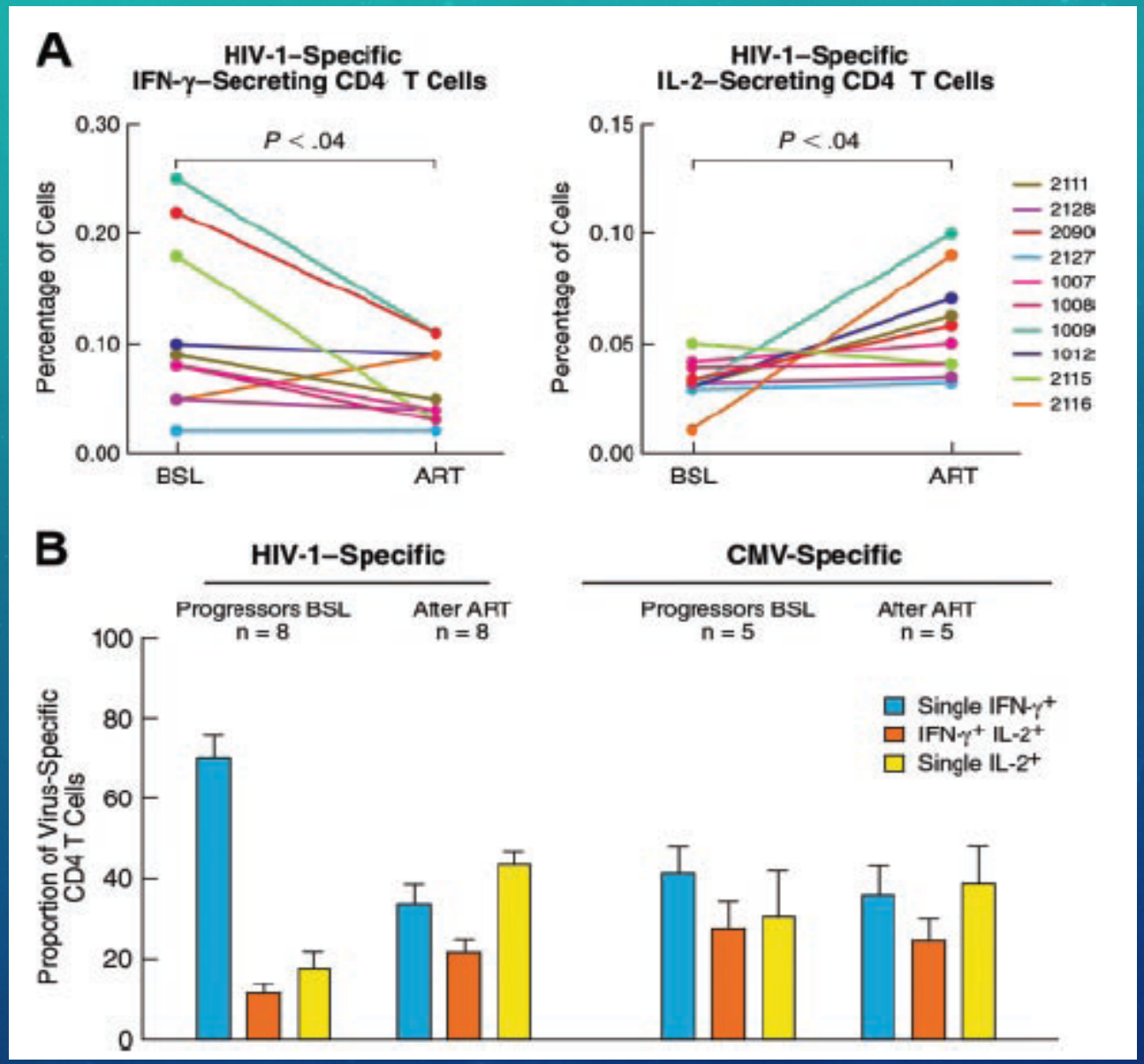
RESIDUAL INFLAMMATION PERSISTS AFTER VIRAL SUPPRESSION ON CART…….

.....HAMPERS CD4+ RECOVERY.....



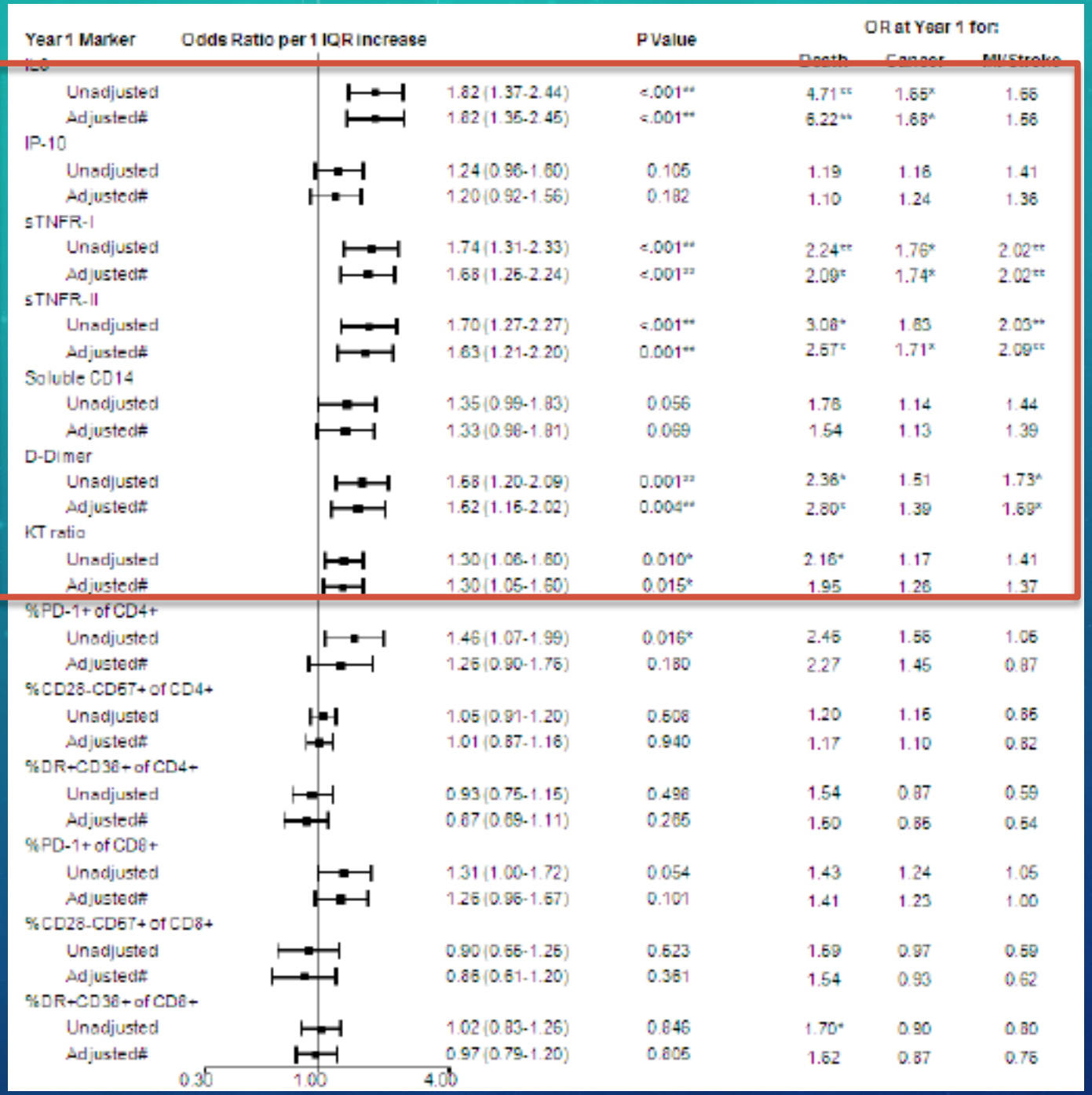
Hunt *et al. JID* 2003;187(10):1534–43

… IMMUNE FUNCTION….



Harari A Blood 2004

Odds ratio of non-AIDS events according to marker at year 1 of HAART

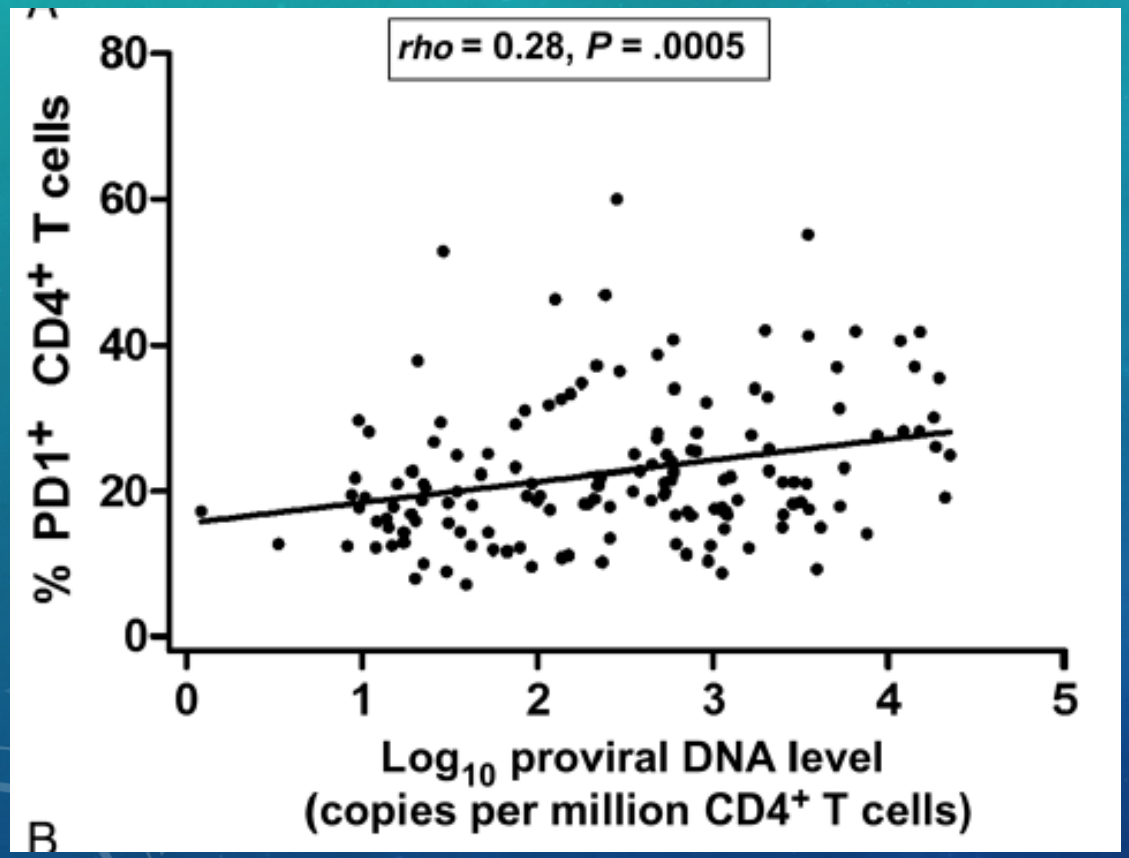


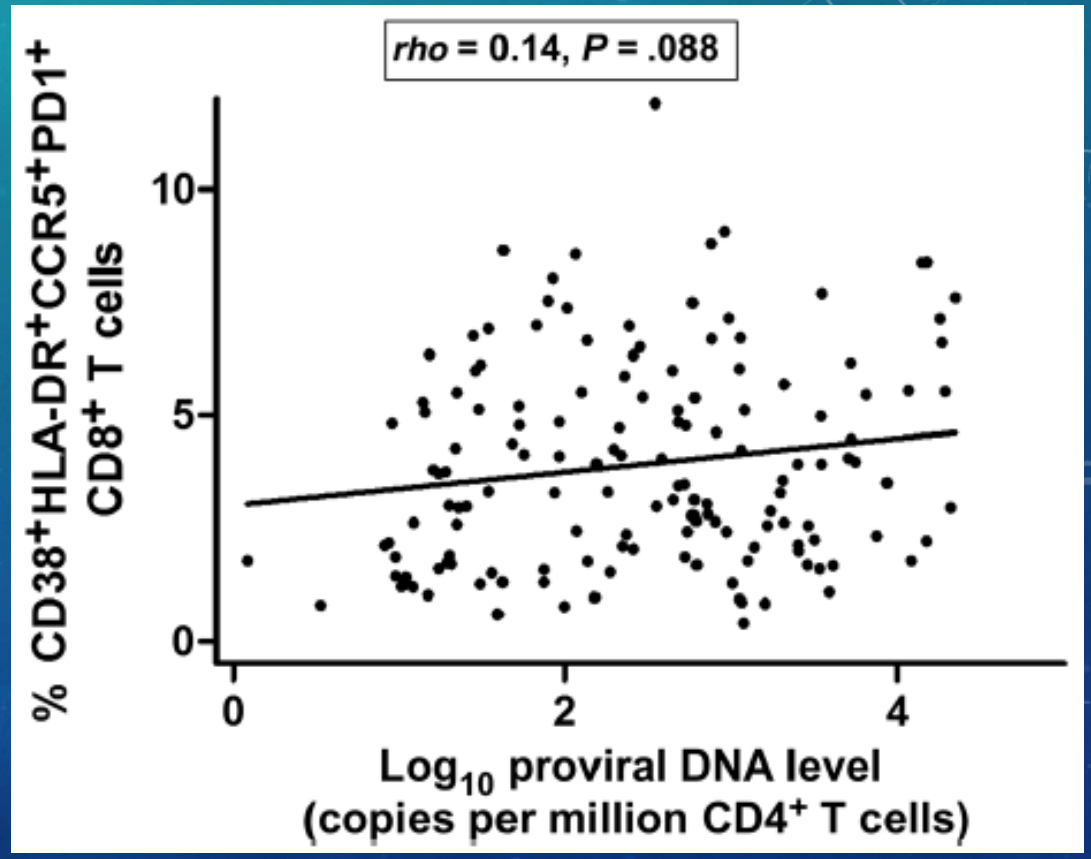
...AND DRIVES RESIDUAL DISEASE

Tenorio et al. JID 2014

ASSOCIATIONS BETWEEN PD1-EXPRESSING ACTIVATED T CELLS AND HIV PROVIRAL DNA IN PERIPHERAL BLOOD

190 HIV+ on virally-effective cART



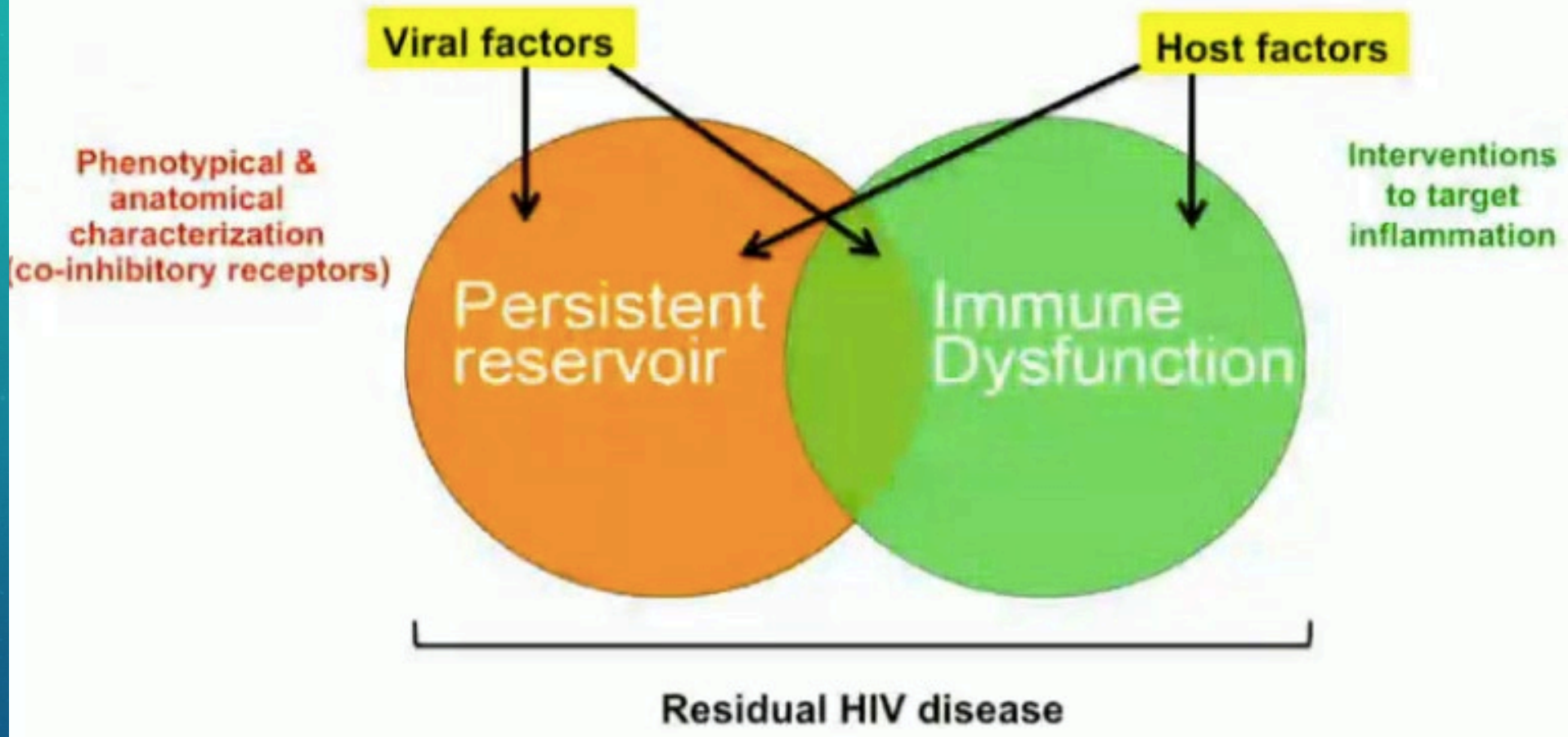


Hatano et al. JID 2013

Residual inflammation during cART is associated to viral persistence

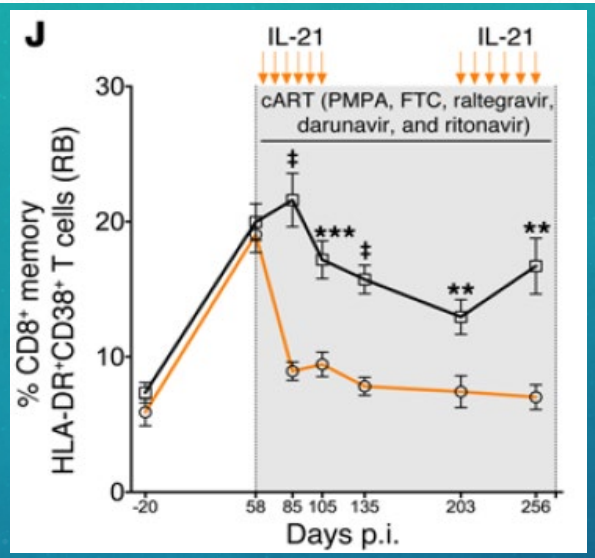
What causes what?

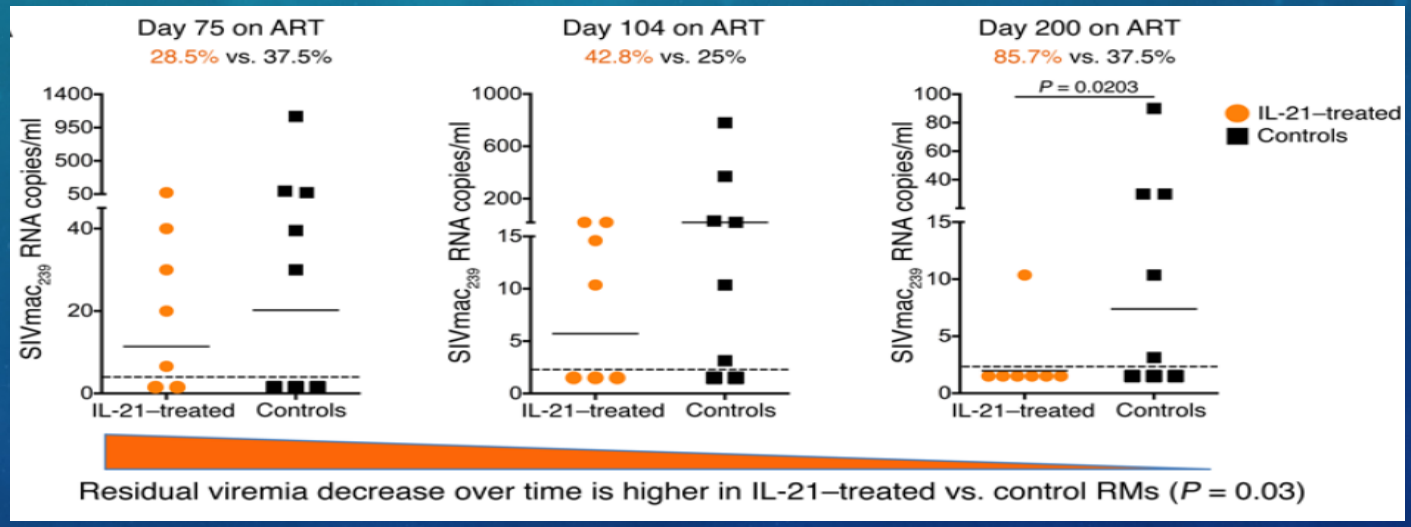
Residual disease in ART-treated HIV-infection



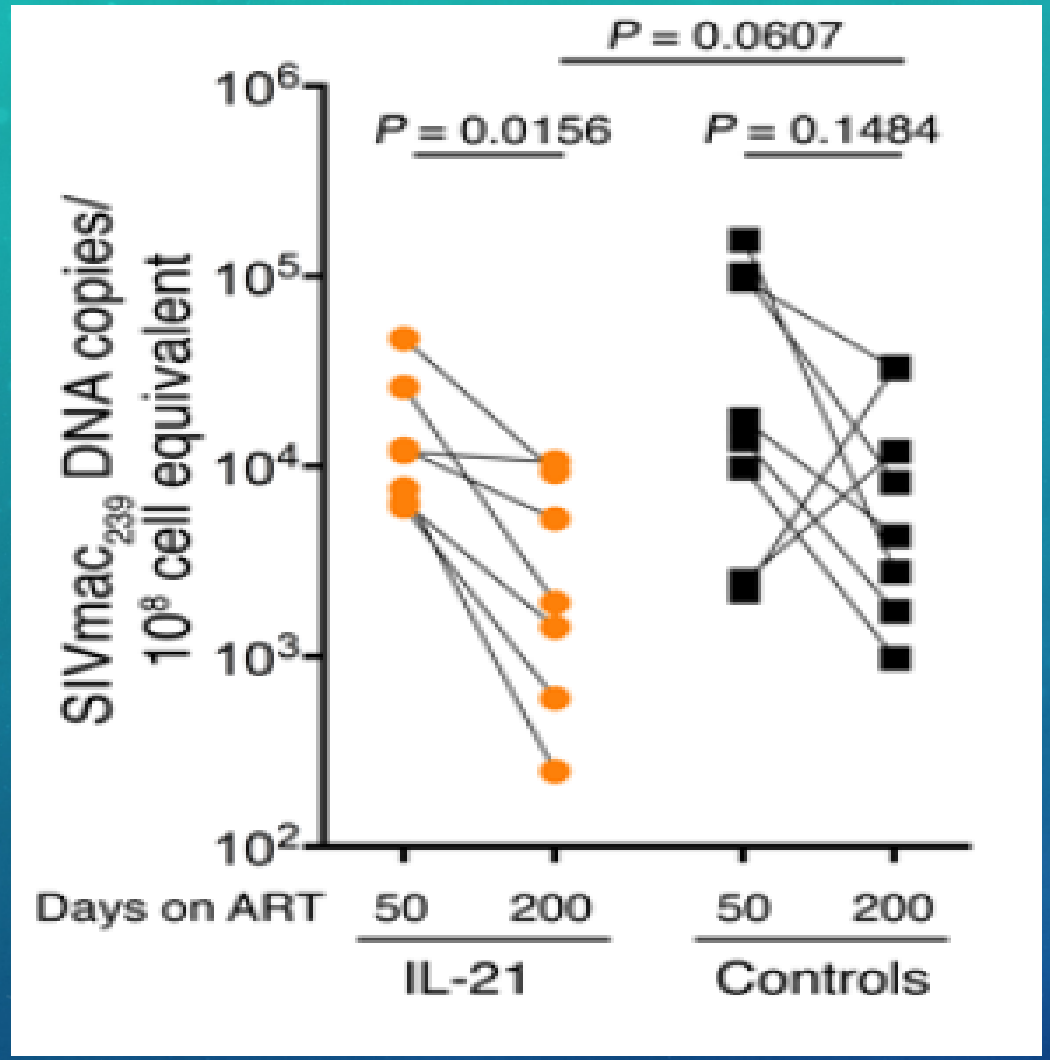
- Curing HIV infection is a virological AND immunological problem
- It is possible that eliminating the "last copy" of HIV in the body will not "cure" the immune dysfunction (inflammation, loss of mucosal integrity, immune senescence, fibrosis, etc.)

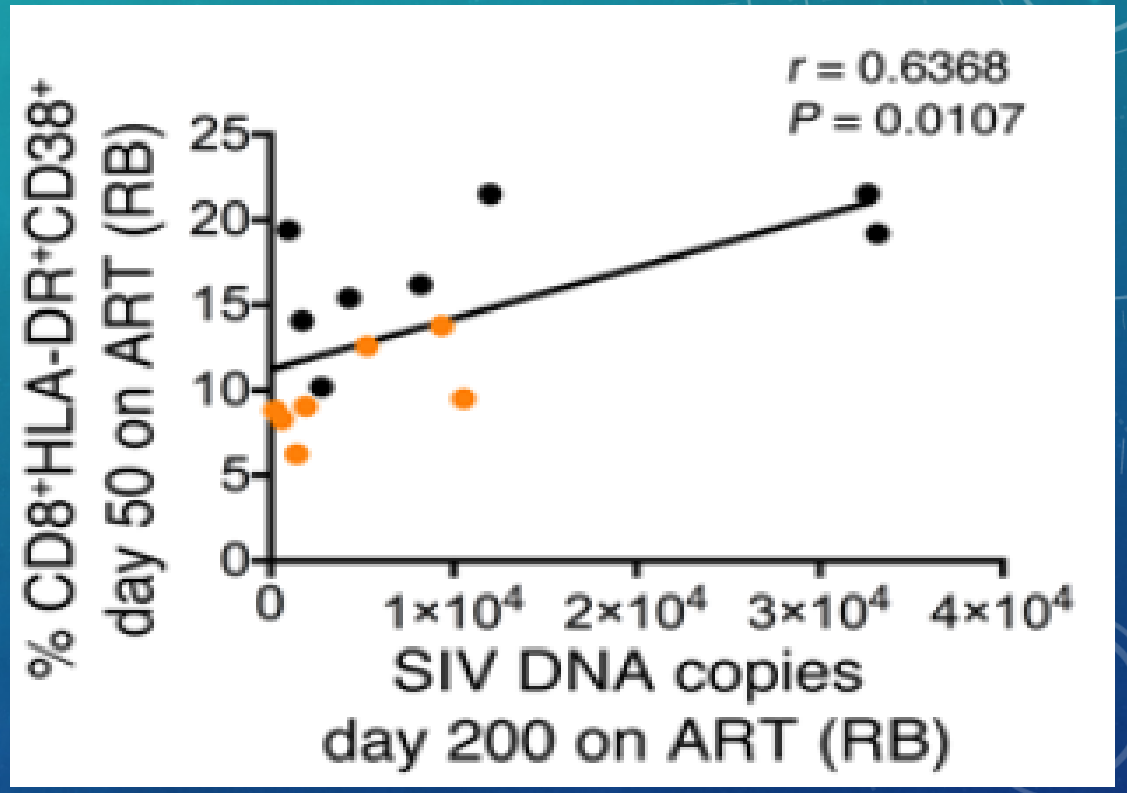
Interventions that reduce inflammation/immune suppression may also be beneficial in containing viral persistence





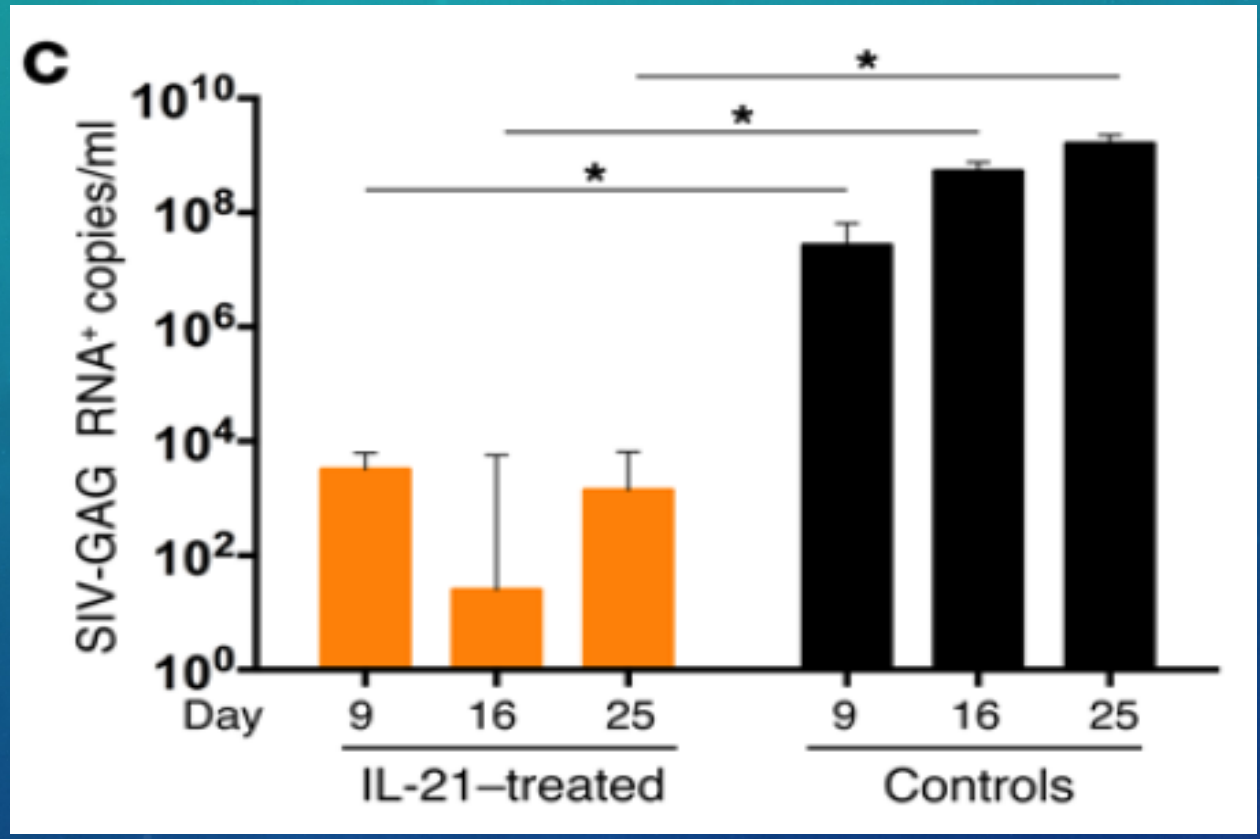
Micci et al. JCI 2015



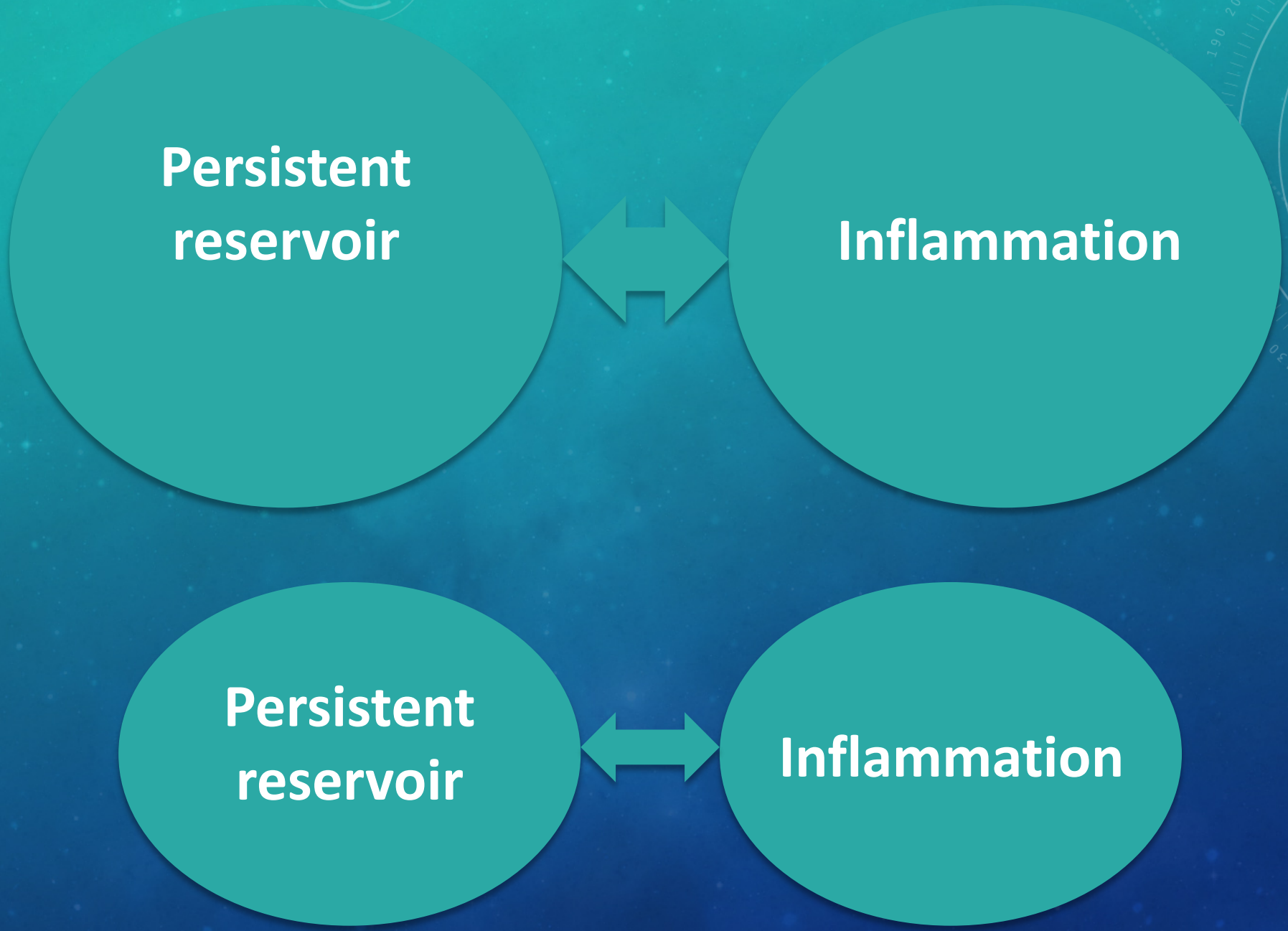


Micci et al. JCI 2015

REDUCTION IN REPLICATION-COMPETENT VIRUS BY IL-21

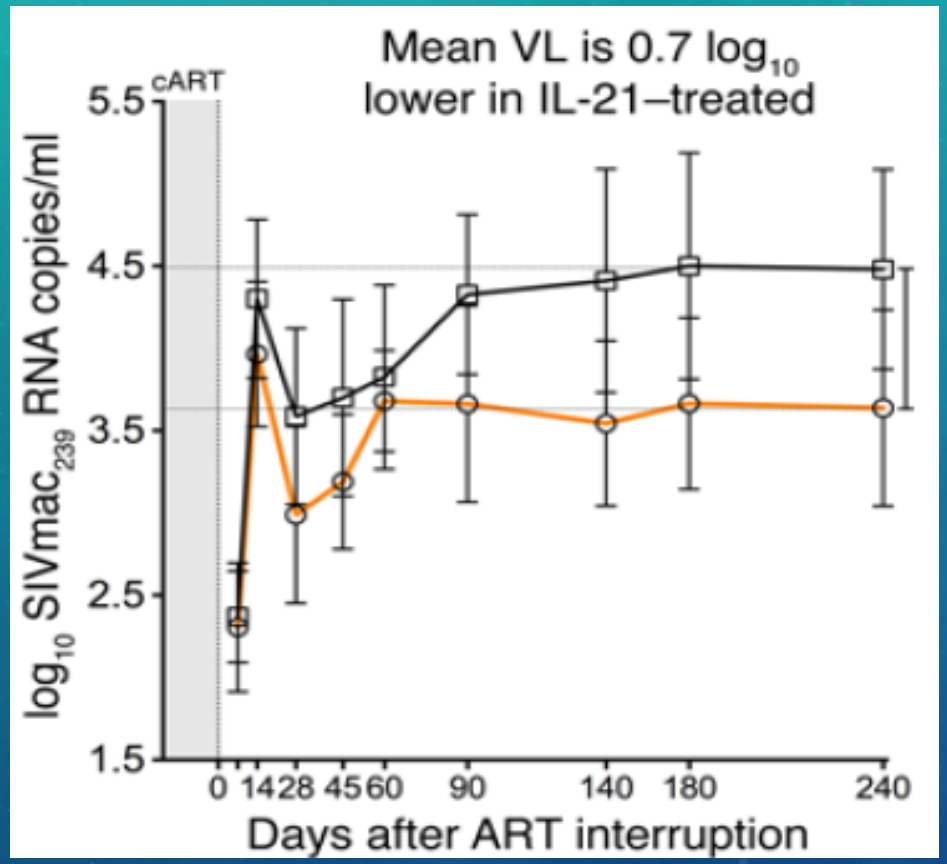


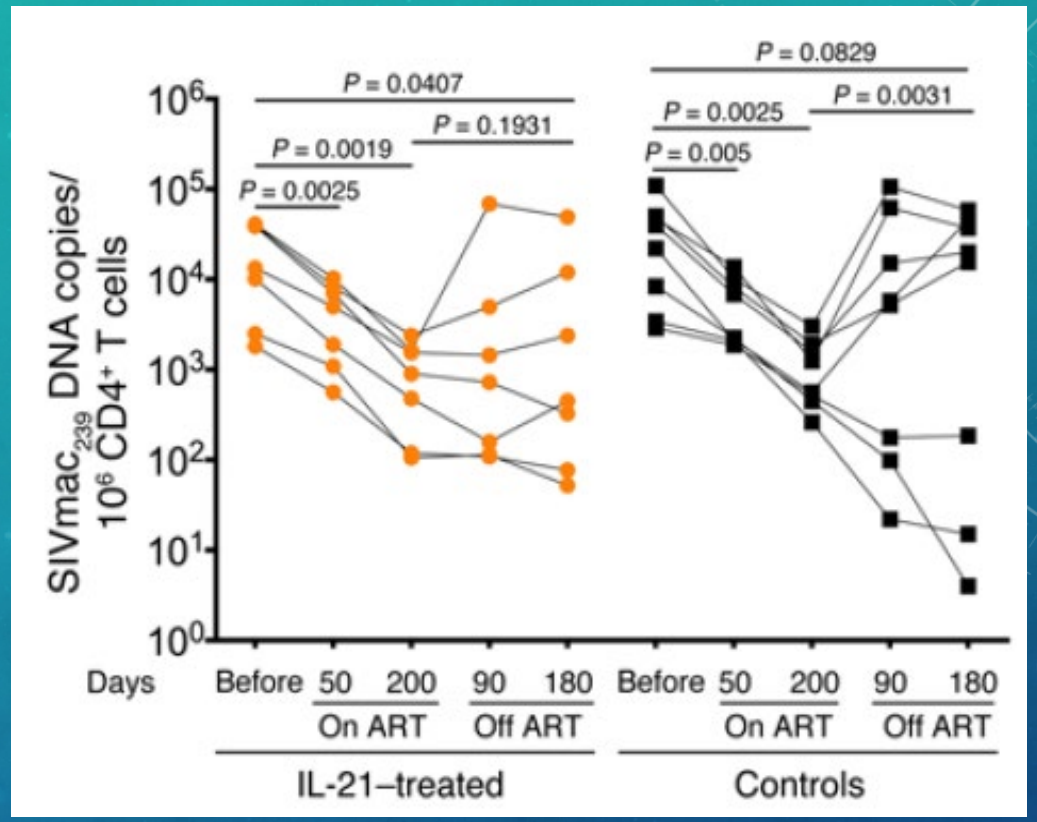
Micci et al. JCI 2015

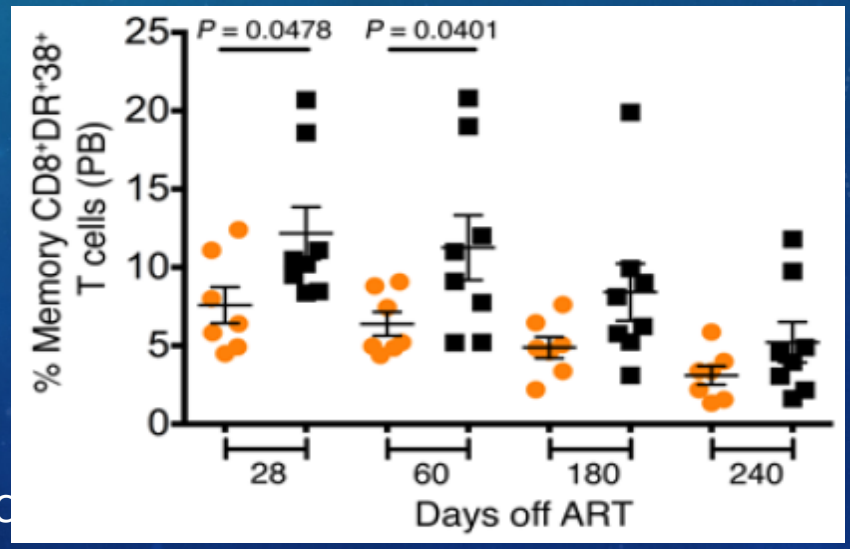

Persistent reservoir
Inflammation
Persistent reservoir
Inflammation

IL-21 in the course of cART limits inflammation and HIV persistence

However,

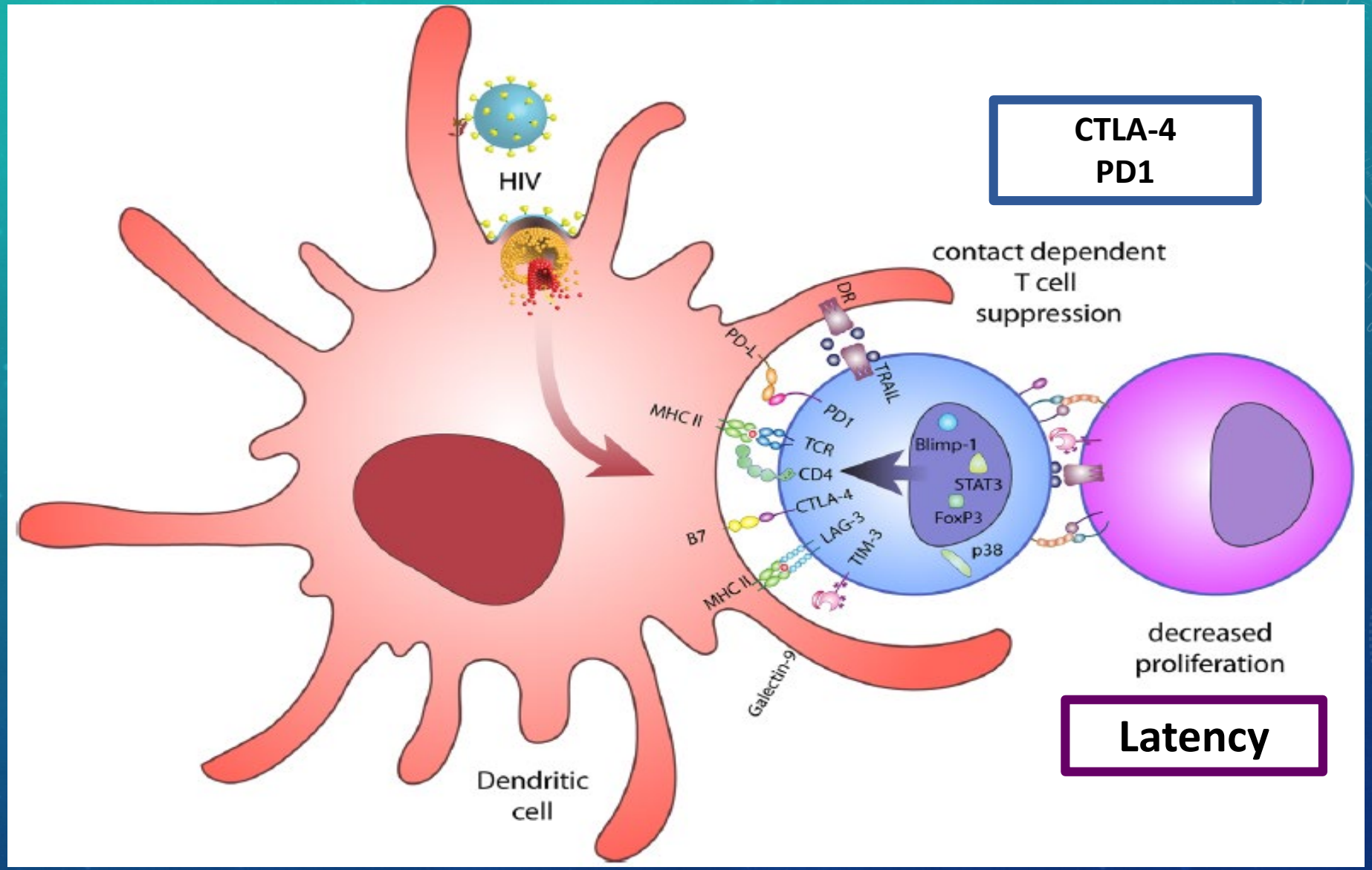






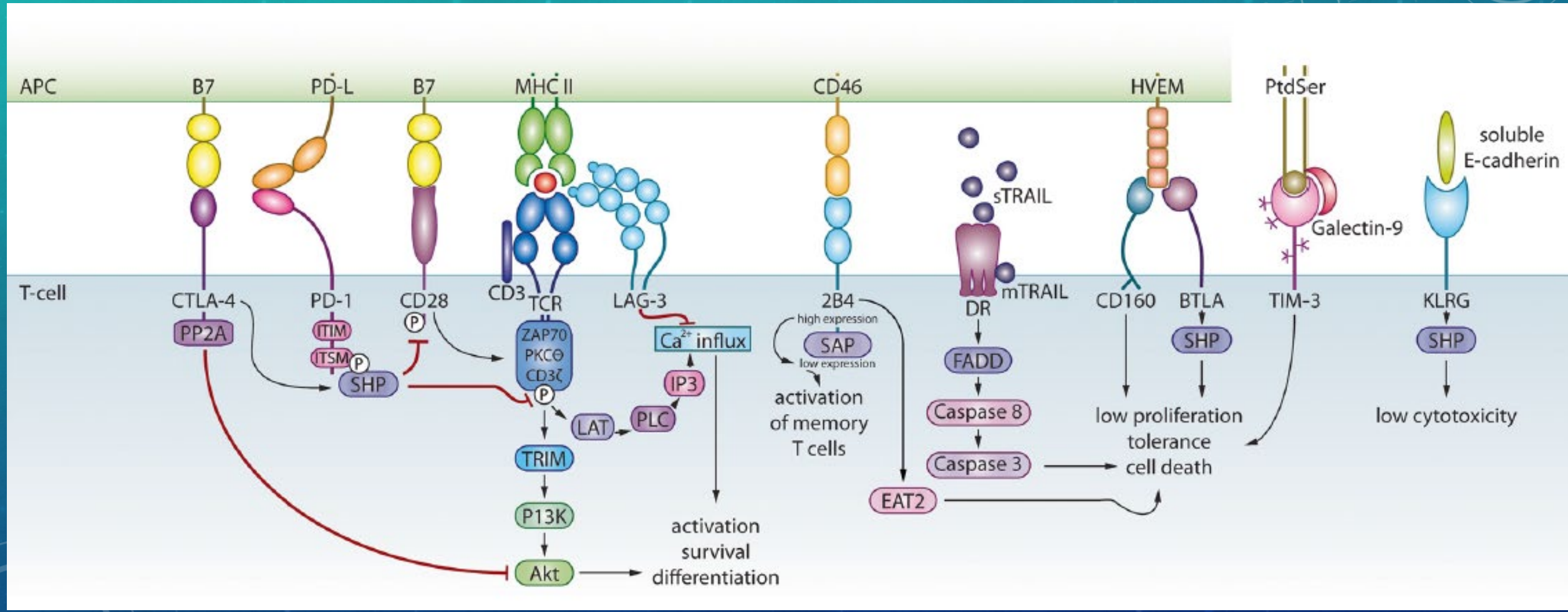
Micci et al. JC

CO-INHIBITORS RECEPTORS AND HIV

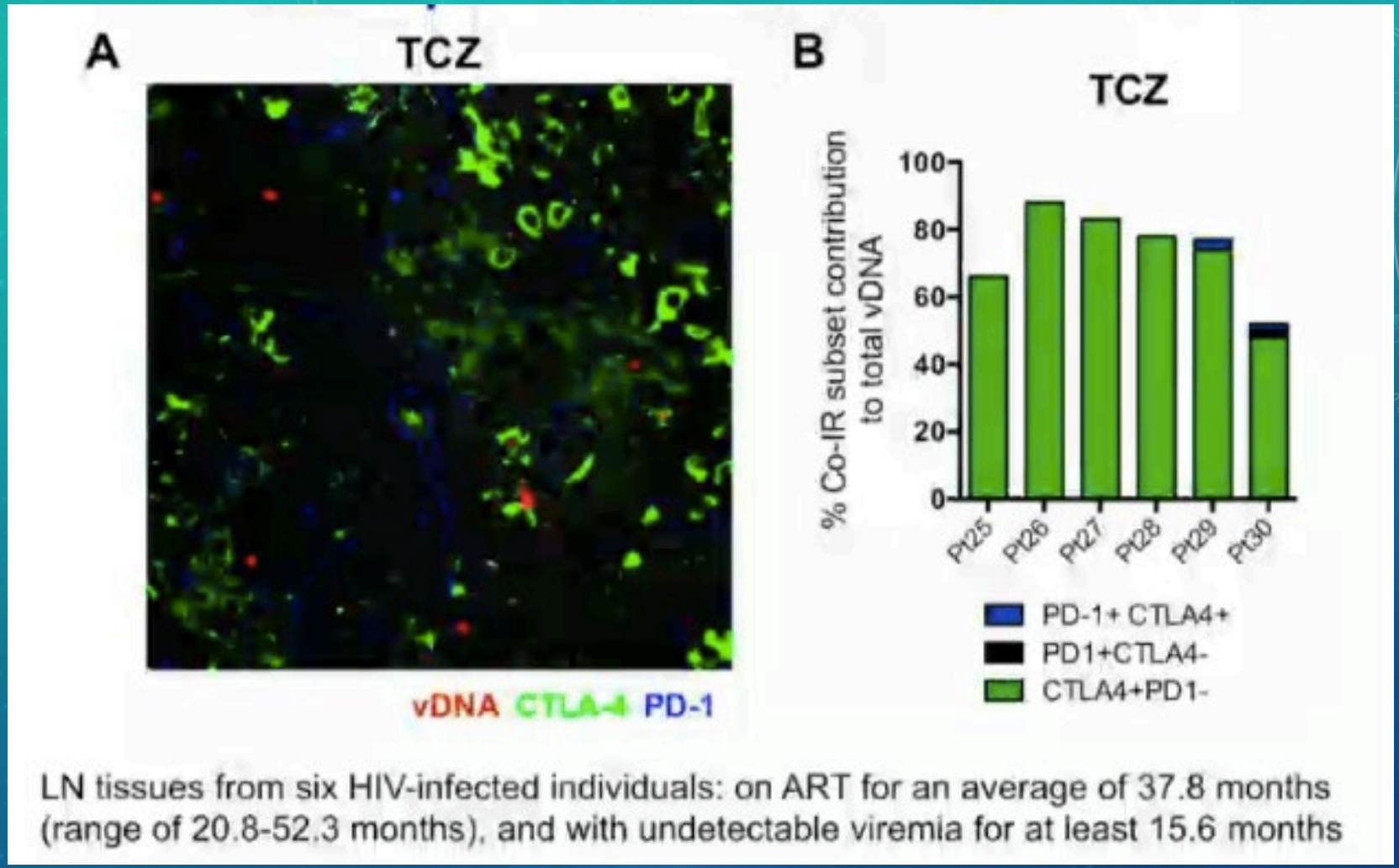


Larrson et al. Retrovirology 2013

INHIBITORY SIGNALS AT APC/T-CELL JUNCTIONS RESULTING IN T-CELL INHIBITION IN HIV



Larrson et al. Retrovirology 2013



LN tissues from six HIV-infected individuals: on ART for an average of 37.8 months (range of 20.8-52.3 months), and with undetectable viremia for at least 15.6 months

CTLA-4-pos PD-1-neg are Treg critical for viral persistence and should be target of cure strategies

Paiardini CROI 2017

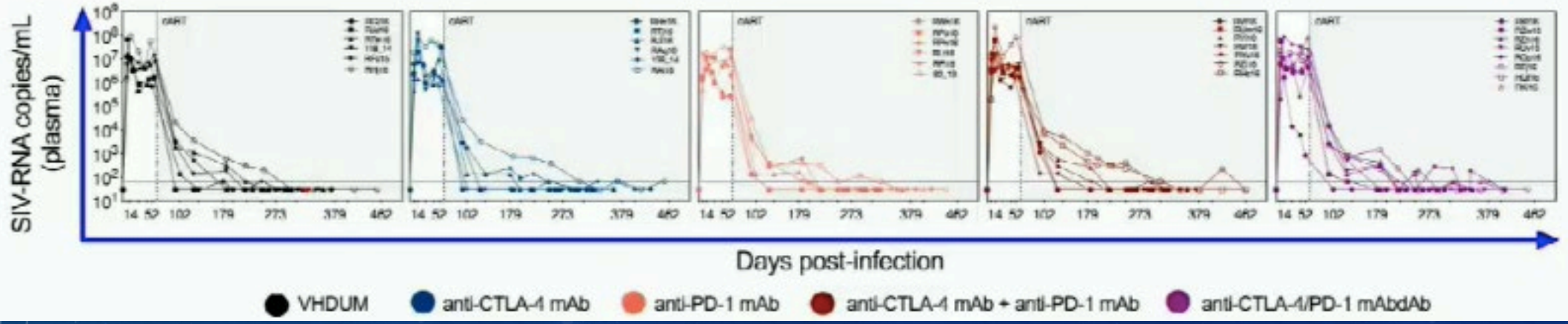
ANY EFFECT FOR CTLA-4/PD-1 BLOCKADE ON SIV PERSISTENCE DURING ART AND AFTER ART STOP?

Paiardini CROI 2019

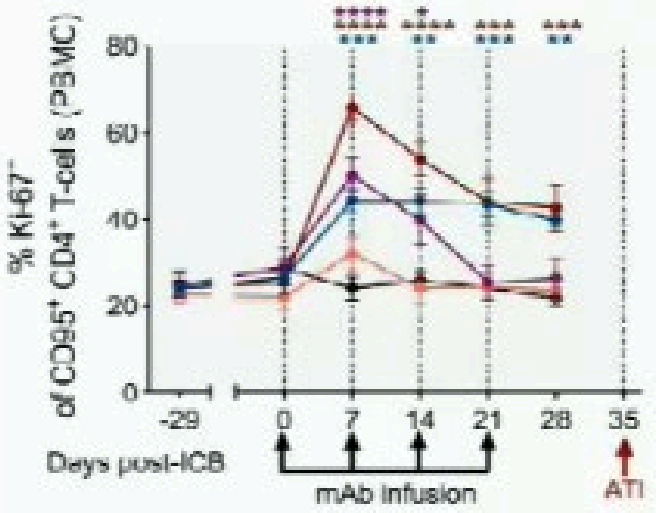
high barrier for cure (to mimic issues pertaining to treatment in PLWH):

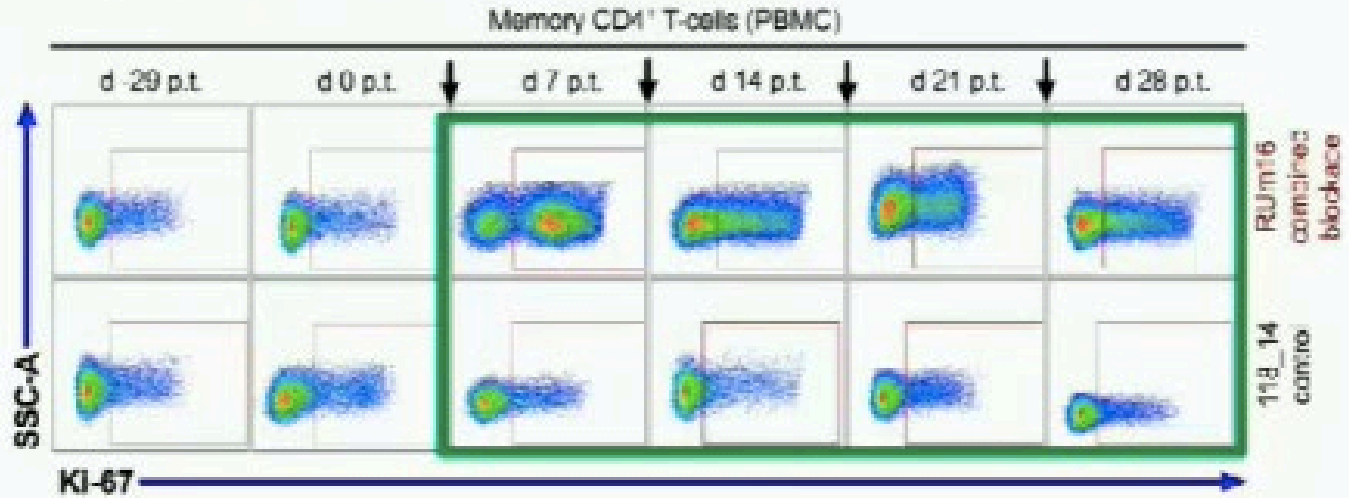
- ART initiation at 60 days p.i., with complete seeding of the viral reservoir and development of T-cell exhaustion

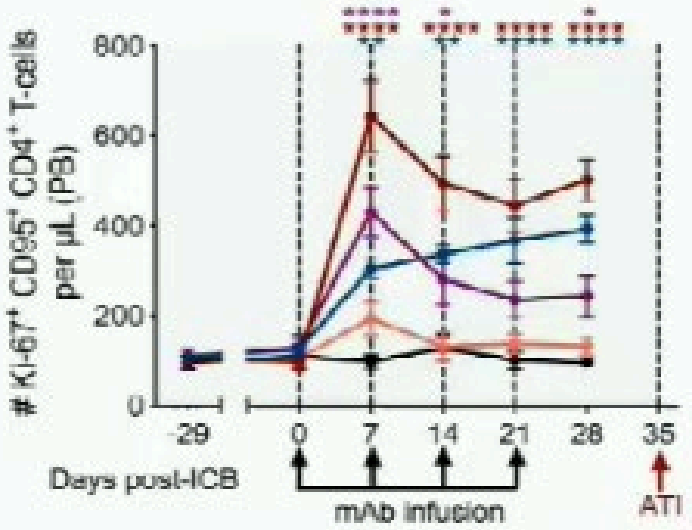
- ICB conducted in the context of sustained aviremia during long-term (> 1 year) ART

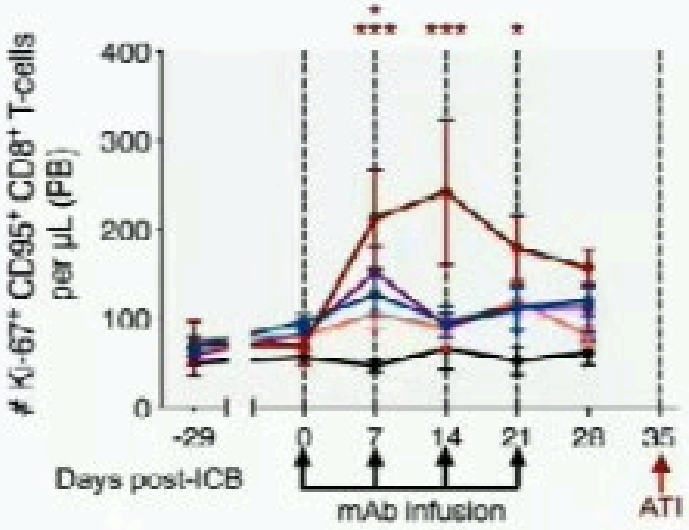


Combined PD-1 and CTLA-4 blockade expands cycling memory CD4⁺ and CD8⁺ T-cells



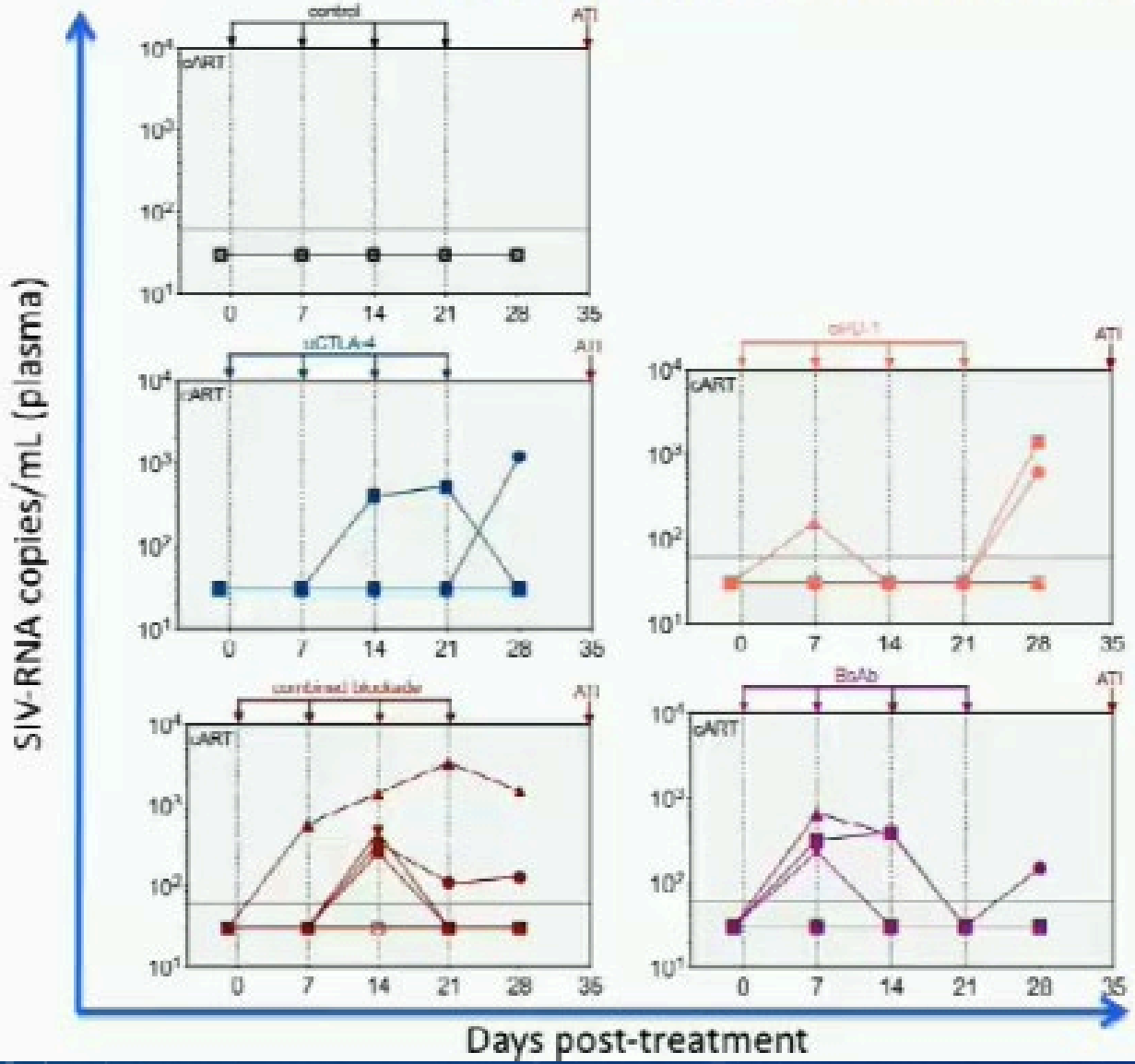


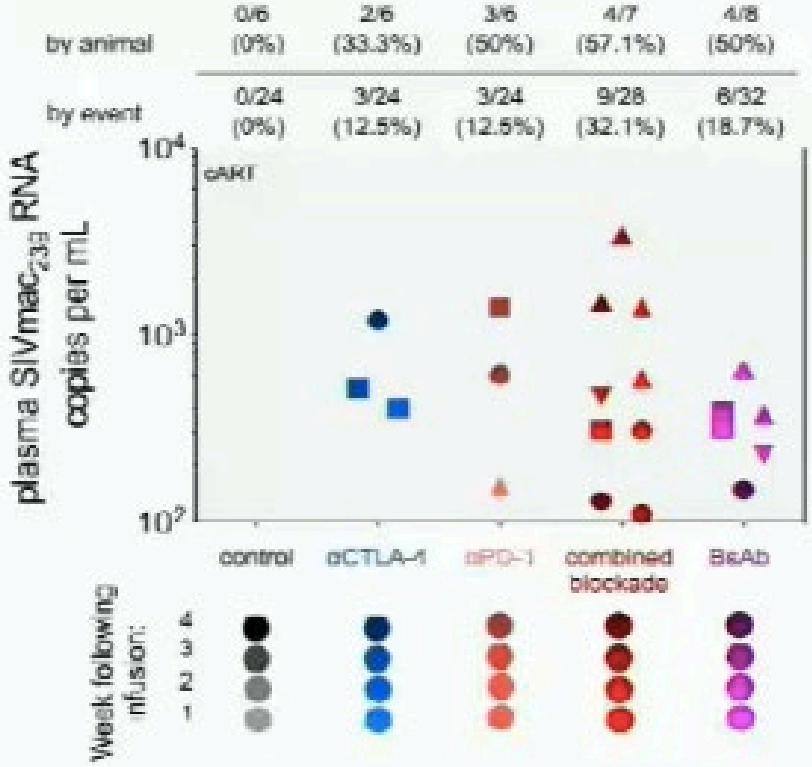




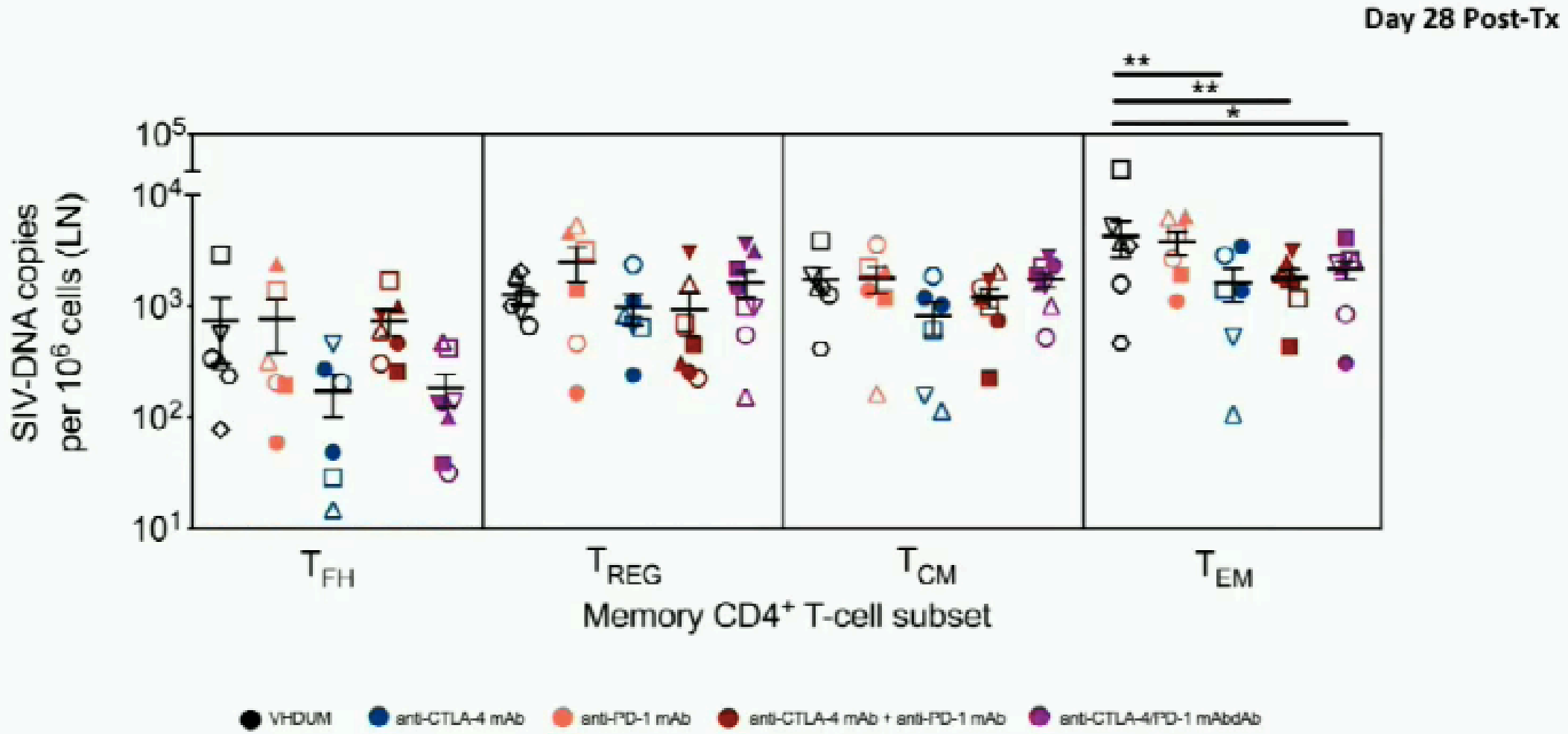
VHDUM · anti-CTLA-4 mAb · anti-PD-1 mAb · anti-CTLA-4 mAb + anti-PD-1 mAb · anti-CTLA-4/PD-1 mAbdAb

Combined PD-1 and CTLA-4 blockade enhances viral reactivation in ART-suppressed RMs

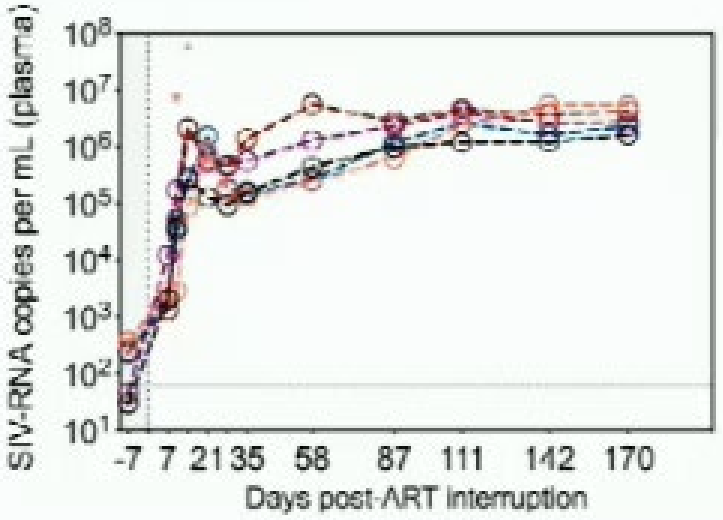


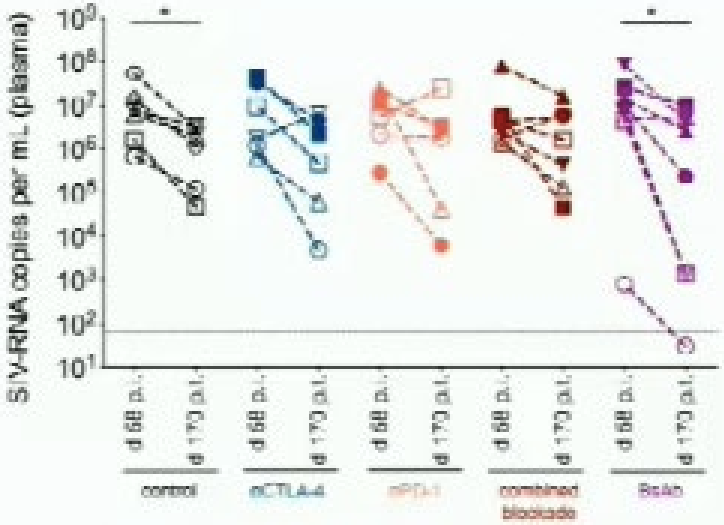


Combined blockade reduces SIV-DNA content in LN CD4+ T_{EM} cells



VIRAL REBOUND POST-TREATMENT INTERRUPTION



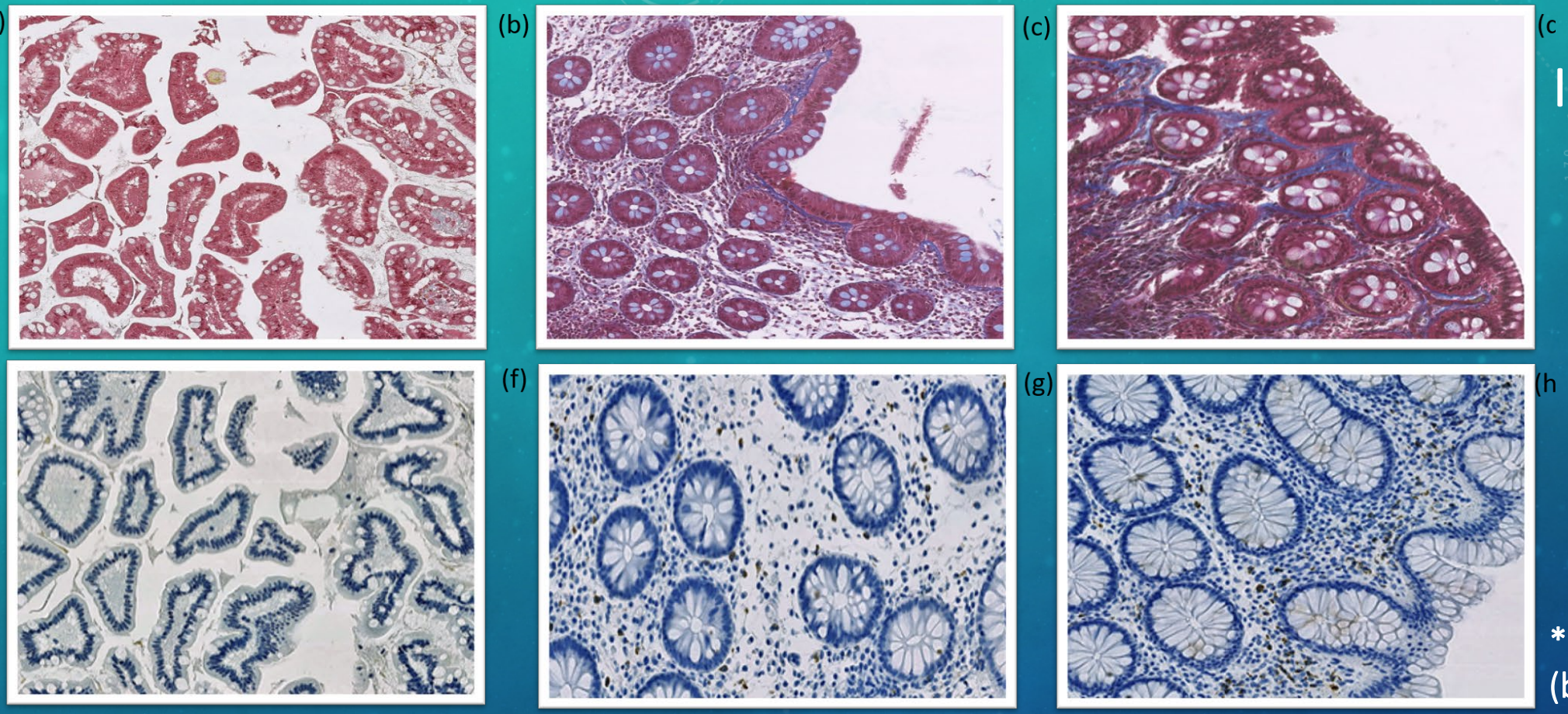


- No "functionally cured" animals
- No significant reduction in set point relative to controls

CONTROLS

ACUTE INFECTION

CHRONIC INFECTION

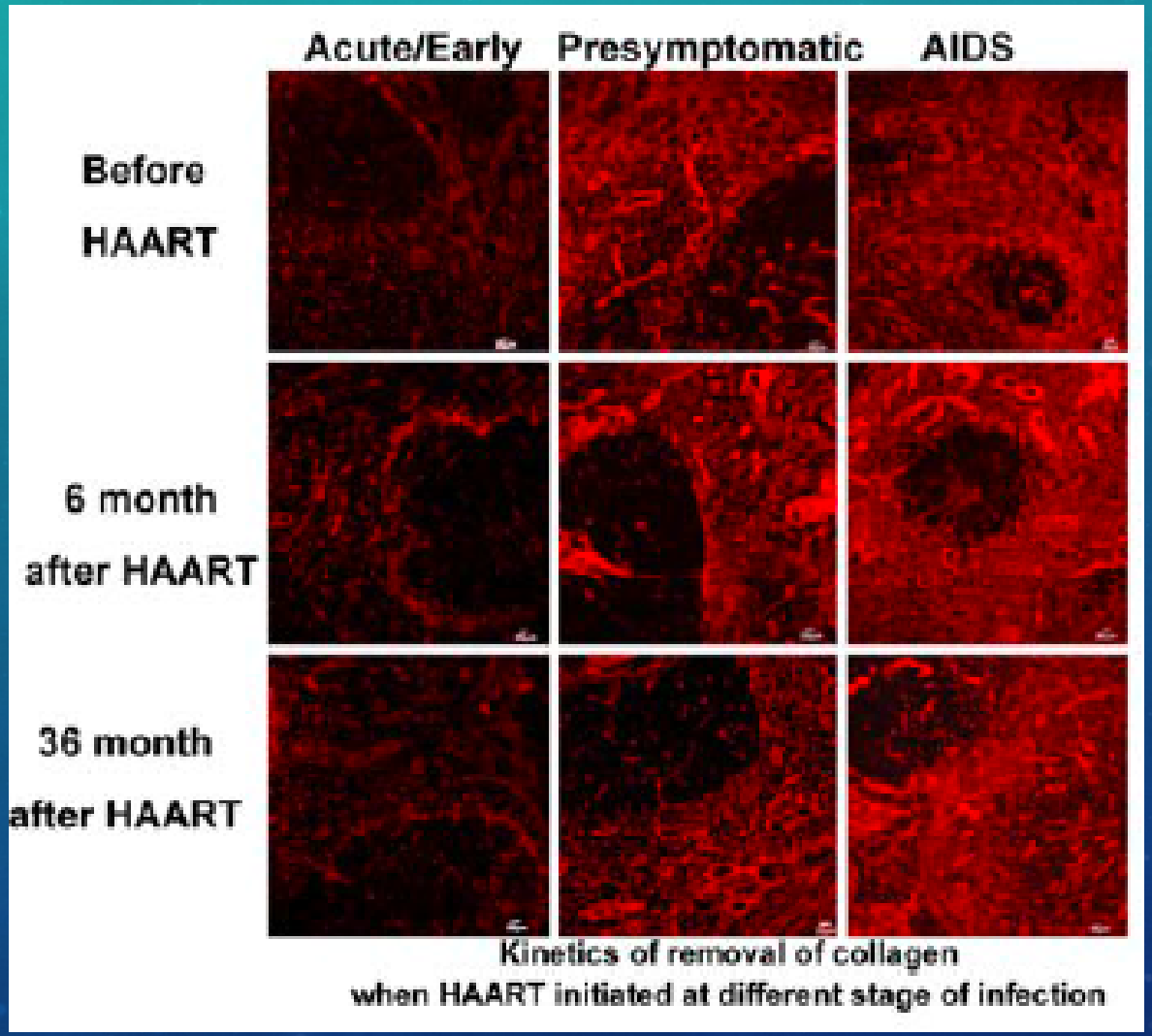


GUT INFLAMMATION * AND FIBROSIS** IS ALREADY DETECTED IN ACUTE HIV INFECTION

*Neutrophil infiltration (brown) ; **collagen (red)

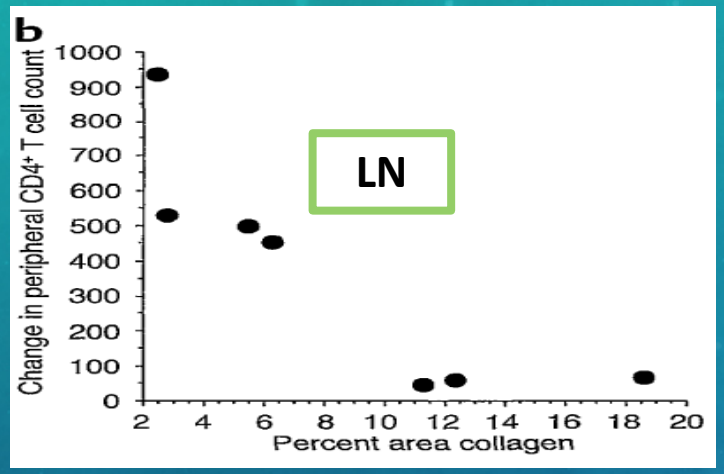
Cannizzo et al. CROI 2018

COLLAGEN DEPOSITION IS NOT RECOVERED BY CART

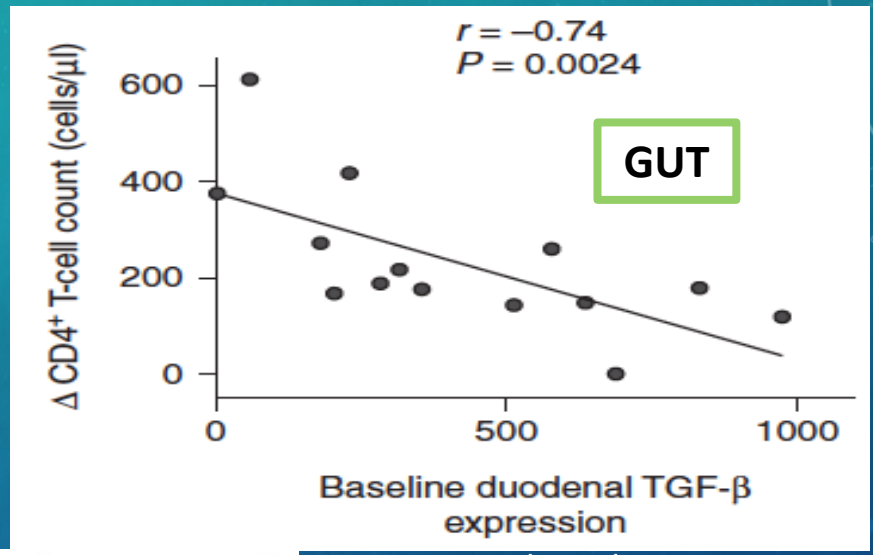


Zeng et al. PlosPathogens 2012

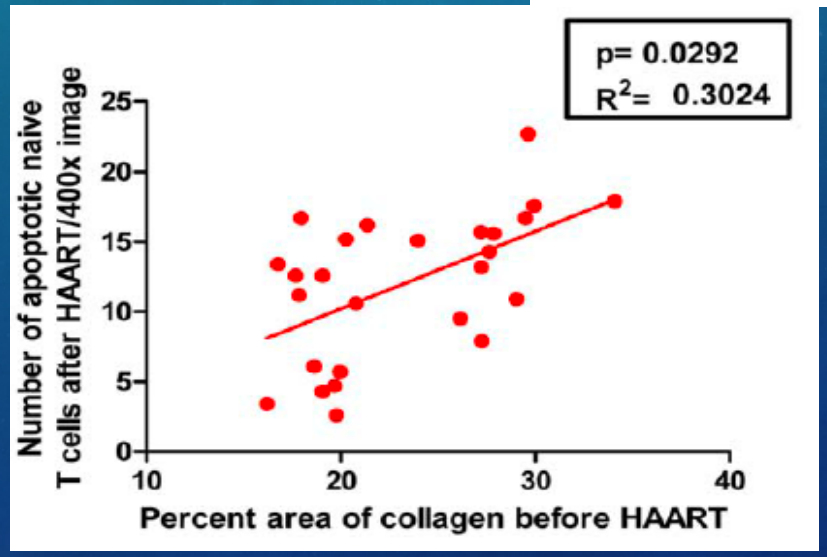
COLLAGEN DEPOSITION IN LYMPHOID TISSUES BEFORE CART SUBSTANTIALLY IMPACTS THE DYNAMICS OF T-LYMPHOCYTE RECONSTITUTION



Schaker et al. JID 2002



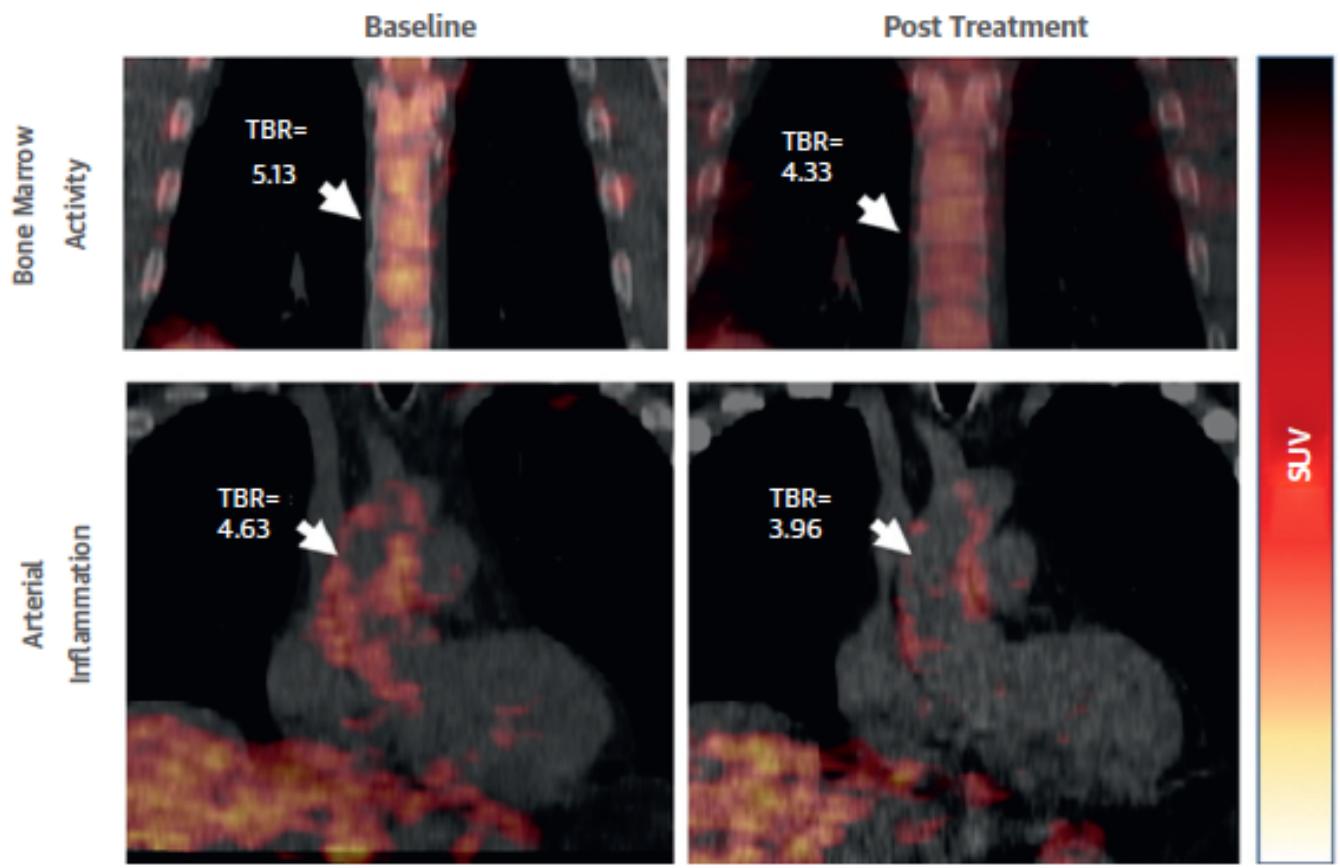
Asmuth et al. AIDS 2015



Zeng et al. PlosPathogens 2012

CANAKINUMAB: IL1-BETA INHIBITION

FIGURE 1 Fluorodeoxyglucose-Positron-Emission Tomography/Computed Tomography Before and After Interleukin-1β Inhibition With Canakinumab



A single dose of canakinumab significantly lowered aortic activity (measure of arterial inflammation) and bone marrow metabolism (measure of leukopoietic tissue activity) as assessed using fluorodeoxyglucose-positron-emission tomography/computed tomography (FDG-PET/CT). Higher activity shown in **yellow/red**. IL = interleukin; TBR = target-to-background ratio.

Hsue et al. JAAC 2018

FUNCTIONAL CURE – WHAT DO WE NEED? (AND WHERE HAVE WE FAILED?)

- Low viral burden
- Low inflammation
- Sustained host responses, that are primed, reside in tissues, target susceptible epitopes

3. ENHANCING IMMUNITY

Therapeutic T-cell HIV-1 vaccines and HIV reservoir

ERAMUNE 02	ART intensification (raltegravir or maraviroc) ± immunomodulation (DNA + HIV-rAd5 vaccine) did not significantly reduce the HIV DNA reservoir in blood or rectal tissue
RISVAC 03	MVA-B vaccination increased Gag- and Env-gp120-specific T-cell responses but had only marginal impact on VL rebound after cART interruption
ACTG A5197	rAd5 HIV-1 Gag vaccine showed positive correlation between Gag-specific cells and lower viral rebound during treatment interruption, although the effect decreased over time
NCT00659789	Vacc-4x, a p24Gag HIV-1 vaccine, lowered VL but did not affect the proportion of participants resuming cART before end of study or change in CD4 counts during treatment interruption
NCT00751595	HIV-1 Tat protein was safe, well tolerated and induced anti-Tat Abs in most patients. Vaccination promoted a durable and significant restoration of T, B, NK cells, and CD4+ and CD8+ central memory subsets. A significant reduction of blood proviral DNA was seen after Week 72
HVTN 090	rVSV vaccine recipients became seropositive for VSV after two vaccinations. Gag-specific T-cell responses were detected in 63% of participants by interferon-γ enzyme-linked immunospot at the highest dose postboost

No impact on HIV reservoir

Do we need to reverse latency?

"Shock and kill strategies"

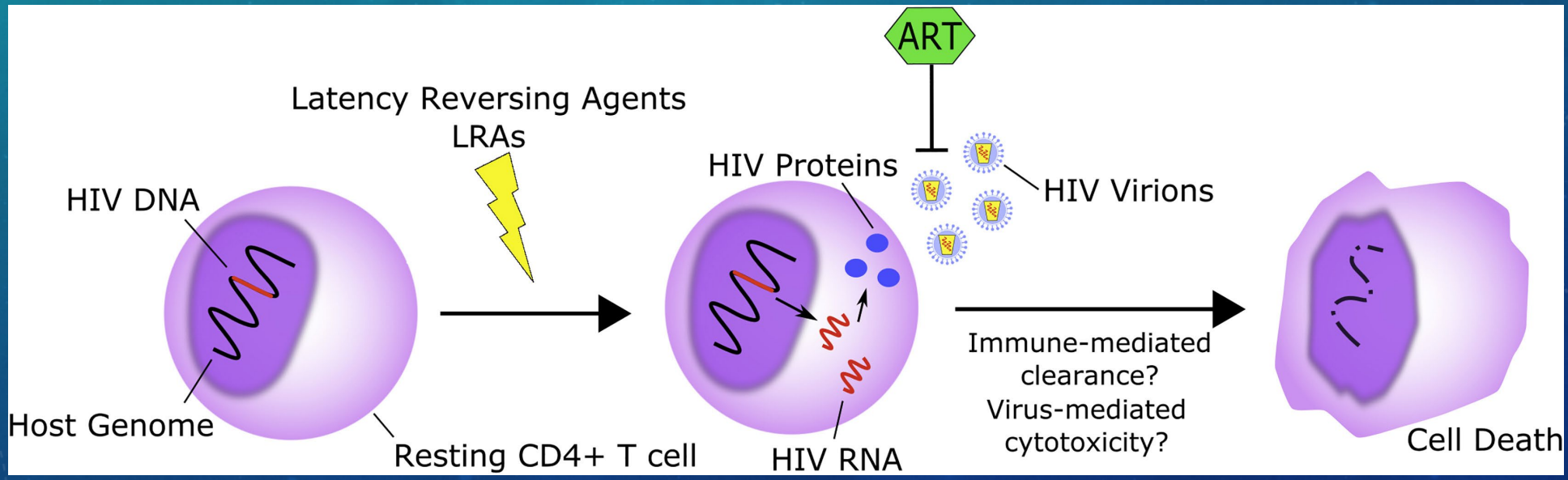


Table 1. Clinical Trials of Latency-Reversing Agents

	Drug Dosing (doses)	HIV-1 Transcription (fold > baseline)	Plasma HIV-1	Post Dosing Viral Effect	T cell Activation	Reservoir Size	Refs
Vorinostat							
Archin *et al.* (2012)	400 mg (1)	4.8	No change	ND[c]	ND	ND	[14]
Elliott *et al.* (2014)	400 mg daily (14)	2.7	No change	Yes	No change	No change	[18]
Archin *et al.* (2014)	400 mg TIW[c] (22)	1.3	No change	ND	ND	No change	[15]
Panobinostat							
Rasmussen *et al.* (2014)	20 mg TIW (12)	2.9	Increased[a]	Yes	Increased	No change	[17]
Romidepsin							
Sogaard *et al.* (2015)	5 mg/m^2 (3)	3.8	Increased	No	Increased	No change	[19]
Disulfiram							
Spivak *et al.* (2014)	500 mg daily (14)	ND	Increased[b]	Yes	ND	No change	[16]
Elliot *et al.* (2015)	Dose escalation (3)	~2	Increased	Yes	ND	ND	[20]

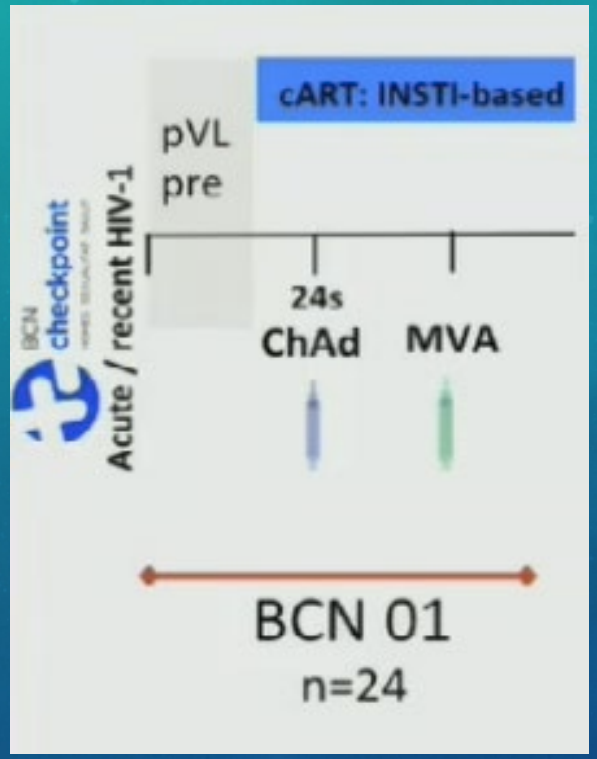
[a]Determined nonquantitatively by nucleic acid testing (NAT) using a transcription-mediated amplification (TMA)-based assay (Procleix Ultrio Plus®, Novartis).
[b]The subgroup of patients with a measurable metabolite had an increase in low-level viremia.
[c]Abbreviations: ND, not determined; TIW, three times a week.

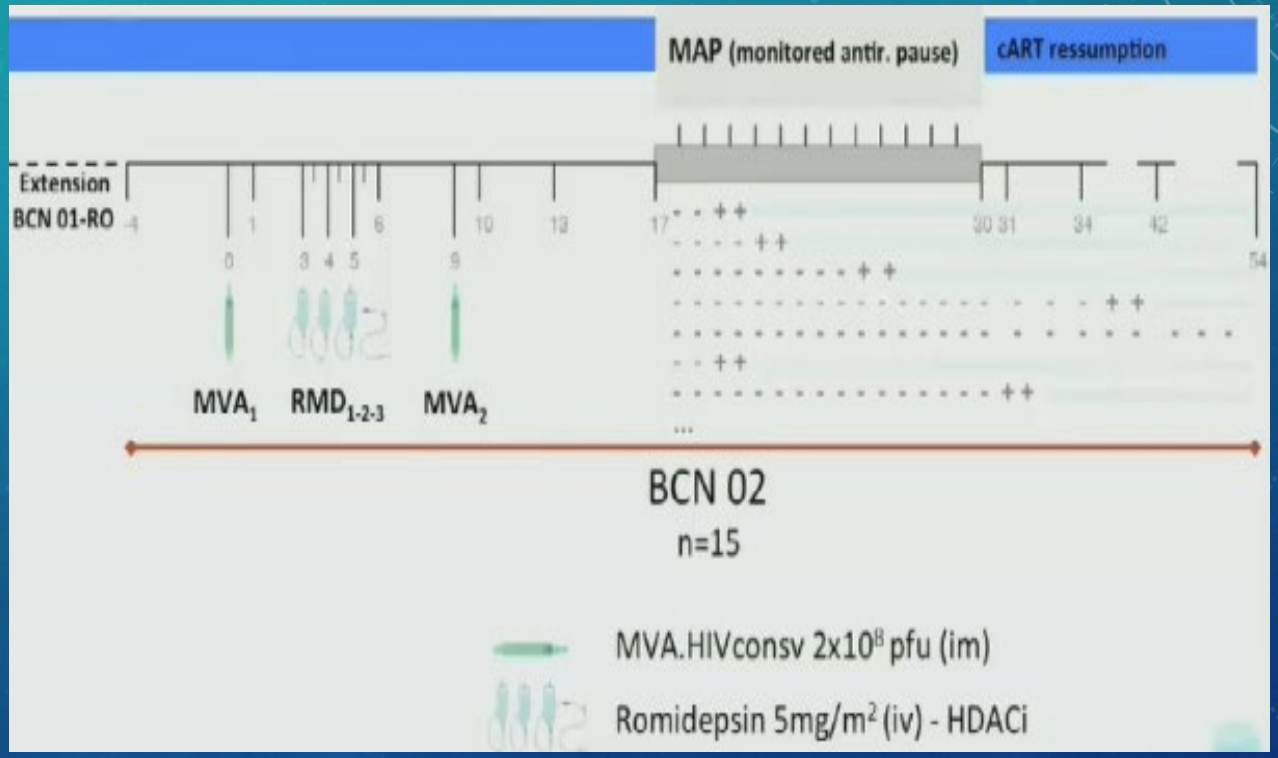
Rasmussen et al. Trends Mircobiol 2016

BCN02- PILOT SINGLE-ARM OPEN LABLE

HIV+ treated within 3 months from acute infection, fully suppressive cART for 3yrs

- **cART interruption 8 weeks after last vaccine boost**
- **cART re-started if VL>2000**



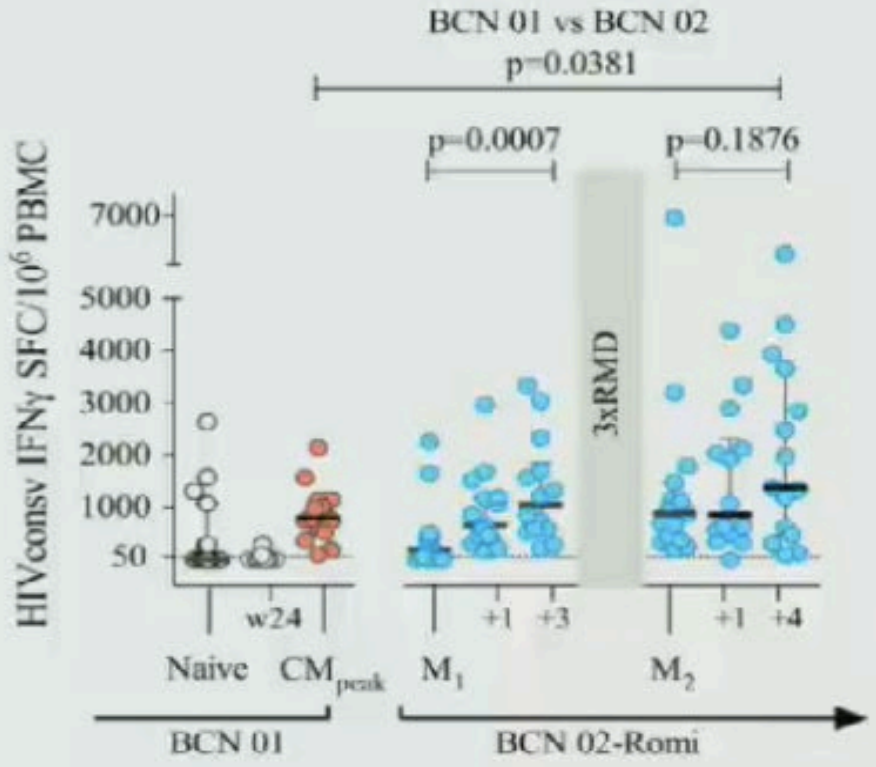


Recombinant modified Ankara-based (MVA-B) HIV-1 vaccine expressing gp120 and fused Gag-Pol-Nef polyprotein of clade B

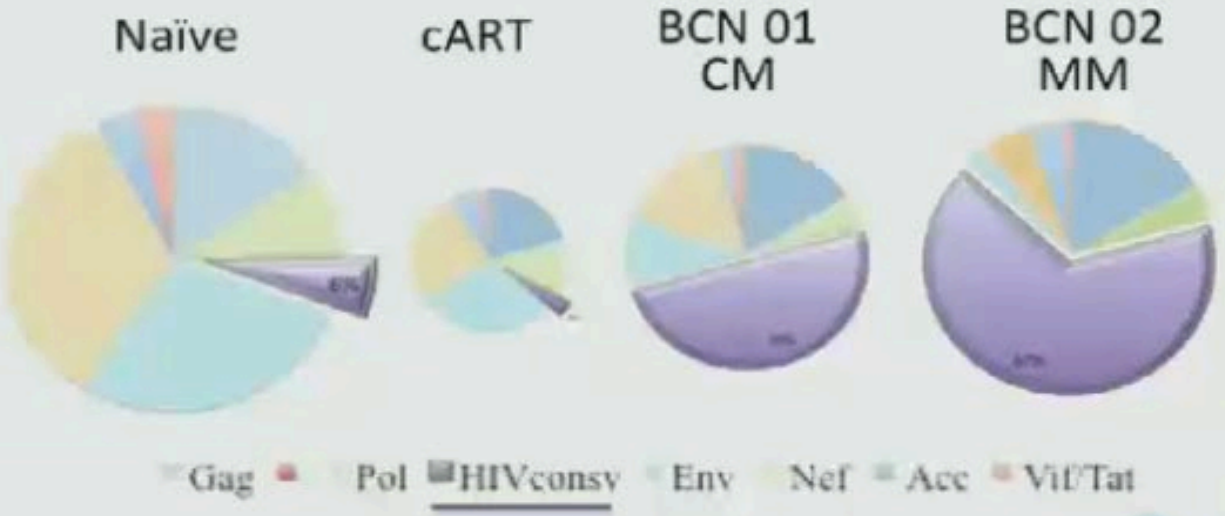
Mothe V #119 LB CROI 2017

n=15

- HIVconsv responses were effectively boosted after >2years from 1st CM



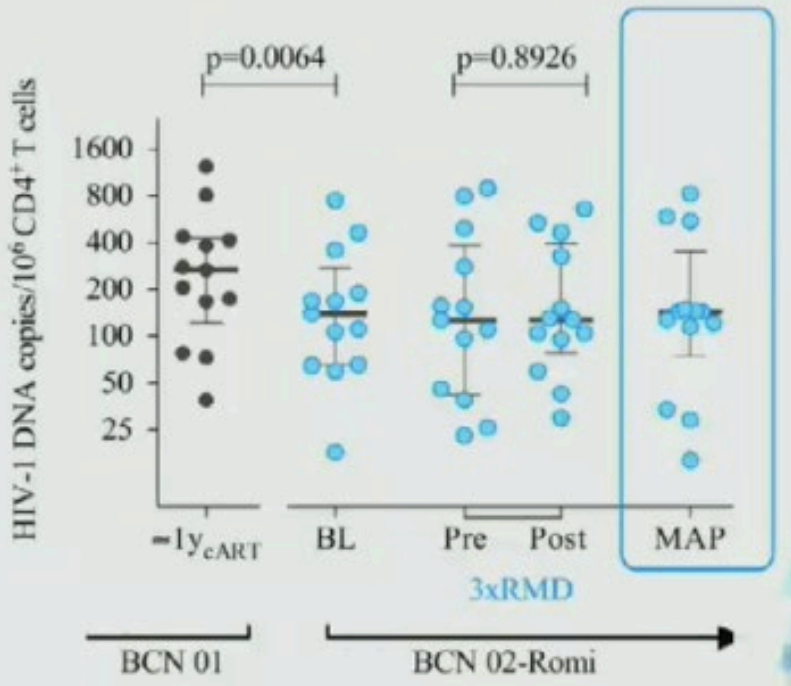
- Change in CTL immunodominance pattern towards conserved regions



Mothe V #119 LB CROI 2017

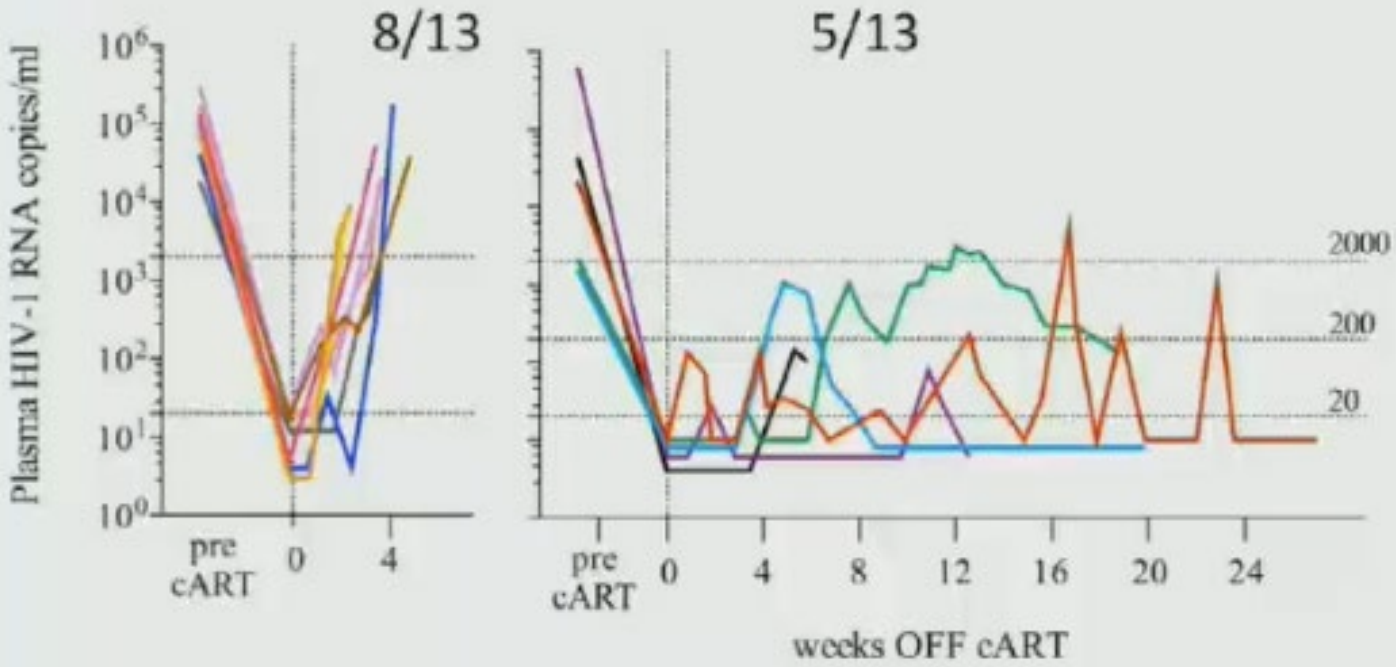
n=13
Feb 15th

- Up to 3years on cART, continues reduction in levels of proviral DNA.
- No further decrease with RMD.
- At MAP, median (range) of
  144 (16-829) copies/10^6 $CD4^+$ T cells
- Detectable in all patients.



Mothe V #119 LB CROI 2017

- 13 participants have interrupted cART to date.



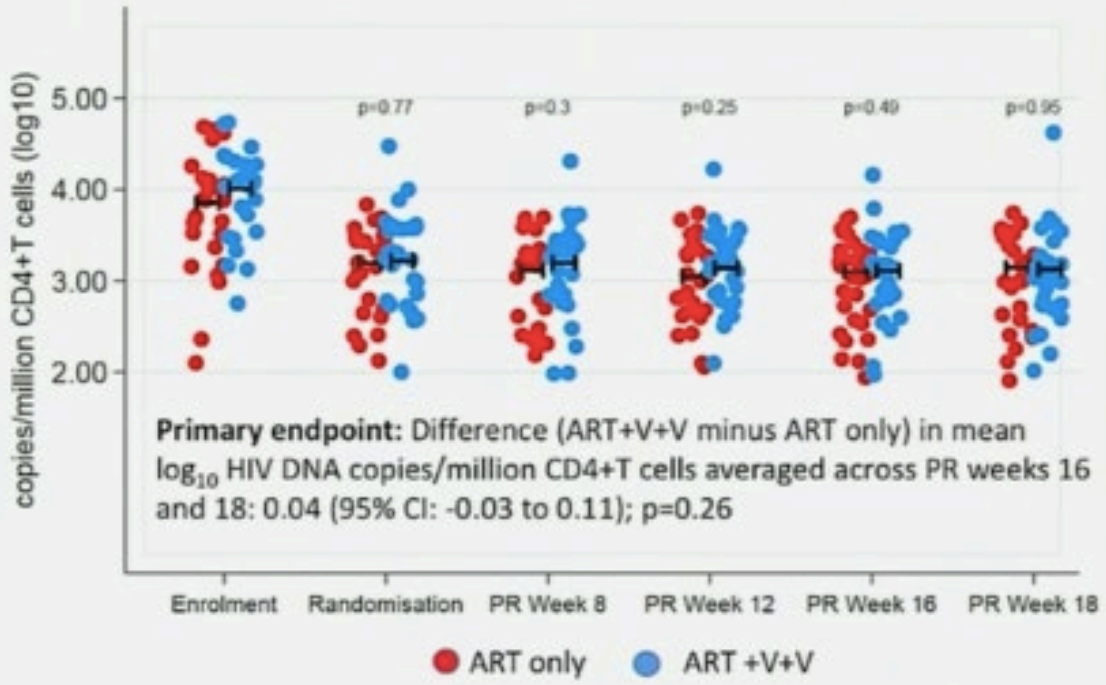
Mothe V #119 LB CROI 2017

Study design: 1:1 randomized control trial

AIDS 2018

A randomised controlled trial comparing the impact of antiretroviral therapy (ART) with a 'Kick-and-Kill' approach to ART alone on HIV reservoirs in individuals with primary HIV infection (PHI); RIVER trial

Sarah Fidler, Imperial College London, United Kingdom



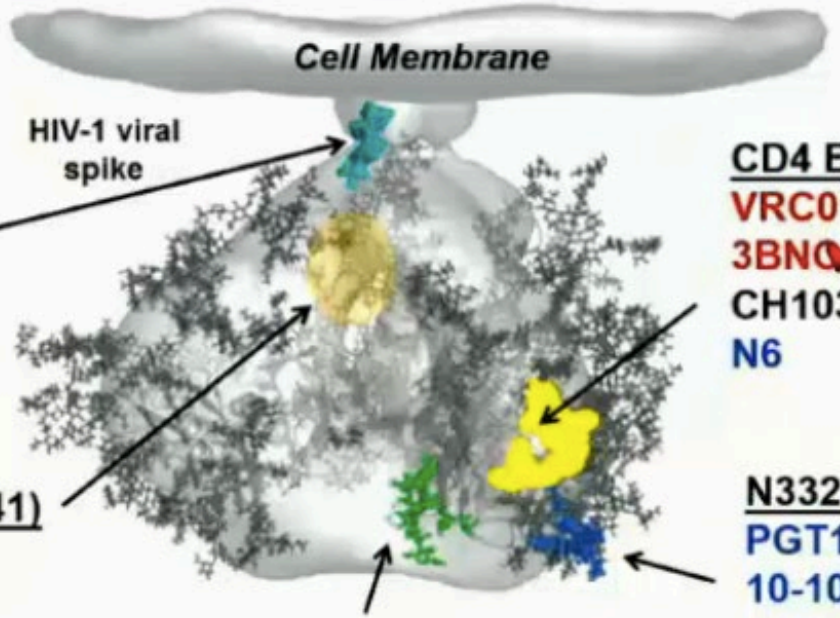
Primary endpoint: Difference (ART+V+V minus ART only) in mean log_{10} HIV DNA copies/million CD4+T cells averaged across PR weeks 16 and 18: 0.04 (95% CI: -0.03 to 0.11); p=0.26

Well over 100 immunotherapeutic studies have been performed in people (most involving therapeutic vaccines) and all have been essentially negative

PHI patients: ART vs ART + Vorinostat + HIV prime boost vaccine

Latency reversal using HDACi may be inadequate or T-cell vaccine epitopes may not recognize the correct viral sequences

Neutralizing Monoclonal Antibodies
plans for clinical trials



gp41 MPER:
2F5, 4E10
10e8

CD4 Binding Site:
VRC01, PG04, CH31
3BNC117, 12A12
CH103, VRC07-523,
N6

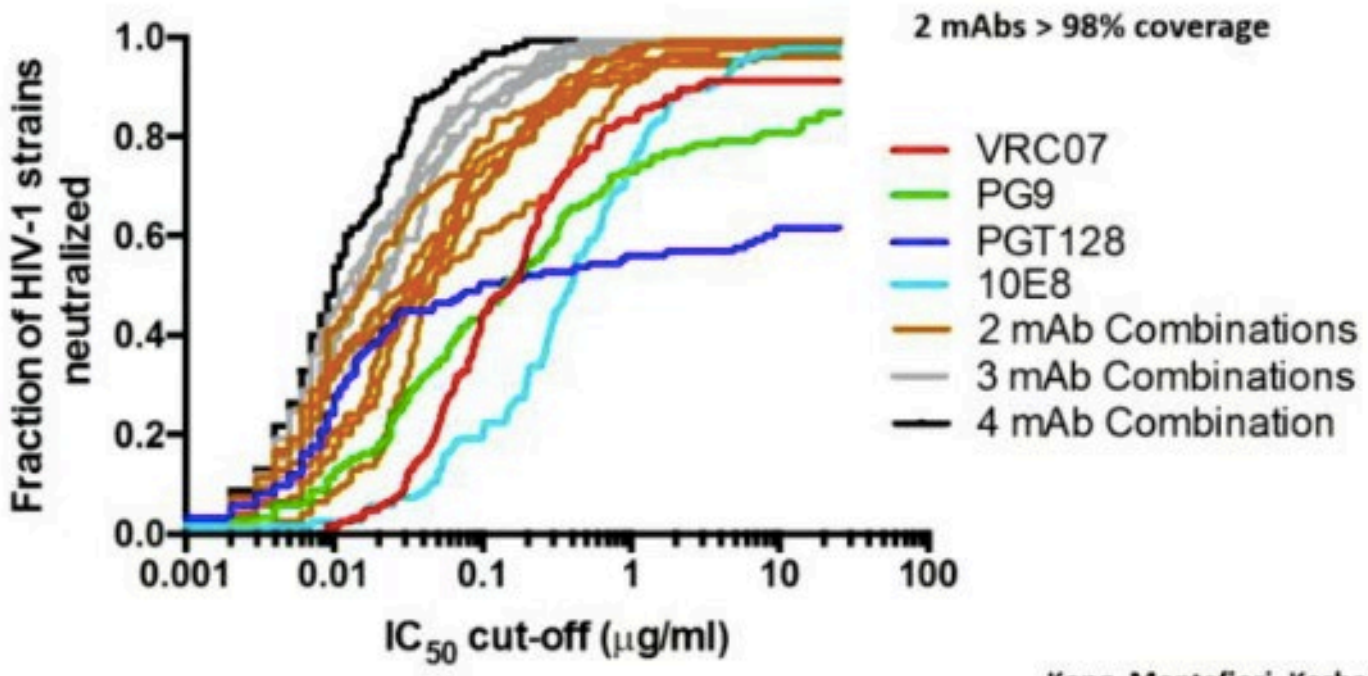
Trimer (gp120/41)
8ANC195
PGT151
35022

N332 Glycan Supersite:
PGT121, PGT128
10-1074

V1V2 Glycan:
PG6, PG16, CH01-04
PGT141-45, PGDM1400
CAP256-VRC26

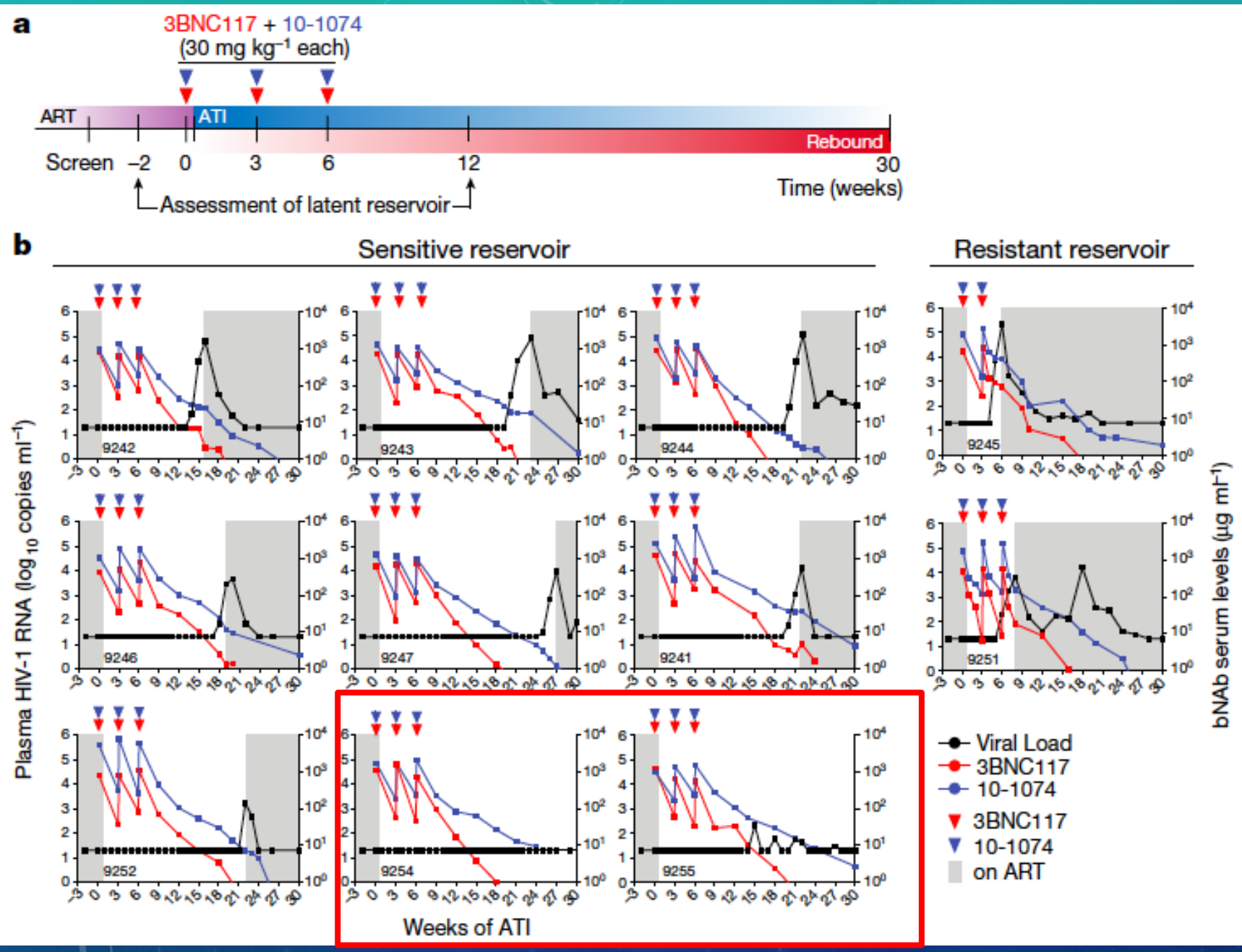
Cryo-EM of viral spike by Subramaniam group. Fit with atomic level structures from Kwong and Wilson groups

Combined Antibodies:
Improved Potency and Breadth



Kong, Montefiori, Korber et al.
J. Virol (2015)

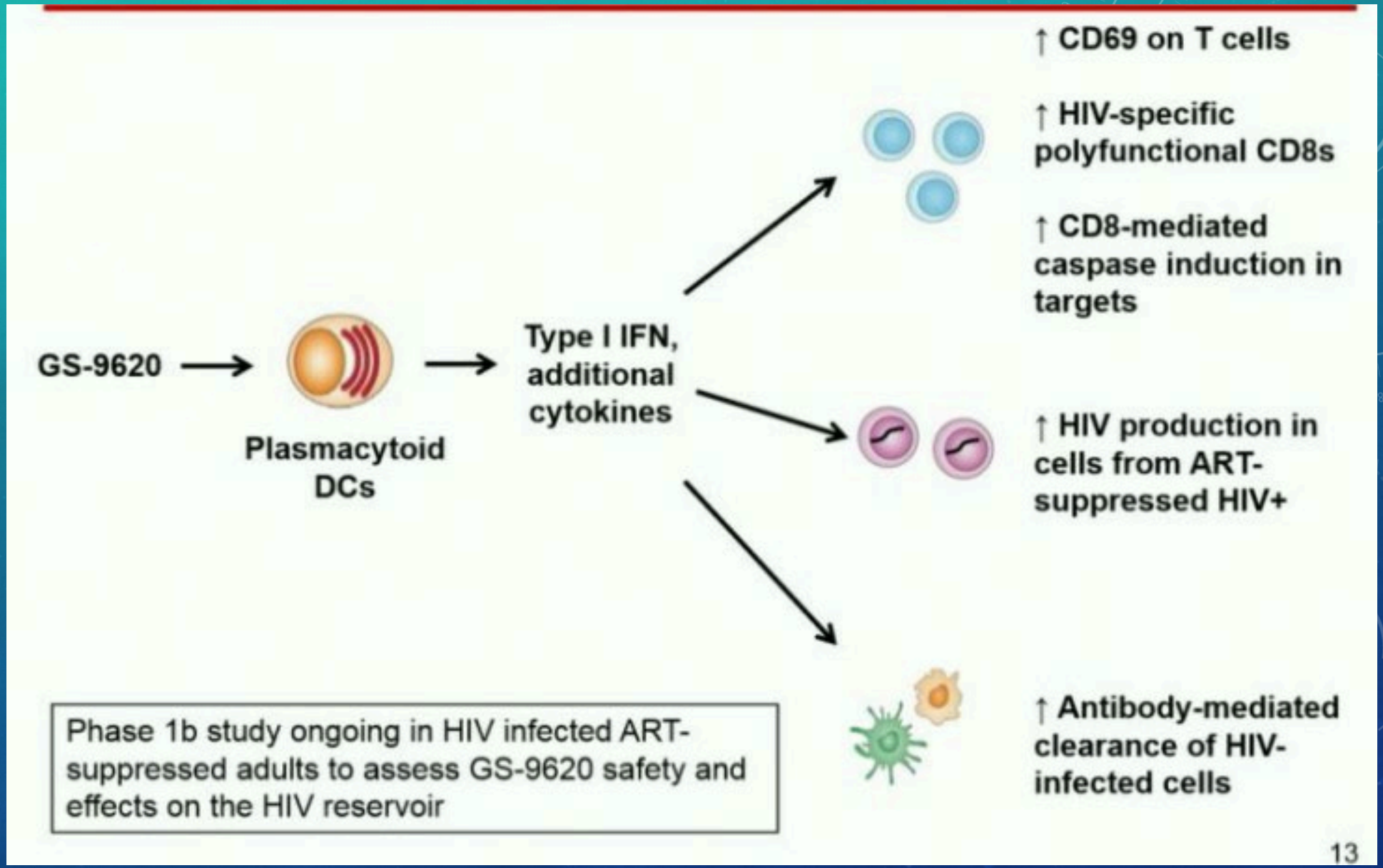
Mendoza et al. Nature 2018



Phase 1b: on virally-effective cART (at least 24 months), CD4>500

2/9 individuals maintained undetectable viremia when bNAbs levels waned – "vaccinal effect"

TLR-7 AGONIST



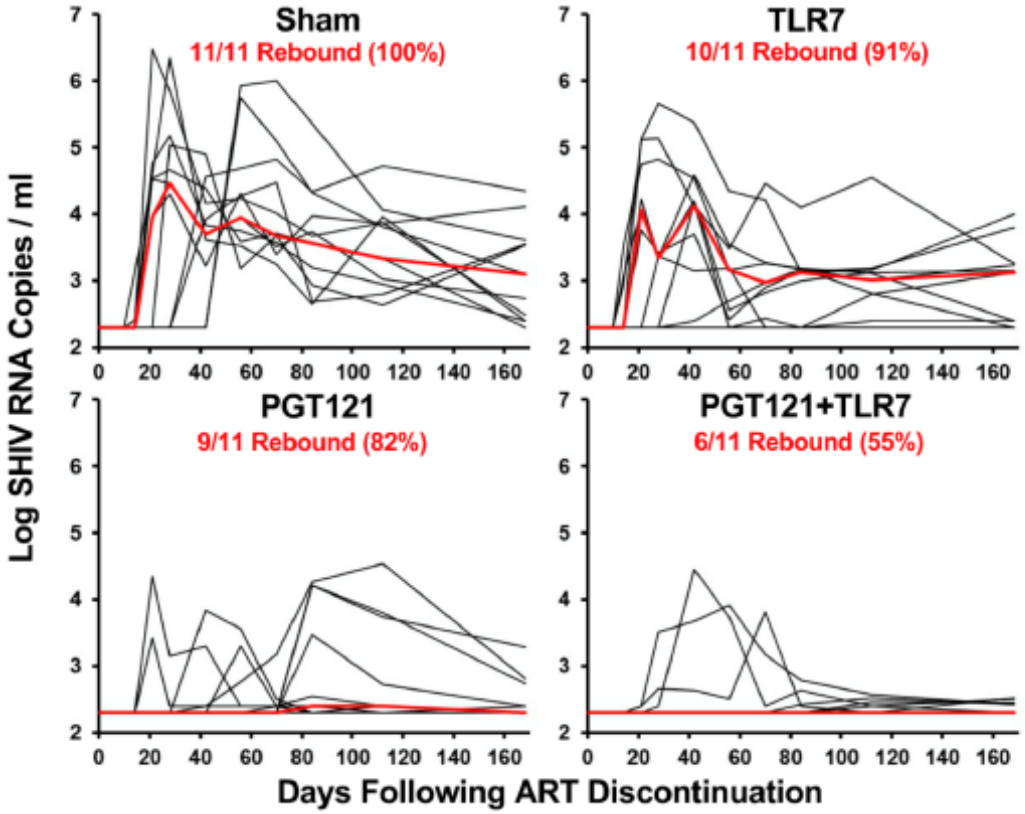
Murry et al. CROI 2017

Monkey: V3 glycan-dependent bNab PGT121 + TLR7 agonist Vesatolimod given during ART initiated during acute infection

nature

Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys

Erica N. Borducchi[1,6], Jinyan Liu[1,6], Joseph P. Nkolola[1,6], Anthony M. Cadena[1,6], Wen-Han Yu[2], Stephanie Fischinger[2], Thomas Broge[2], Peter Abbink[1], Noe B. Mercado[1], Abishek Chandrashekar[2], David Jetton[1], Lauren Peter[1], Katherine McMahan[1], Edward T. Moseley[1], Elena Bekerman[3], Joseph Hesselgesser[3], Wenjun Li[4], Mark G. Lewis[5], Galit Alter[2], Romas Geleziunas[3] & Dan H. Barouch[1,2]*



- SHIV-162P3
- Day 7 ART
- ART maintained for two years
- Reservoir reduced or eliminated during ART
- Mechanism not known: no blips observed with GS-9620 (although CD4+ T cell activation observed)

2. E 3. REDUCING INFLAMMATION , ENHANCING IMMUNITY

Immunotherapy for HIV infection

Two decades of largely failed approaches

- T cells ineffective
  - Target immunodominant (often escaped epitopes)
  - Upregulation of PD-1 and other pathways
- Inflammation and counter-regulatory immunosuppression

WHAT'S IN THE PIPELINE

- Angiotensin II blockers (anti-fibrotic, anti-inflammatory actions): losartan, telmisartan
- mTOR inhibitor inhibitor: Sirolimus, Everolimus
- JAK inhibitors
- Anti-PD-1: Nivolumab, Pembrolizumab
- TLR agonists: TLR7, TLR9

*Dept of Health Sciences- Clinic of Infectious Diseases - Univ of Milan, San Paolo H

Elvira S Cannizzo
Esther Merlini
Camilla Tincati
Giuseppe Ancona
Francesca Bai
Antonella d'Arminio Monforte

***all the patients and staff

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I. C. O. N. A.