

Top 10 of Basic Science

Moderator: Georg Behrens, Germany

Charlotte Charpentier, France
Agnès Libois, Belgium

1st Author	Title of “TOP 8 ARTICLES“	Reference
Lederman MM	A cure for HIV infection: "Not in My Lifetime" or "Just Around the Corner"?	Pathog Immun (1), Spring 2016
Barouch DH	Rapid inflammasome activation following mucosal SIV infection of rhesus monkeys	Cell (165), April 2016
Lorenzo-Redondo R	Persistent HIV-1 replication maintains the tissue reservoir during therapy	Nature (530), February 2016
Gautam R	A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges	Nature (533), May 2016
Scheid JF	HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption	Nature (535), July 2016
Bar KJ	Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption	N Engl J Med, November 2016
Byrareddy SN	Sustained virologic control in SIV+ macaques after antiretroviral and $\alpha 4\beta 7$ antibody therapy	Science (354), October 2016
Borducchi EN	Ad26/MVA Therapeutic Vaccination with TLR7 Stimulation in SIV-Infected Rhesus Monkeys	Nature, November 2016

COMMENTARY

A CURE FOR HIV INFECTION: “NOT IN MY LIFETIME” OR “JUST AROUND THE CORNER”?

AUTHORS

Michael M. Lederman¹, Paula M. Cannon², Judith S. Currier³, Carl H. June⁴, Hans-Peter Kiem⁵, Daniel R. Kuritzkes⁶, Sharon R. Lewin⁷, David M. Margolis⁸, Joseph M. McCune⁹, John W. Mellors¹⁰, Timothy W. Schacker¹¹, Rafick P. Sekaly¹, Pablo Tebas⁴, Bruce D. Walker¹², Daniel C. Douek¹³

- Michael Lederman and Daniel Douek (editors of *Pathogens and Immunity*) ask whether curing HIV is a realistic, scalable objective: overview perspective

Complete HIV eradication will not be simple and may not even be possible

- **Virus quiescence** in host cell genomes → Reservoir, infected cells not easily visible to host defenses.
- HIV-specific CD8: frequency ↓ with ↓ VL with ART and « exhausted » phenotype
- Infected cells broadly distributed in numerous tissues, including **sites** relatively **inaccessible** to host defenses or treatment strategies
- **“Functional cure”**: broadly defined as a state in which ART may be withdrawn without subsequent virus recrudescence

- The “Berlin patient”: only one person cured
- Elite controllers
 - Sustained low levels of plasma viremia in the absence of ART
 - But inflammation and coagulation > in ART-controlled HIV+
 - Progressive CD4 decline occurs
 - ATCG trial ongoing in elite controllers: effect of ART on inflammation and CD4

→ Is the goal of achieving functional cure as defined by elite controller status good enough?

- Current ART: durable control of HIV with once daily well-tolerated single pill
- With early start treatment, sustain normal CD4 and predicted survival similar to that of uninfected persons

→ The bar for a cure strategy is high!

Article

Cell

Rapid Inflammasome Activation following Mucosal SIV Infection of Rhesus Monkeys

Dan H. Barouch, Khader Ghneim, William J. Bosche, Yuan Li, Brian Berkemeier, Michael Hull, Sanghamitra Bhattacharyya, Mark Cameron, Jinyan Liu, Kaitlin Smith, Erica Borducchi, Crystal Cabral, Lauren Peter, Amanda Brinkman, Mayuri Shetty, Hualin Li, Courtney Gittens, Chantelle Baker, Wendeline Wagner, Mark G. Lewis, Arnaud Colantonio, Hyung-Joo Kang, Wenjun Li, Jeffrey D. Lifson, Michael Piatak Jr.8, Rafick-Pierre Sekaly

- **Background**

- The earliest events following mucosal HIV-1 infection, prior to measurable viremia, remain poorly understood

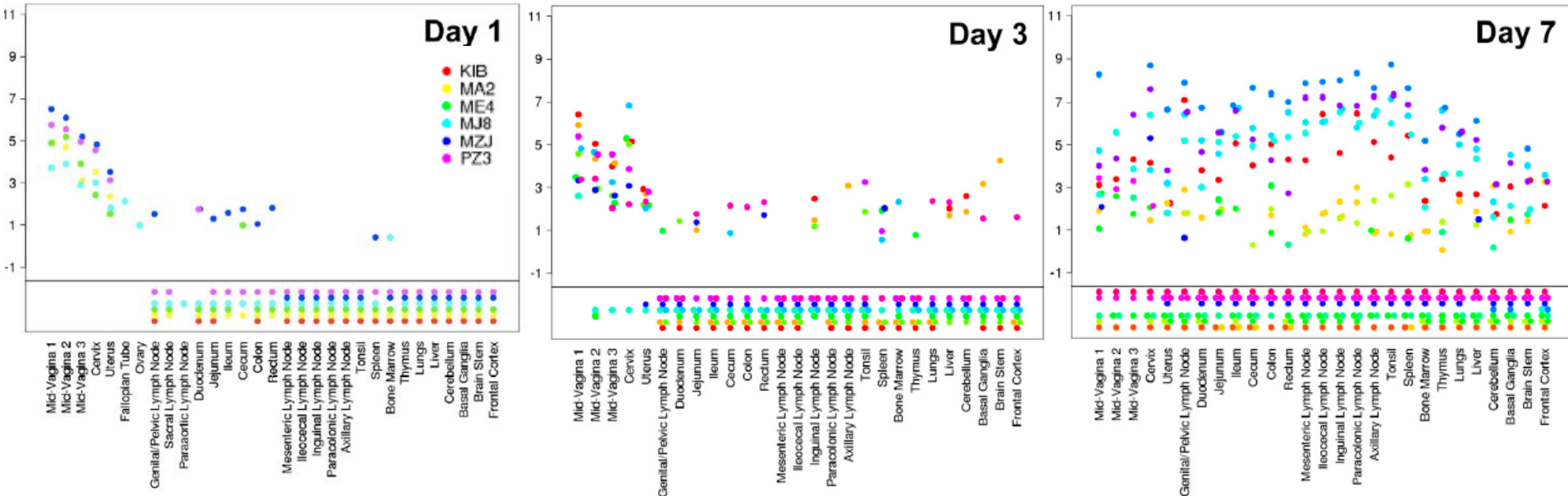
- **Objectives**

- Evaluate the kinetics of virus dissemination to distal tissues
- Evaluate the initial innate and adaptive host immune responses following intra-vaginal SIV infection of rhesus monkeys

- **Material & method**

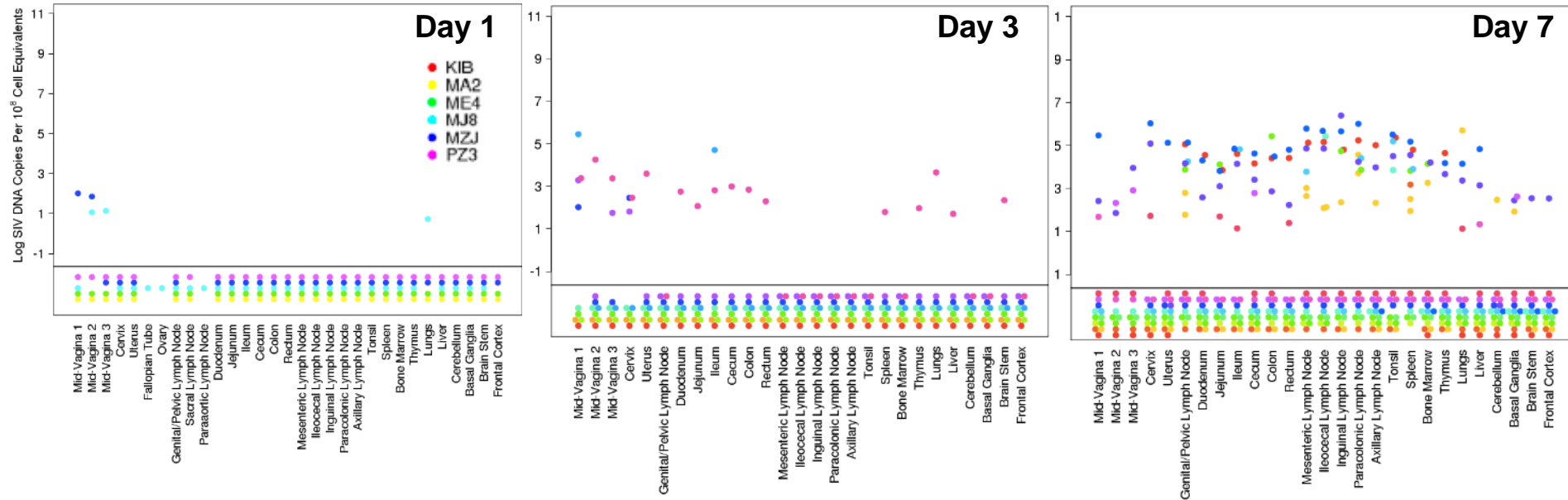
- 44 female rhesus monkeys (*Macaca mulatta*)
- Infection with SIVmac251 (5×10^4 TCID₅₀) by the intravaginal route
- Necropsies on days 0, 1, 3, 7 or 10 following infection (multiple samples of 23-37 tissues)
- SIV RNA and DNA quantification (real time PCR)
- Transcriptomic analysis: Illumina[©] platform

Viral RNA in tissues (\log_{10} SIV RNA c/ 10^6 cells)

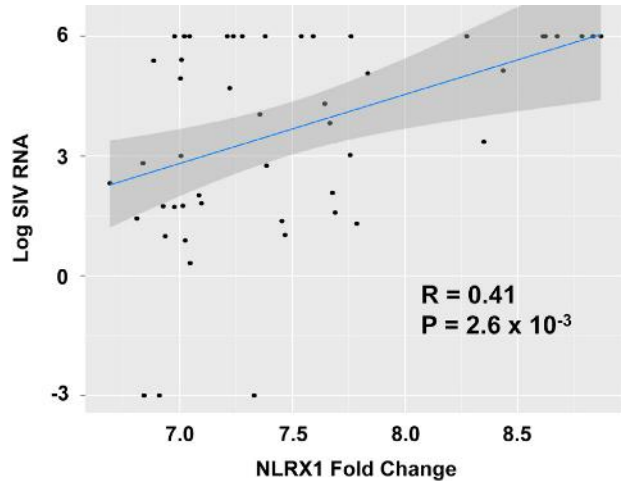


➔ Rapid kinetics of virus dissemination from the site of inoculation to distal tissues

Tissue viral DNA (\log_{10} SIV DNA c/ 10^8 cells)



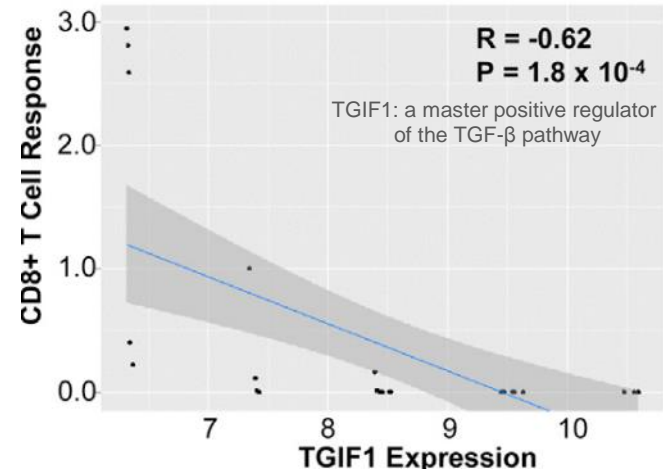
Correlation analysis between NLRX1 expression and viral RNA levels in GALT



- NLRX1 is a negative regulator of the inflammasome pathway that inhibits expression of antiviral ISGs, IRF7, RIG-I, and TLRs
- NLRX1 suppresses antiviral restriction factors

➔ ⚓ Innate immunity

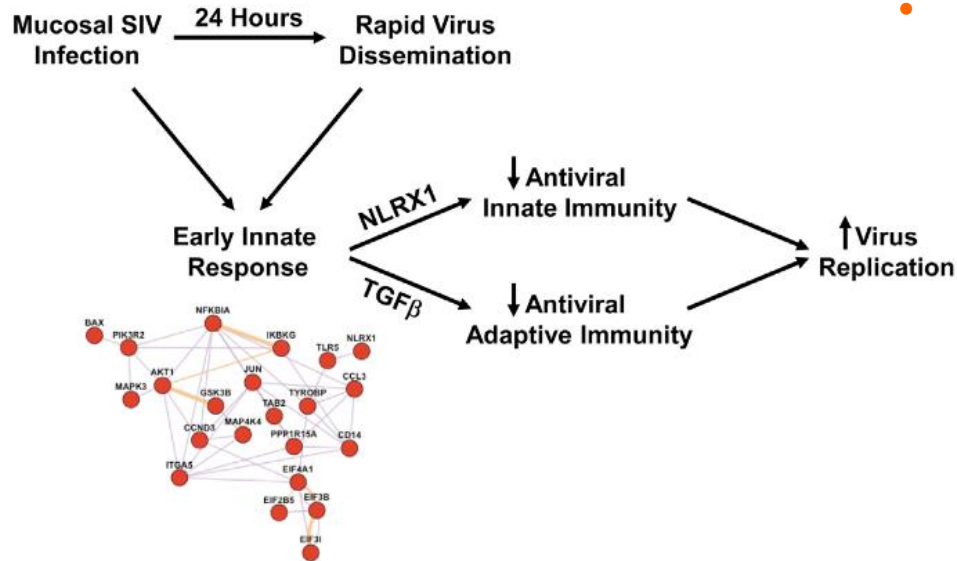
CD8+ T lymphocyte responses in tissues are correlated inversely with TGIF1 expression



- Triggering of the TGF- β pathway was an early event
- Inverse correlation with the magnitude of CD4+ and CD8+ T responses in GALT

➔ ⚓ Adaptive immunity

Conclusions



- The virus triggers initial proinflammatory host responses that
 - downregulate innate antiviral immunity
 - inhibit adaptive cellular immunity
 - facilitate viral replication at sites of early distal dissemination

Persistent HIV-1 replication maintains the tissue reservoir during therapy

Ramon Lorenzo-Redondo^{1*}, Helen R. Fryer^{2*}, Trevor Bedford³, Eun-Young Kim¹, John Archer⁴, Sergei L. Kosakovsky Pond^{5†}, Yoon-Seok Chung⁶, Sudhir Penugonda¹, Jeffrey G. Chipman⁷, Courtney V. Fletcher⁸, Timothy W. Schacker⁹, Michael H. Malim¹⁰, Andrew Rambaut¹¹, Ashley T. Haase¹², Angela R. McLean² & Steven M. Wolinsky¹

FEBRUARY 2016 | VOL 530 | NATURE | 51

- **Background:** low-level viremia can result from

Reactivation of a small proportion of latently infected CD4+ T cells

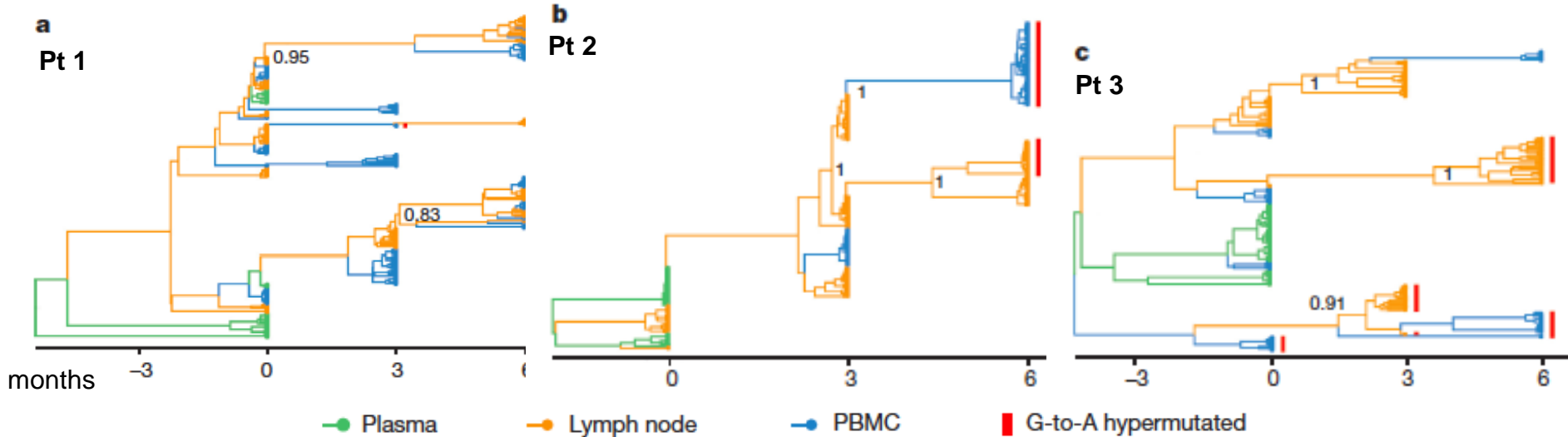
- No replication
- No mutation and no viral genetic divergence over time

Low levels of ongoing replication

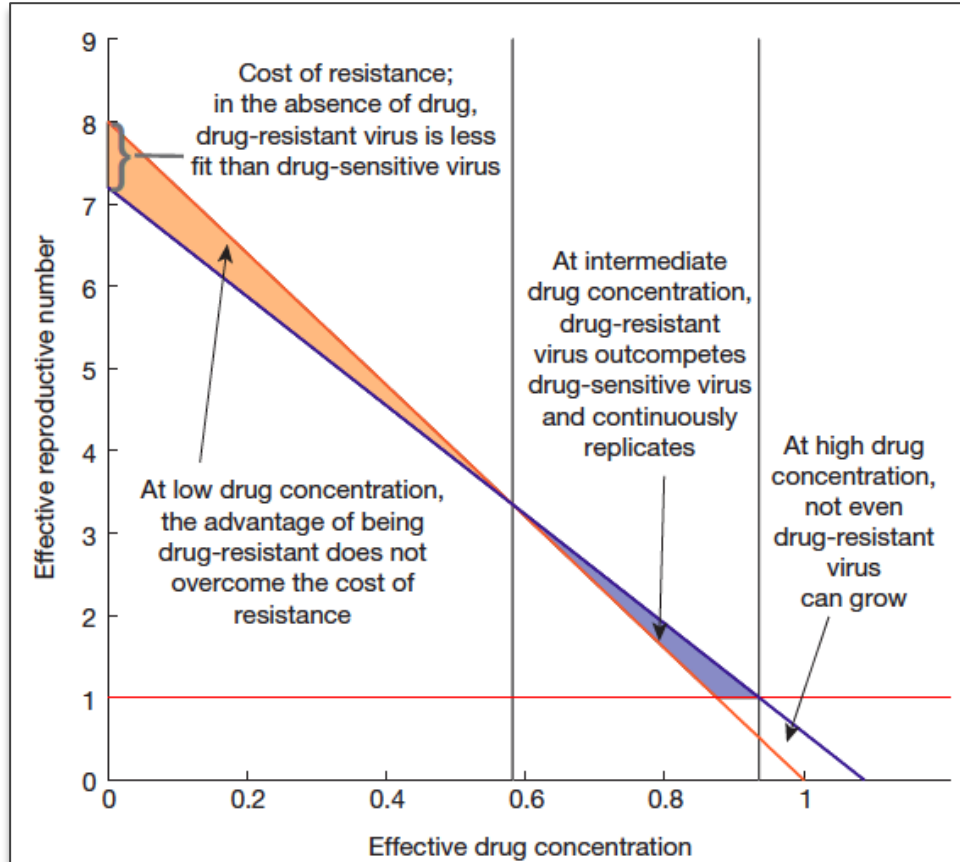
- Replication
- Introduction of mutations → genetic evolution
- However, **NO** emergence of resistance mutations

- **Objective:** to assess whether cART fully suppresses viral replication in lymphoid tissue reservoirs
- **Material & method**
 - 3 patients at 3 different time points (baseline, M3 and M6 after ARV initiation):
 - 2 were in first-line and the remaining was ARV-experienced but ARV were stopped since 1 year
 - Sampling of plasma, PBMC and inguinal lymph nodes
 - Ultra-deep sequencing

Time-structured phyloanatomic history of sequences in lymph nodes and blood (gag region)



Virus evolution and trafficking between tissue compartments continues in patients with undetectable levels of virus in the plasma compartment



Hypothetical fitness landscape, which portrays the relationship between the fitness of each strain and the evolutionary adaptation across a range of drug concentrations

Results & Conclusions

- These findings showed a continued virus production from infected cells in lymphoid tissue sanctuary sites, where drug concentrations are not fully suppressive, that can continue to replenish the viral reservoir and traffic to blood or lymphoid tissue
- This viral production does not inevitably develop resistance to ARV because the lower concentration of drugs in the sanctuary sites (not sufficient to confer a competitive advantage upon drug-resistant strains)
- These findings explain the failure of ARV intensification to fully suppress *de novo* infections
- ➔ Achieving optimal cellular PK and spatial distribution of ARV in lymphoid tissue to fully suppress viral replication and preserve immune function would be a prerequisite to the elimination of the viral reservoir and ultimately a step towards a cure for HIV-1 infection

A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges

Rajeev Gautam^{1*}, Yoshiaki Nishimura^{1*}, Amarendra Pegu², Martha C. Nason³, Florian Klein^{4,5,6}, Anna Gazumyan⁴, Jovana Golijanin⁴, Alicia Buckler-White¹, Reza Sadjadpour¹, Keyun Wang², Zachary Mankoff², Stephen D. Schmidt², Jeffrey D. Lifson⁷, John R. Mascola², Michel C. Nussenzweig^{4,8} & Malcolm A. Martin¹

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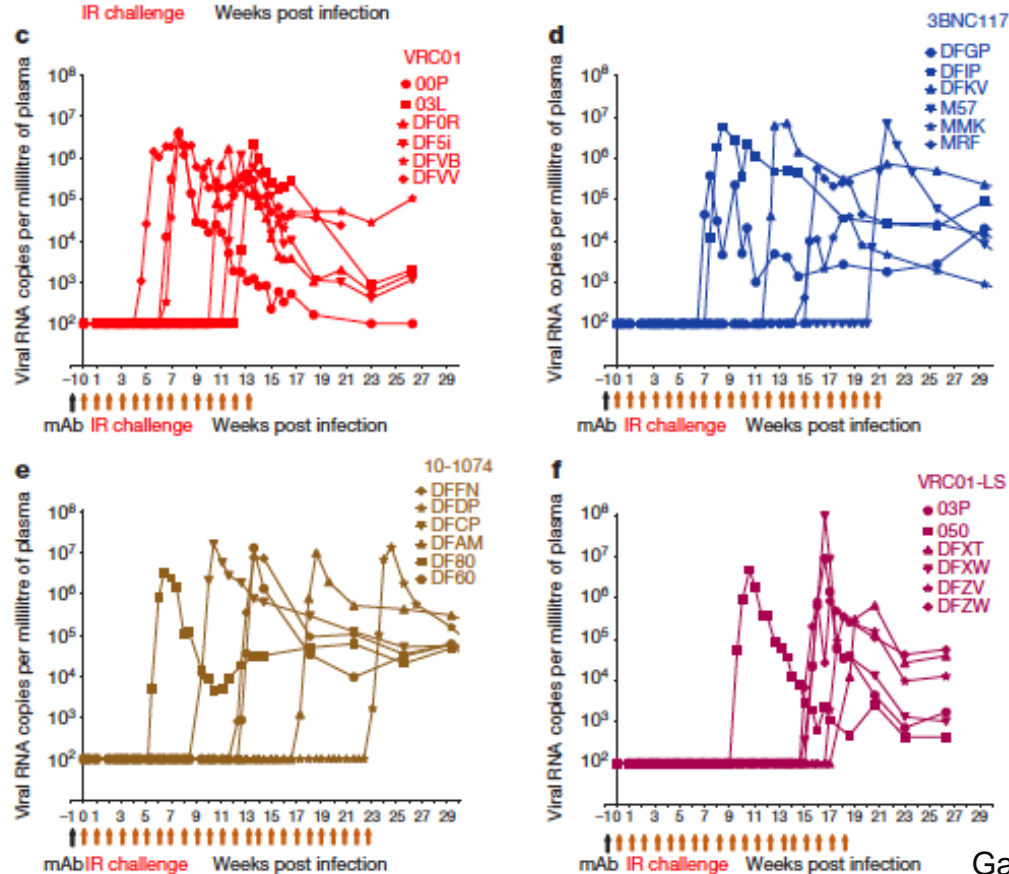
- **Objective:** to explore the possibility that a single administration of a potent neutralizing anti-HIV monoclonal antibodies (MAb), in the setting of repeated low-dose SHIV challenges, might protect for extended periods of time, thereby providing a proof of concept for periodic administration of MAb as an alternative to HIV-1 vaccination

Material & method

- Individual MAbs (20 mg kg^{-1}) were administered a single time intravenously to four cohorts of six macaques
 - **VRC01**: targets the gp120 CD4 binding site
 - **3BNC117**: targets the gp120 CD4 binding site
 - **10-1074**: dependent on the presence of HIV-1 gp120 N332 glycan, located immediately downstream of the V3 loop
 - **VRC01-LS**: introduction of two amino-acid mutations (M428L and N434S, referred to as 'LS') into Fc domain of VRC01 → improvement of the PK profile of VRC01 with an increased half-life in both plasma and tissues

- Starting 1 week later, each group was challenged weekly by the intrarectal route with ten TCID_{50} of SHIV_{AD8-EO}, in the absence of antibody treatment (study design in order to simulate low-dose mucosal transmission in humans)

HIV MAbs delay virus acquisition after repeated low-dose intrarectal SHIV_{AD8-EO} challenges (Figure 1)



- Compared with control animals, which required two to six challenges (median, $n = 3$) for infection, a single broadly neutralizing antibody infusion prevented virus acquisition for up to 23 weekly challenges
- The introduction of a mutation that extends antibody half-life of VRC01 **increased median protection from 8 to 14.5 weeks**
- The duration of protection was directly related to antibody potency and half-life

- ➔ In conclusion, this is a proof of concept that a single administration of potent anti-HIV-1-neutralizing MAbs to naive macaques was protective against repeated low-dose SHIV infection for several months
- ➔ Administered to populations at high risk of HIV-1 transmission, such an immunoprophylaxis regimen could have a major impact on virus transmission

LETTER

doi:10.1038/nature18929

HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption

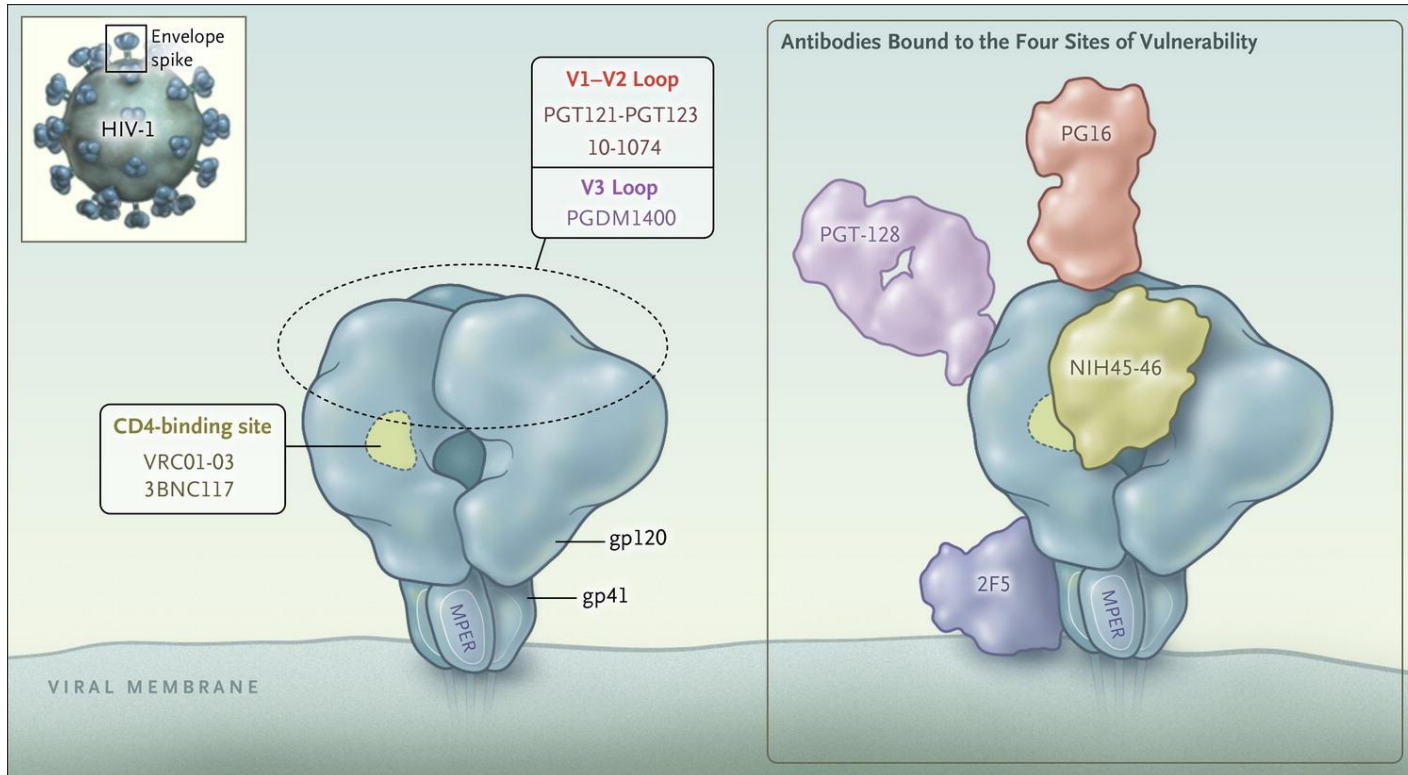
Johannes F. Scheid^{1,2*}, Joshua A. Horwitz^{1*}, Yotam Bar-On¹, Edward F. Kreider³, Ching-Lan Lu¹, Julio C. C. Lorenzi¹, Anna Feldmann⁴, Malte Braunschweig¹, Lilian Nogueira¹, Thiago Oliveira¹, Irina Shimeliovich¹, Roshni Patel¹, Leah Burke⁵, Yehuda Z. Cohen¹, Sonya Hadrihan¹, Allison Settler¹, Maggi Witmer-Pack¹, Anthony P. West Jr⁶, Boris Juelg⁷, Tibor Keler⁸, Thomas Hawthorne⁸, Barry Zingman⁹, Roy M. Gulick⁵, Nico Pfeifer⁴, Gerald H. Learn³, Michael S. Seaman¹⁰, Pamela J. Bjorkman⁶, Florian Klein^{1,11,12}, Sarah J. Schlesinger¹, Bruce D. Walker^{7,13}, Beatrice H. Hahn³, Michel C. Nussenzweig^{1,14} & Marina Caskey¹

Background

- Animal models: bNAbs show potent prophylactic activity, suppress established viraemia, and delay viral rebound during analytical treatment interruption (ATI)
- In humans: a phase I clinical trial showed that 3BNC117 is
 - Safe, well tolerated
 - A single infusion of 3BNC117 ↓ VL by 1.5 log₁₀ cp/ml, with durable activity for 4 weeks

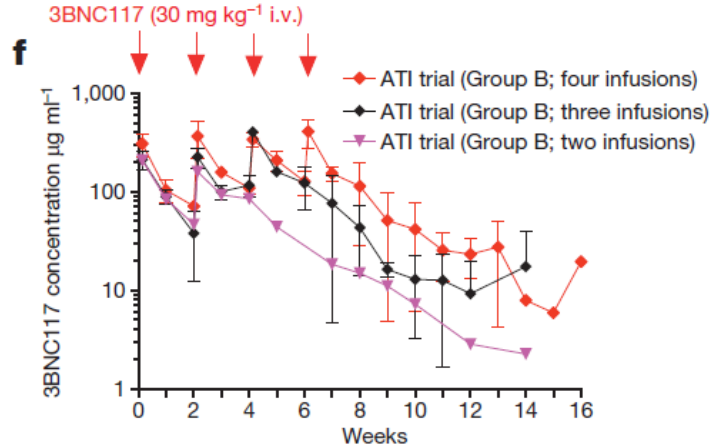
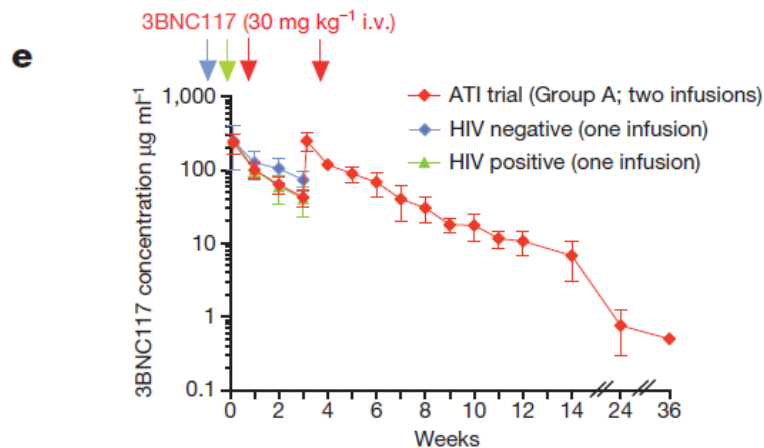
Method

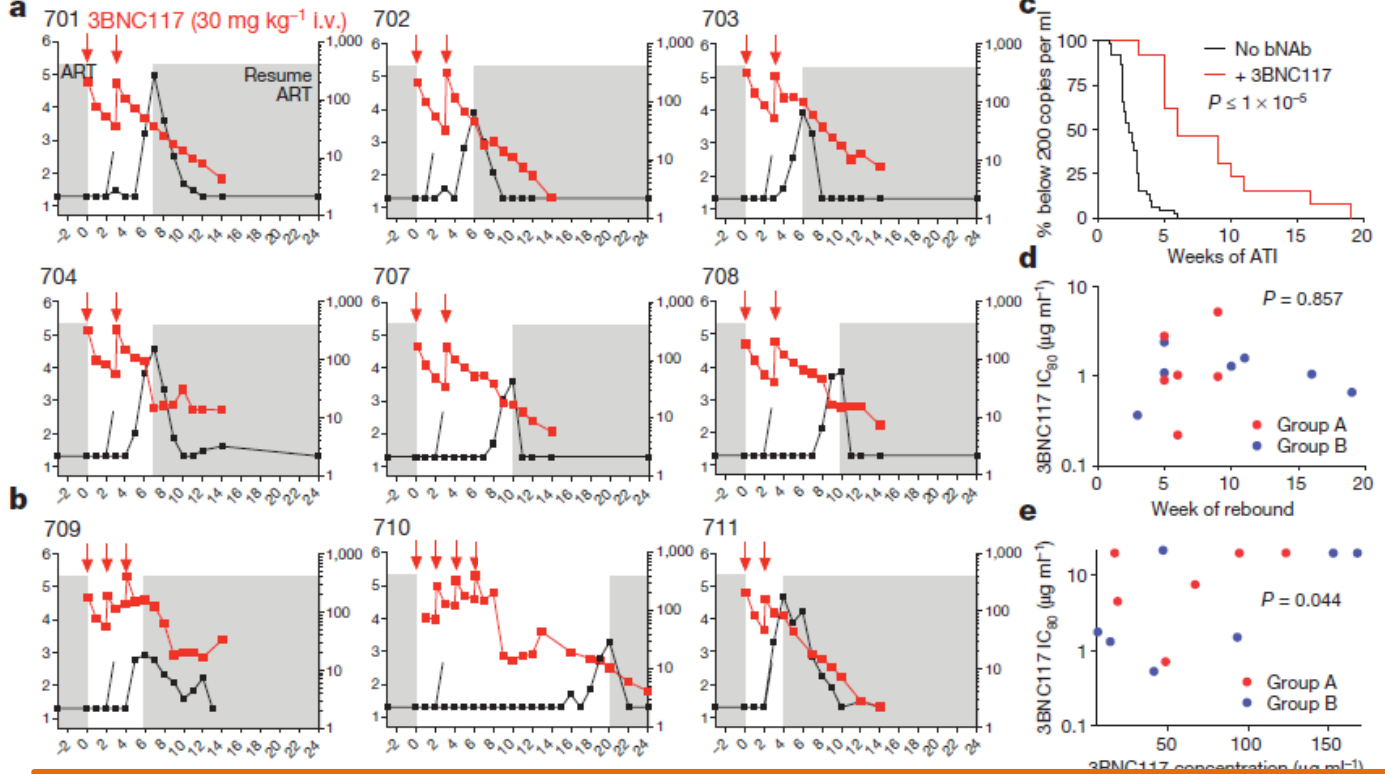
- Phase IIa open label clinical trial
- 3BNC117: broad and potent neutralizing antibody against the CD4 binding site of the HIV-1 Env protein
- 63 individuals screened for sensitivity to 3BNC117: 11% resistant, 65% sensitive
- 13 patients (VL <20 cp/ml >12 months, CD4 >500/mm³, 3BNC117-sensitive). 2 groups with different schedule of infusions
- Analytical treatment interruption (ATI) 2 days after the first 3BNC117 infusion
- ART reinitiated and infusions stopped after 2 consecutive VL >200 cp/ml



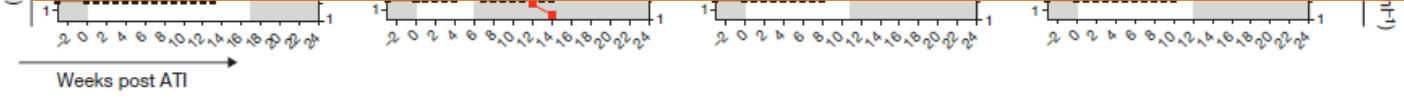
Results (1)

- Well tolerated, safe (no acute retroviral syndrome during rebound, VL<20 cp/ml within 2–7 weeks after restarting ART)
- Half-life of 3BNC117 during ATI: 2-3 weeks





- Average time to rebound 8.4 weeks compared with 2.6 weeks for matched historical non-infused control individuals ($P < 0.00001$)
- 6/13 (46%) individuals remained suppressed until at least 9 weeks after ATI



Results (2)

- A majority (8/13) of participants had rebound viruses more resistant to 3BNC117
- Rebound viruses arise predominantly from a single provirus (5/8 individuals)
- Time to rebound did not correlate with the amount of viral DNA in PBMCs (but this is a poor measure of the reservoir, since most integrated proviruses in patients on ART are defective)

Conclusion

- Administration of bNAb 3BNC117 exerts strong selective pressure on HIV-1 emerging from latent reservoirs during analytical treatment interruption in humans
- Immunotherapy will probably require combinations of bNAbs that target different sites to increase the frequency of individuals that remain suppressed by antibody during ATI

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption

K.J. Bar, M.C. Sneller, L.J. Harrison, J.S. Justement, E.T. Overton, M.E. Petrone, D.B. Salantes, C.A. Seamon, B. Scheinfeld, R.W. Kwan, G.H. Learn, M.A. Proschan, E.F. Kreider, J. Blazkova, M. Bardsley, E.W. Refsland, M. Messer, K.E. Clarridge, N.B. Tustin, P.J. Madden, K.S. Oden, S.J. O'Dell, B. Jarocki, A.R. Shiakolas, R.L. Tressler, N.A. Doria-Rose, R.T. Bailer, J.E. Ledgerwood, E.V. Capparelli, R.M. Lynch, B.S. Graham, S. Moir, R.A. Koup, J.R. Mascola, J.A. Hoxie, A.S. Fauci, P. Tebas, and T.-W. Chun

Aim & method

- VRC01: bNAb that targets the CD4-binding site
- To determine whether administration of VRC01, can safely prevent or delay plasma viral rebound after the discontinuation of ART in humans
- 2 open-label trials phase I
(ACTG A5340 and NIH15-I-0140)
- Study participants were not prescreened for sensitivity of the virus to neutralization by VRC01

Results

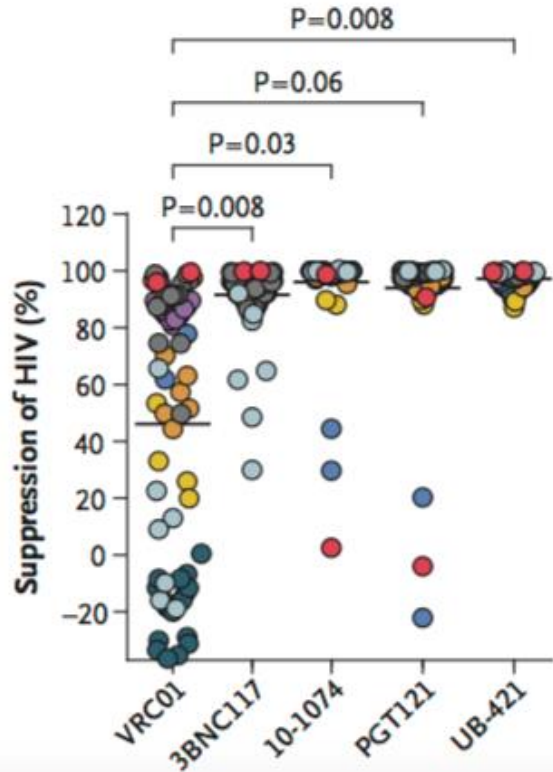
- 24 participants (22 M-2F)
- Safe
- Viral rebound occurred despite high plasma VRC01 concentrations
- Median time to rebound was 4 weeks and 5.6 weeks according to the trial
- Compared to historical controls, participants more likely to have viral suppression at week 4 but the difference was not significant at week 8
 - Slightly delayed viral rebound

Results

- Baseline resistance to VRC01 common
- Nearly all participants who had viral rebound early with high concentrations of plasma VRC01 had rebound with resistant virus
 - ➔ Considerable challenge in the use of bNAbs as therapeutic agents

Study Participants

- N01 ● N03
- N04 ● N05
- N06 ● N08
- N09 ● N10



Other tested bNAbs appeared to have less prevalent archived resistance

RESEARCH ARTICLE

HIV-1 THERAPY

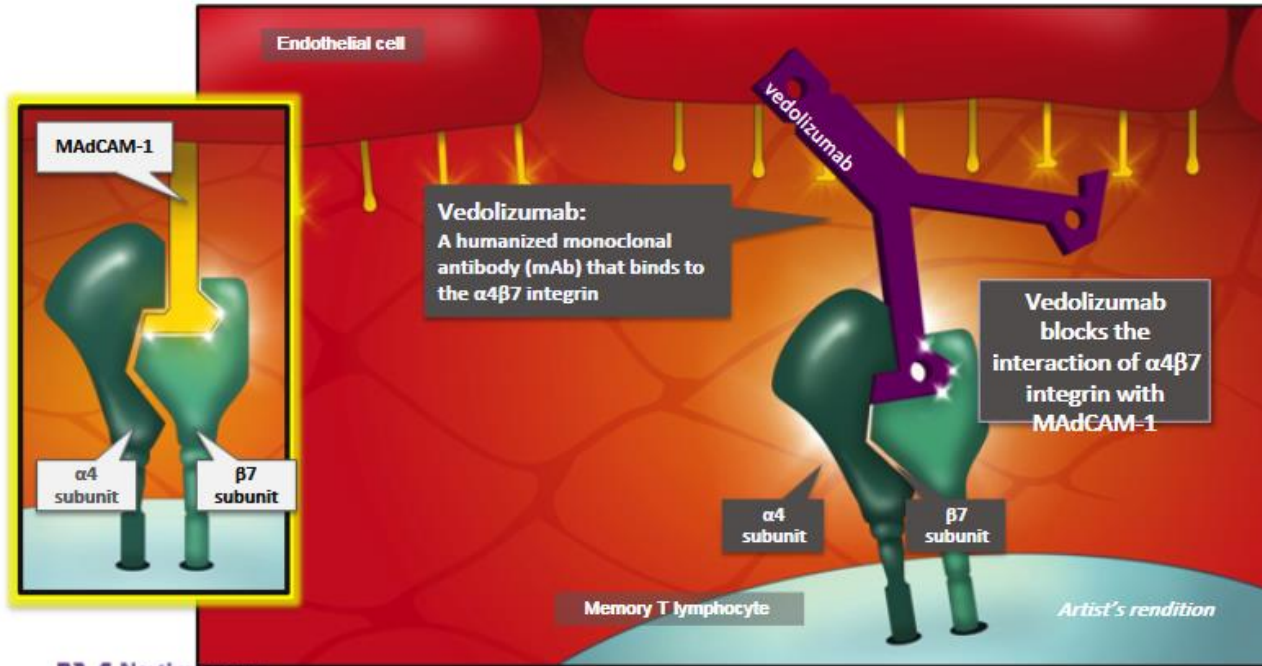
Sustained virologic control in SIV⁺ macaques after antiretroviral and $\alpha_4\beta_7$ antibody therapy

Siddappa N. Byrareddy,^{1*†} James Arthos,^{2*} Claudia Cicala,^{2*} Francois Villinger,^{1,3‡} Kristina T. Ortiz,¹ Dawn Little,¹ Neil Sidell,⁴ Maureen A. Kane,⁵ Jianshi Yu,⁵ Jace W. Jones,⁵ Philip J. Santangelo,⁶ Chiara Zurla,⁶ Lyle R. McKinnon,^{7§} Kelly B. Arnold,⁸ Caroline E. Woody,⁸ Lutz Walter,⁹ Christian Roos,⁹ Angela Noll,⁹ Donald Van Ryk,² Katija Jelacic,² Raffaello Cimbrotto,¹⁰ Sanjeev Gumber,³ Michelle D. Reid,¹ Volkan Adsay,¹ Praveen K. Amancha,³ Ann E. Mayne,¹ Tristram G. Parslow,¹ Anthony S. Fauci,² Aftab A. Ansari^{1||}

Background

- High levels of viral replication in gastrointestinal tissues (GITs) during acute infection
 - Severe depletion of local CD4+ T cells
 - Damage to the gut epithelium
 - Rapid formation of persistent viral reservoirs
- **Hypothesis:** preventing HIV susceptible cells from accessing GITs might reduce damage to the gut and the mucosal immune system?
- CD4+ T use to traffic into GITs involves an interaction between integrin $\alpha 4\beta 7$, expressed on CD4+ T, with a mucosal vascular adhesion molecule 1 (MAdCAM-1), expressed within GITs

Recombinant rhesus monoclonal antibody against $\alpha 4\beta 7$ ($\alpha 4\beta 7$ mAb) that blocks $\alpha 4\beta 7$ binding to MAdCAM \rightarrow Stop susceptible immune cells from entering intestinal tissues

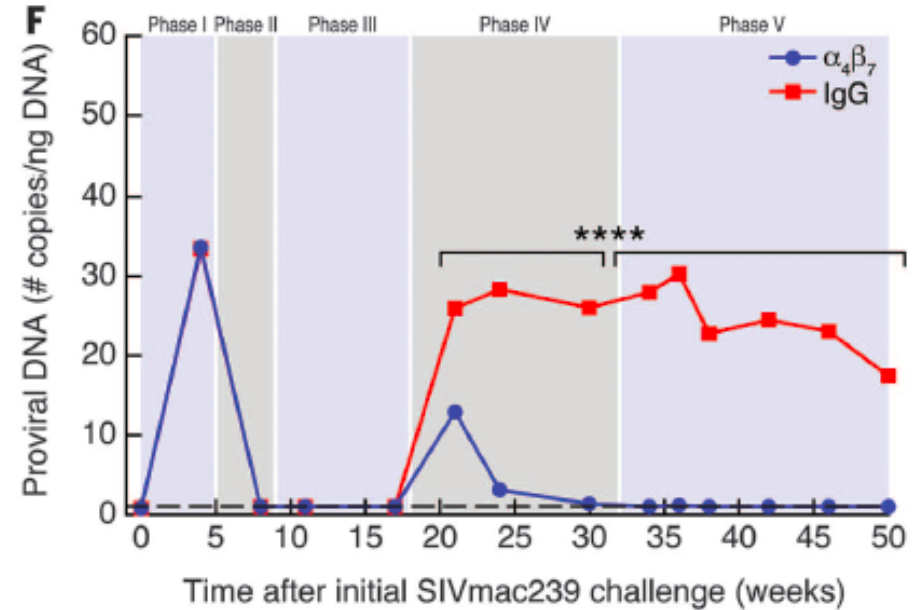
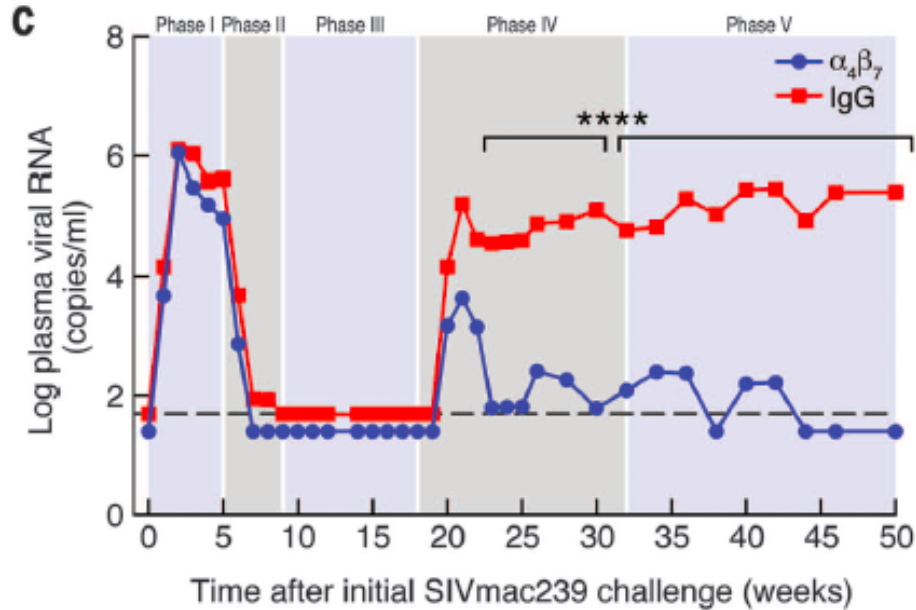


Antibody similar to vedolizumab (approved for IBD treatment)

Method

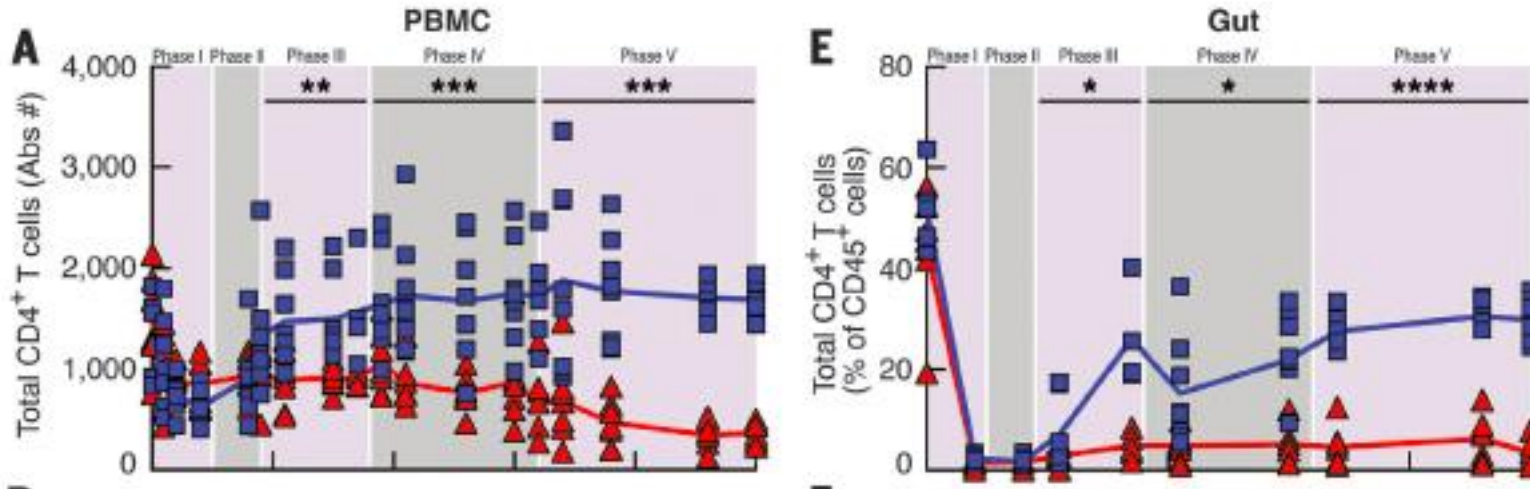
- 18 SIV-infected RMs
 - ART start 5 weeks post-infection (phase II) for 3 months
 - + 8 infusions of $\alpha 4\beta 7$ mAb (n=11) or IgG (control group) (week 9-18)
 - ART stop + $\alpha 4\beta 7$ mAb–IgG 4 weeks
- 3/11 $\alpha 4\beta 7$ mAb–treated animals developed antibodies against the $\alpha 4\beta 7$ mAb → excluded

Results

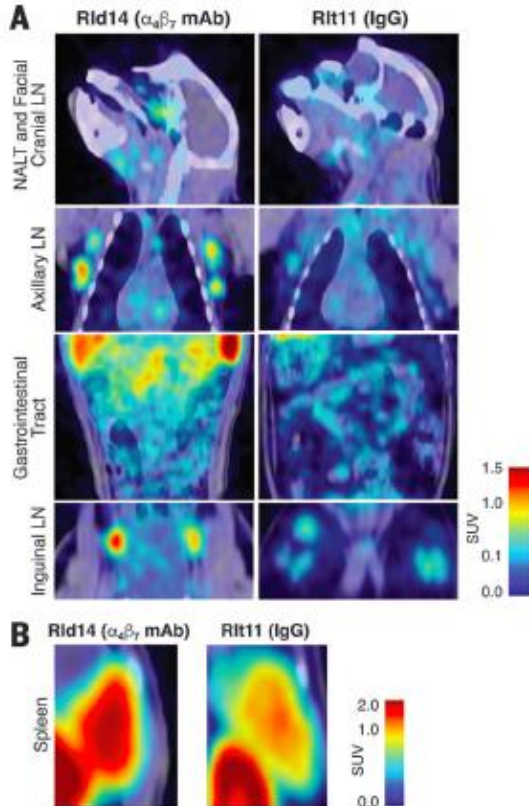


→ Combining $\alpha_4\beta_7$ mAb therapy with short term ART promoted persistent systemic and mucosal virologic control following discontinuation of all therapy.

Results



- In the $\alpha 4\beta 7$ -treated monkeys (blue), gradual restoration of CD4⁺ T cells
- CD4⁺ T cell subsets: apparent recovery of T central memory (CM) and T Effector Memory cells



- Immuno-PET–CT analysis at weeks 50 confirms preservation of CD4+

Conclusion

- Combining ART with $\alpha 4\beta 7$ mAb promoted prolonged virologic control and the restoration of CD4+ T cells.
- Control persisted long after $\alpha 4\beta 7$ mAb treatment was terminated
- *A pilot clinical trial, testing the safety of vedolizumab and its effect on HIV in human, has begun at NIAID*

nature

Accelerated Article Preview

LETTER

doi:10.1038/nature20583

**Ad26/MVA Therapeutic Vaccination with TLR7 Stimulation in
SIV-Infected Rhesus Monkeys**

Erica N. Borducchi, Crystal Cabral, Kathryn E. Stephenson, Jinyan Liu, Peter Abbink, David Ng'ang'a, Joseph P. Nkolola, Amanda L. Brinkman, Lauren Peter, Benjamin C. Lee, Jessica Jimenez, David Jetton, Jade Mondesir, Shanell Mojta, Abishek Chandrashekar, Katherine Molloy, Galit Alter, Jeff M. Gerold, Alison L. Hill, Mark G. Lewis, Maria G. Pau, Hanneke Schuitemaker, Joseph Hesselgesser, Romas Geleziunas, Jerome H. Kim, Merlin L. Robb, Nelson L. Michael and Dan H. Barouch

Background and aim

- Little evidence exists that the viral reservoir can be sufficiently targeted to improve virologic control following discontinuation of ART
- Is a therapeutic vaccine will be able to induce cellular immune responses with sufficient potency and breadth to control viral rebound following ART discontinuation?
- TLR7 (toll-like receptor) triggering: activate dendritic cells and lymphocytes and lead to innate immune activation
- **Aim:** evaluation of Ad26/MVA therapeutic vaccination and TLR7 agonist administration in ART suppressed, SIV-infected rhesus monkeys

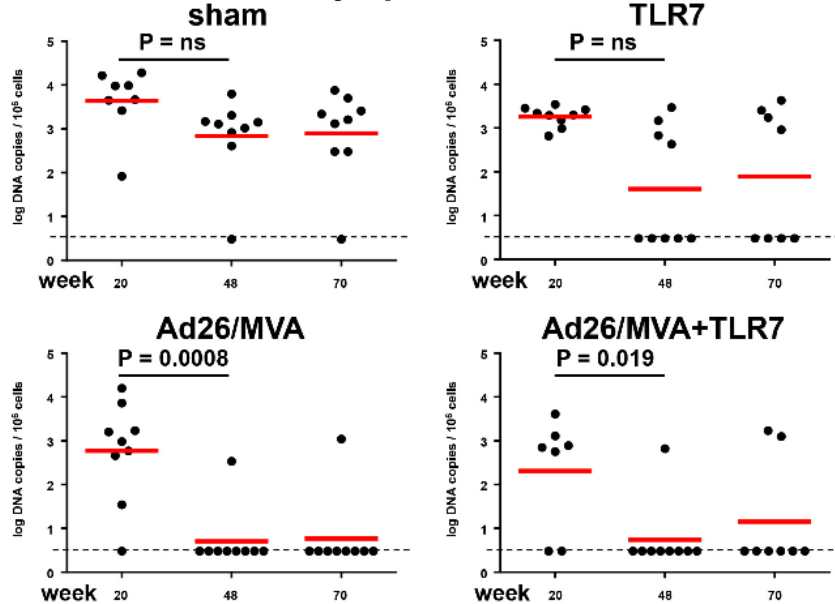
Method

- 36 monkeys, ART at 7 days post-infection
- Interventions after 24 weeks of suppressive ART
- 4 groups
 - (1) Ad26/MVA vaccines alone
 - (2) Ad26/MVA vaccines +TLR7 agonist GS-986
 - (3) TLR7 agonist GS-986 alone
 - (4) sham (control)

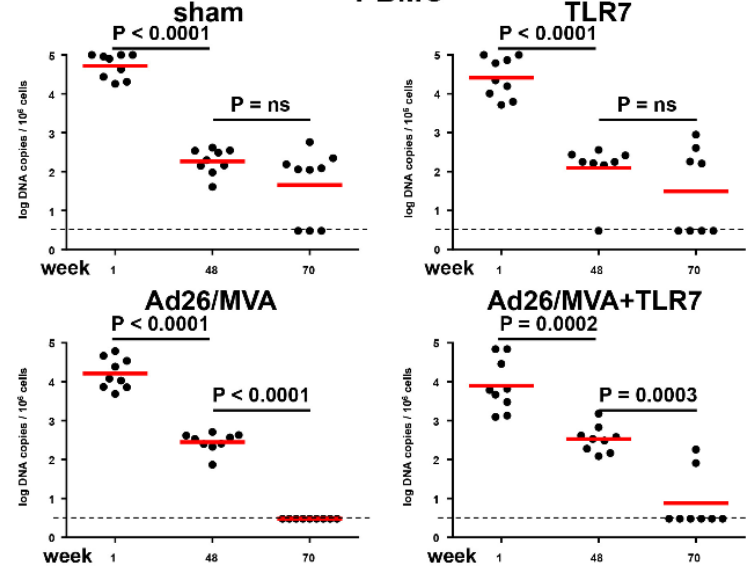
Results

- Immunogenicity of the Ad26/MVA vaccine
 - >100-fold ↑ in the magnitude of Gag-, Pol-, Env- specific cellular immune responses (compared with prevaccination responses)
 - Expanded cellular immune breadth by 10x → induction of responses to a large number of epitopes that were not detectable before vaccination
 - Modest humoral immune responses

lymph nodes



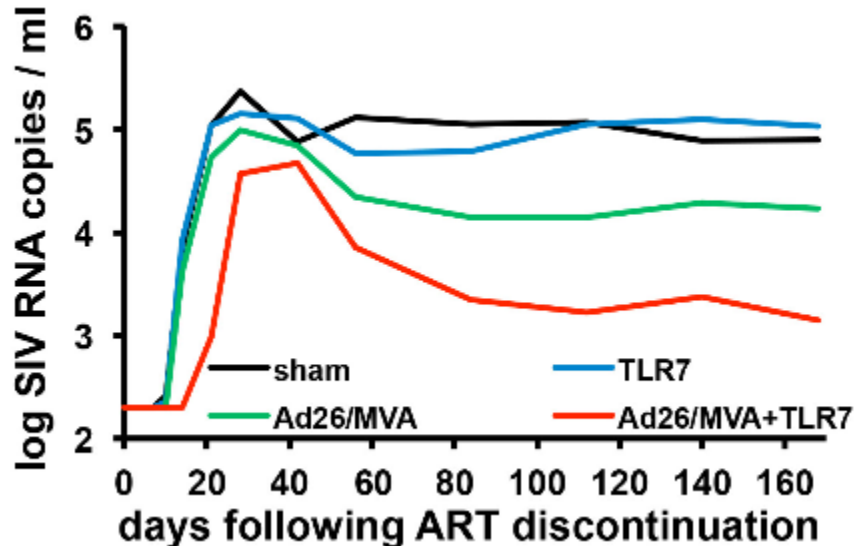
PBMC



Ad26/MVA vaccine: reductions of viral DNA to undetectable levels in the majority of animals by week 70 in lymph nodes and PBMC

→ Suggest ↓ CD4 infected

Stop ART at w72



- 3/9 animals in the combined group: virologic control to undetectable levels in the absence of ART
- Viral DNA in lymph nodes and PBMC correlated poorly with virologic control following ART discontinuation and time to viral rebound ($P=0.03$) → viral DNA assays are not sufficiently sensitive to predict functional cure

Vaccine+ TLR7: ↓ viral set-point 1.7 log and delay of viral rebound 2.5x

Conclusion

- The combination of Ad26/MVA vaccination and TLR7 stimulation
 - ↓ viral DNA in lymph nodes and peripheral blood
 - Improved virologic control and delayed viral rebound following ART discontinuation in SIV-infected RM that initiated ART during acute infection
- Potential of therapeutic vaccination with innate immune stimulation as a HIV-1 functional cure strategy