An overview of basic science discoveries that will impact clinical practice

John Frater

University of Oxford

Expecting the unexpected…..

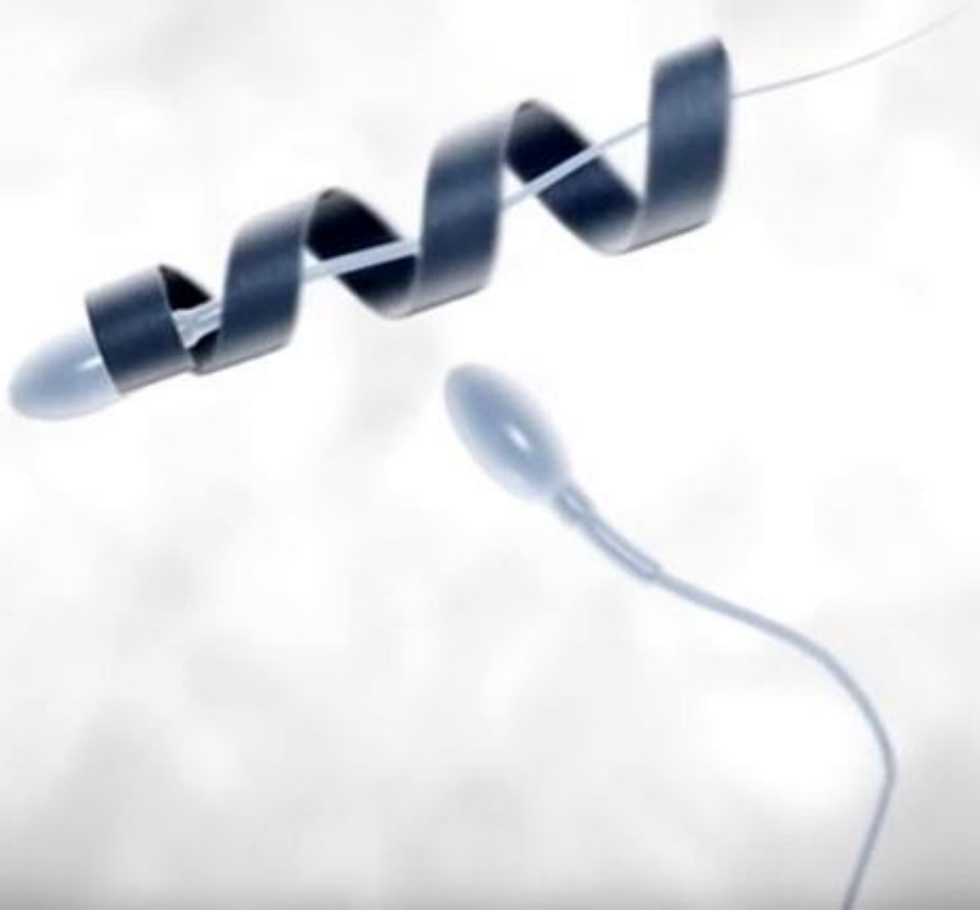
- Few scientific discoveries are predictable.
- In retrospect, many are obvious
  - At the time, not so.
  - The ‘black swans’
- This talk is about guessing where the next black swan will come from….

The funky stuff……

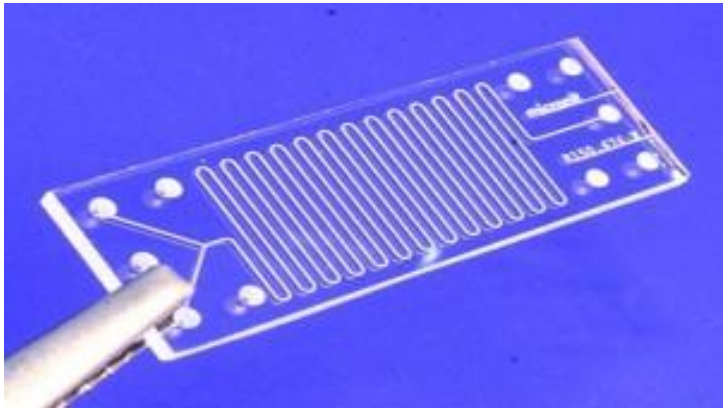
- Single Cell Technologies
- Gene Editing
- Manipulating Immunity

Single Cell Technology – Lab on a Chip

SPERMBOT



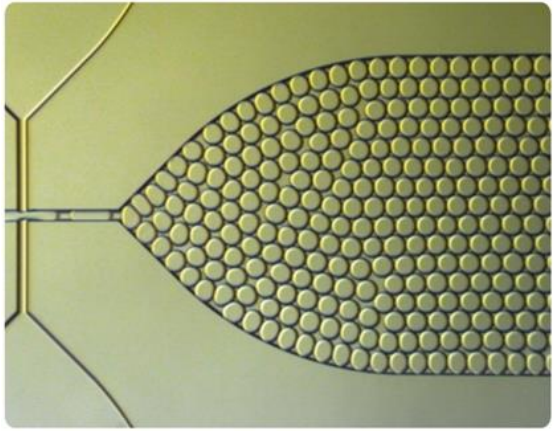
THE MICROFLUIDICS REVOLUTION



Today:
100ul

Single reaction tube

Vs



Tomorrow
1 picolitre

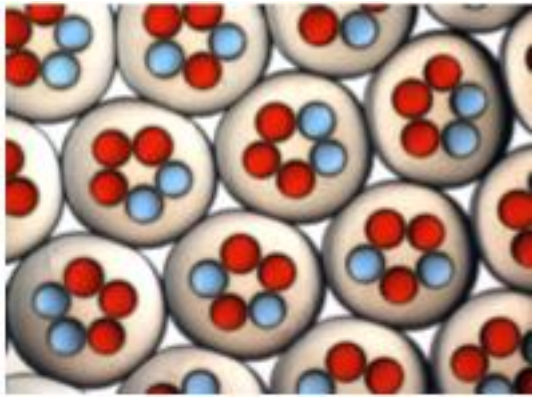
1000s of reactions per second

Lab-on-a-Chip Microfluidics

- Massive scaling up of capacity
- Reactions occur at picolitre volumes, thousands of times
- Greater sensitivity
- Fast – 1000s of experiments per second
  - Great for screening 10,000s of drug compounds – fast and less reagents
- Ability to study very rare cell populations
- Ability to carry out complex processes without massive lab facilities
  - PCR, qPCR, Cell sorting, Bacterial culture….

Applications

- Revolutionise PoC bedside diagnostics and monitoring
- Revolutionise single cell research
  - Clinical algorithms for patient stratification
- Massive opportunities for understanding:
  - HIV immunity,
  - drug discovery,
  - vaccine design
  - Latency and the reservoir



The funky stuff……

- Single Cell Technologies
- **Gene Editing**
- Manipulating Immunity

The Berlin Patient – over 5 years ago…….

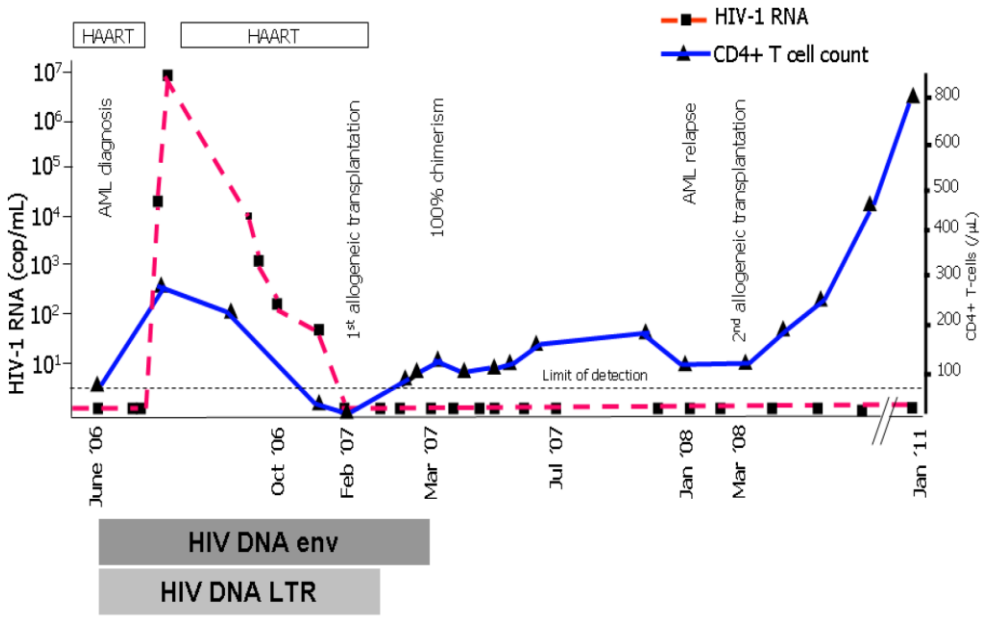


Table 1. Men with Human Immunodeficiency Virus Type 1 (HIV-1) Infection Who Received an Allogeneic Transplant from a Stem-Cell Donor Who Was Homozygous for the *CCR5* delta32/delta32 Mutation.*

Location of Transplantation	Age of Patient	Type of Cancer	Type of Graft	Outcome after Transplantation
	yr			
Berlin†	40	Acute myeloid leukemia	HLA-matched unrelated	Alive after 7 yr, no viral rebound, no ART
Utrecht, the Netherlands‡	53	Myelodysplastic syndrome	Combined haploidentical bridge with umbilical-cord blood	Died from relapse of the myelodysplastic syndrome and pneumonia after 2 mo
Münster, Germany§	51	Non-Hodgkin's lymphoma	HLA-mismatched unrelated	Died from infection after 4 mo
Essen, Germany¶	30	Non-Hodgkin's lymphoma	HLA-matched unrelated	CXCR4-tropic HIV-1 rebound, died from relapse of non-Hodgkin's lymphoma after 12 mo
Minneapolis§	12	Acute lymphoblastic leukemia	Umbilical-cord blood	Died from GVHD after 3 mo
Santiago, Chile§	46	Non-Hodgkin's lymphoma	HLA-matched related	Died from pneumonia shortly afterward
Barcelona§	37	Non-Hodgkin's lymphoma	Combined haploidentical bridge with umbilical-cord blood	Died from relapse of non-Hodgkin's lymphoma after 3 mo

N Engl J Med 2014;371:2437-2438.

Can we inactivate HIV proviral DNA in latently infected cells?

4 gene editing techniques:

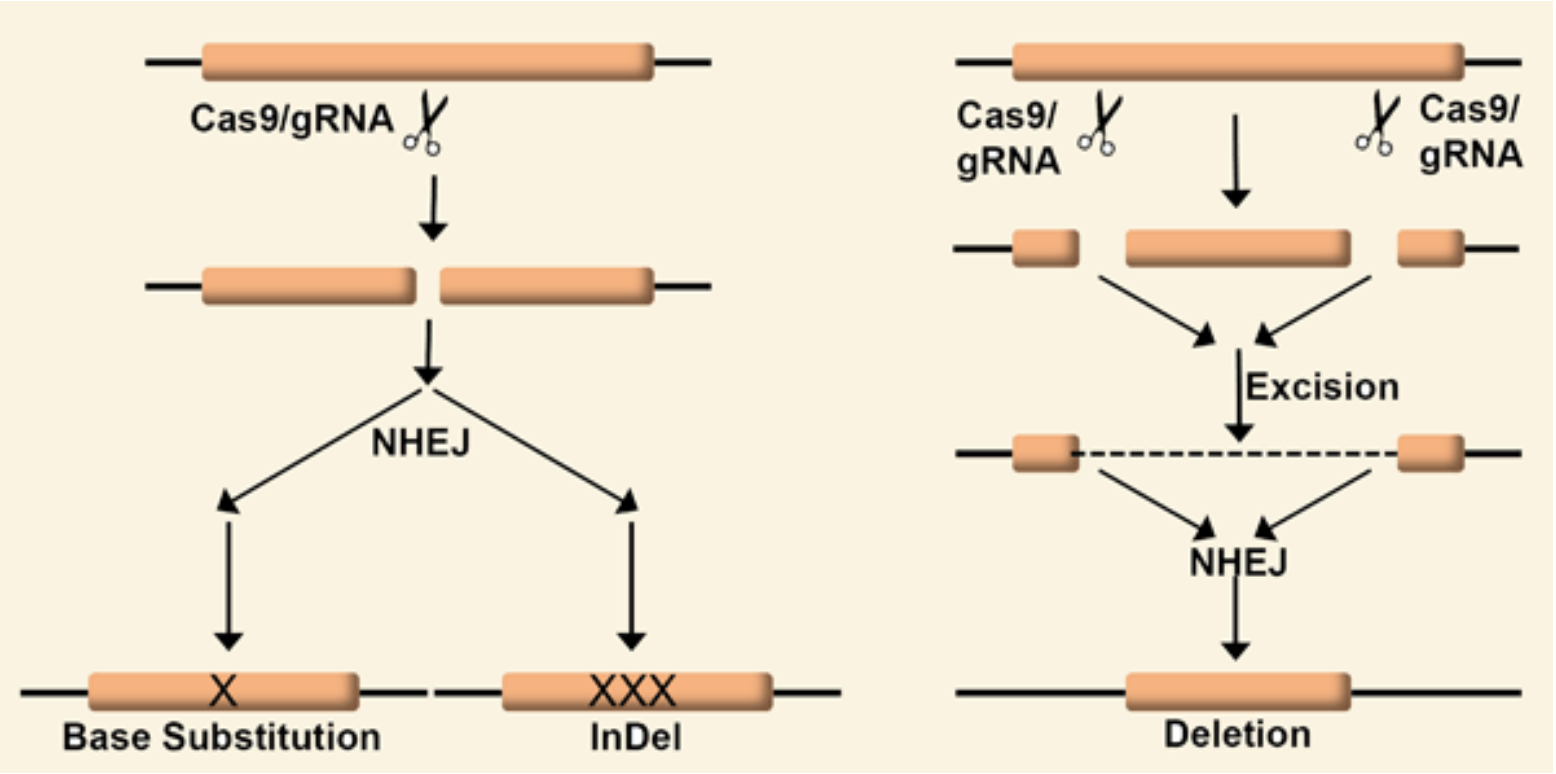
- **Cre recombinase**
  - Site-specific recombinase from bacteriophage enables precise genome editing by recombination between two DNA recognition sites (LoxP sites)
- **Zinc-finger nuclease**
  - Fusion proteins of nonspecific endonuclease cleavage domain of the FokI restriction enzyme with a custom-designed zinc-finger protein.
- **TALEN**
  - Transcription activator-like effector nuclease - from Xanthomonas TAL effector proteins
- **CRISPR-Cas9**
  - Most powerful gene-editing tool

“Clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9)”

- CRISPR loci and Cas proteins are present in ~90% of archaea and ~50% of bacteria
- Evolved as a defense against viruses
- A flexible and precise gene-editing tool. Two components
  - a short guide RNA (gRNA) is used to direct the sequence-specific cleavage of a specific target DNA.
  - and an endonuclease (Cas9) that cleaves both strands of the target DNA.

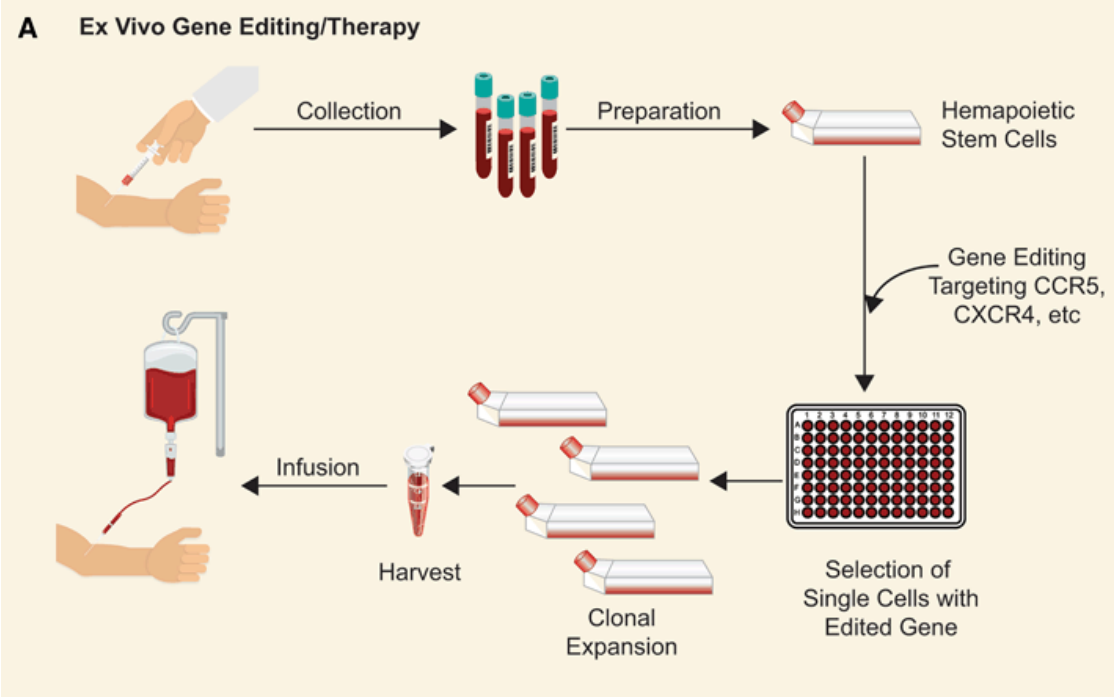
- Successful binding of Cas9 to the target and subsequent endonucleolytic cleavage causes a double-strand break (DSB).
- Repair is by ‘Non Homologous End Joining’ (NHEJ)

THE PRINCIPLE OF CRISPR/Cas9



How to apply gene-editing to the clinic?

- Ex vivo
- In vivo



How can we utilise gene editing for HIV?

- *Ex vivo* versus *in vivo*
- Only *ex vivo* trialed so far for HIV:
  - Zn Finger nucleases

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MARCH 6, 2014 VOL. 370 NO. 10

Gene Editing of *CCR5* in Autologous CD4 T Cells of Persons Infected with HIV

Pablo Tebas, M.D., David Stein, M.D., Winson W. Tang, M.D., Ian Frank, M.D., Shelley Q. Wang, M.D., Gary Lee, Ph.D., S. Kaye Spratt, Ph.D., Richard T. Surosky, Ph.D., Martin A. Giedlin, Ph.D., Geoff Nichol, M.D., Michael C. Holmes, Ph.D., Philip D. Gregory, Ph.D., Dale G. Ando, M.D., Michael Kalos, Ph.D., Ronald G. Collman, M.D., Gwendolyn Binder-Scholl, Ph.D., Gabriela Plesa, M.D., Ph.D., Wei-Ting Hwang, Ph.D., Bruce L. Levine, Ph.D., and Carl H. June, M.D.

ABSTRACT

BACKGROUND

CCR5 is the major coreceptor for human immunodeficiency virus (HIV). We investigated whether site-specific modification of the gene ("gene editing") — in this case, the infusion of autologous CD4 T cells in which the *CCR5* gene was rendered permanently dysfunctional by a zinc-finger nuclease (ZFN) — is safe.

From the Perelman School of Medicine, University of Pennsylvania, Philadelphia (P.T., I.F., M.K., R.G.C., G.B.-S., G.P., W.-T.H., B.L.L., C.H.J.); Albert Einstein College of Medicine, Bronx, NY (D.S.); and Sangamo

How can we utilise gene editing for HIV?

- *Ex vivo* versus *in vivo*
- Only *ex vivo* trialed so far for HIV:
  - Zn Finger nucleases
- Recent data in humanised mice not discouraging
- Next step…human clinical trials??

Gene Therapy (2016) **23,** 690–695

www.nature.com/gt

SHORT COMMUNICATION

Excision of HIV-1 DNA by gene editing: a proof-of-concept *in vivo* study

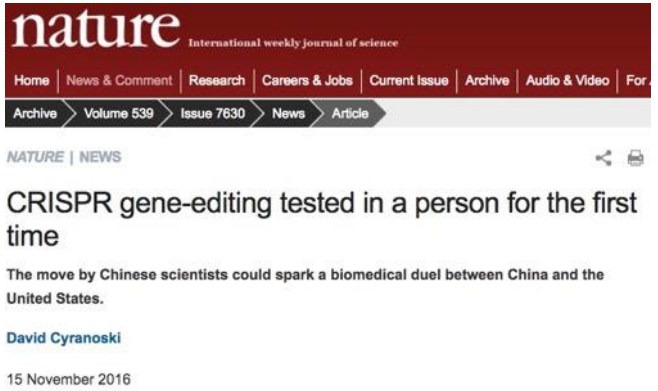
R Kaminski[1], R Bella[2], C Yin[1], J Otte[1], P Ferrante[2], HE Gendelman[3], H Li[4], R Booze[4], J Gordon[1], W Hu[1] and K Khalili[1]

A CRISPR/Cas9 gene editing strategy has been remarkable in excising segments of integrated HIV-1 DNA sequences from the

CRISPR in Clinical Trials

- Chinese scientists to pioneer first human CRISPR trial. Nature. 2016 Jul.
  - PD-1 to be targeted in patients with metastatic non-small cell lung Ca.
  - Similar study recently approved by NIH and FDA.

- Conditions under consideration:
  - Malaria
  - Muscular dystrophy
  - Retinitis pigementosa
  - HIV
  - ……and others.



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NATURE | NEWS

CRISPR gene-editing tested in a person for the first time

The move by Chinese scientists could spark a biomedical duel between China and the United States.

David Cyranoski

15 November 2016

The funky stuff……

- Single Cell Technologies
- Gene Editing
- **Manipulating Immunity**

Biologics – Can we improve on Nature?

- **Phase 1: HIV-1-specific mAbs (Now)**
  - Monoclonal, broadly neutralising antibodies:
  - VRCO1, 3BNC117, $\alpha 4\beta 7$ integrin

- **Phase 2: Engineered antibodies (Tomorrow)**
  - Dual-affinity re-targeting (DART) proteins
  - Bispecific T cell engagers (BiTES)
  - Chimeric Antigen Receptor (CAR) T cells
  - Immune-mobilizing monoclonal T-cell receptors against viruses (ImmTav)

VRC01

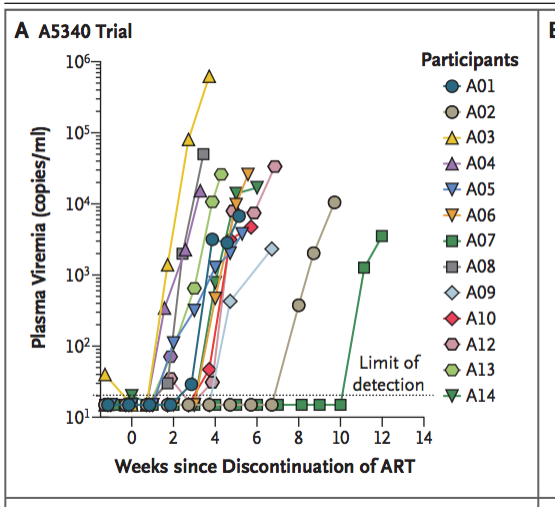
The NEW ENGLAND JOURNAL of MEDICINE

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

Nov 2016



- 24 participants with chronic infection.
  - 3 & 8 doses, overlapping TI.
- All rebound after TI – after 4 or 5.6 weeks (mean)
- Significant delay at 4 weeks vs historic controls. Lost by 8 weeks
- Rebound virus showed evidence of resistant variants

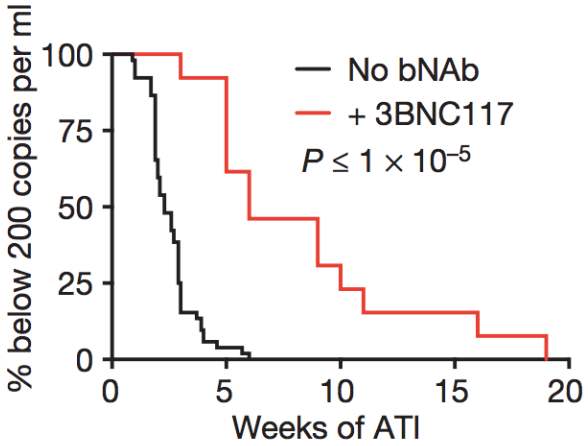
LETTER

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-On[1], Edward F. Kreider[3], Ching-Lan Lu[1], Julio C. C. Lorenzi[1], Anna Feldmann[4], Malte Braunschweig[1], Lilian Nogueira[1], Thiago Oliveira[1], Irina Shimeliovich[1], Roshni Patel[1], Leah Burke[5], Yehuda Z. Cohen[1], Sonya Hadrigan[1], Allison Settler[1], Maggi Witmer-Pack[1], Anthony P. West Jr[6], Boris Juelg[7], Tibor Keler[8], Thomas Hawthorne[8], Barry Zingman[9], Roy M. Gulick[5], Nico Pfeifer[4], Gerald H. Learn[3], Michael S. Seaman[10], Pamela J. Bjorkman[6], Florian Klein[1,11,12], Sarah J. Schlesinger[1], Bruce D. Walker[7,13], Beatrice H. Hahn[3], Michel C. Nussenzweig[1,14] & Marina Caskey[1]

July 2016



- N=13 with chronic HIV infection suppressed for >12 months
- Infusions of 3BNC117. TI 2 days later
- Up to 19 week delay in rebound vs historical controls (2.6 weeks)
- Rebound occurred with escape variants or once antibody levels had dropped

$\alpha 4\beta 7$ integrin

- $\alpha 4\beta 7$ integrin found on CD4 T cells & NK cells
- Mediates migration and retention of leucocytes in the gut.
- MAsCAM is natural ligand - constitutively expressed in the gut
- $\alpha 4\beta 7$ integrin 'high' cells are preferentially infected by HIV
- Protection against mucosal transmission in SIV

- Question: Can monoclonals vs $\alpha 4\beta 7$ integrin prevent viral rebound after TI?

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 Jelicic,[2] Raffaello Cimbro,[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||]

Oct 2016, Science

- SIVmac239 infected macaques received ART and mAb vs $\alpha 4\beta 7$ integrin.

Post TI:

- CD4 T cell restoration in blood, gut and peripheral tissues
- Two never rebound, 6 blip but then control.
  - Now between 1-2 years without rebound.
- Mechanism unclear - ? related to IgG vs Gp120 V2
- NEXT: chronically-infected humans: N=15; Phase 1 trial with Vedolizumab then ATI; already recruiting.

Fig. 1 Control of plasma and GIT viral loads.

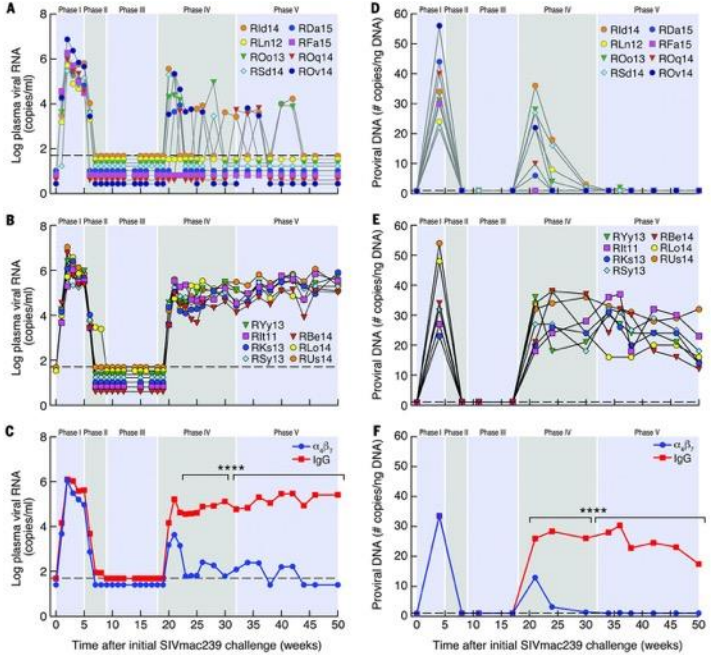
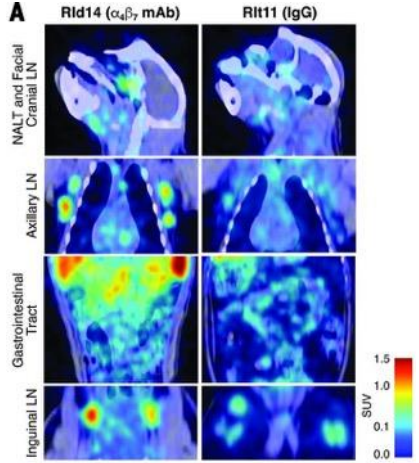


Fig. 3 Immuno-PET–CT analysis confirms the preservation of CD4+ cells.



Siddappa N. Byrareddy et al. Science 2016;354:197-202

Vedolizumab to Cure HIV

- Effective therapy in IBD
- Stops cells homing to the gut
- Induces SIV remission in macaques…
- Takeda working with NIH to conduct clinical trials in HIV in humans
- Mechanism unclear
  - Will know more later this year……

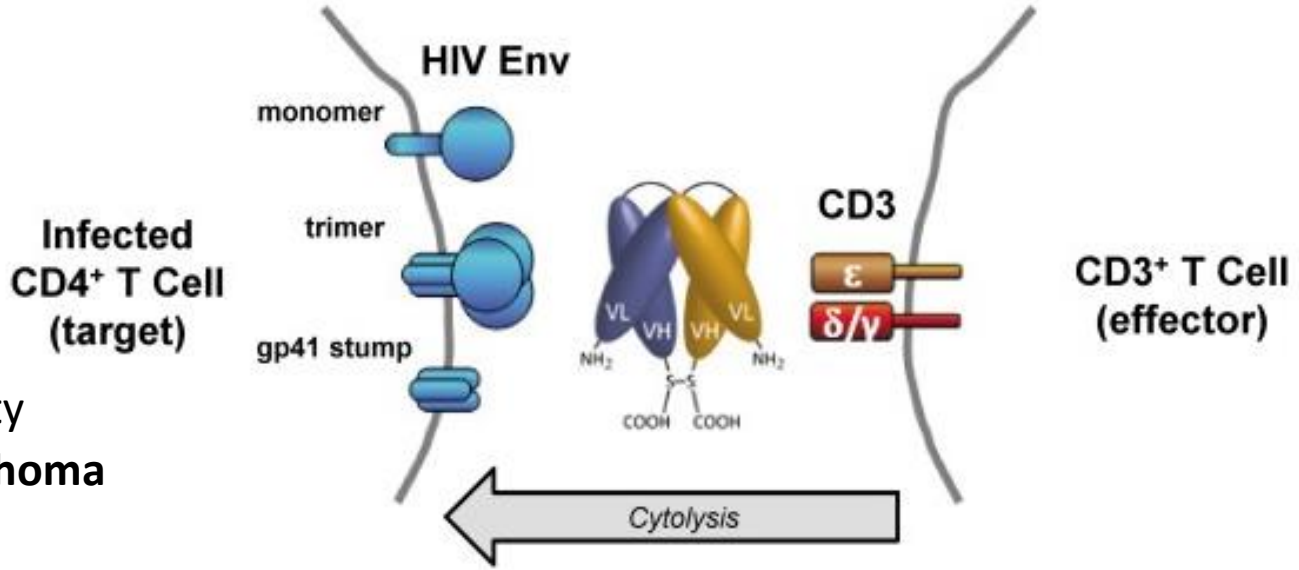
Phase 2: DARTs and BITEs

- ‘Dual-affinity re-targeting’ (DART) molecules
- ‘Bispecific T cell engagers’ (BiTEs)

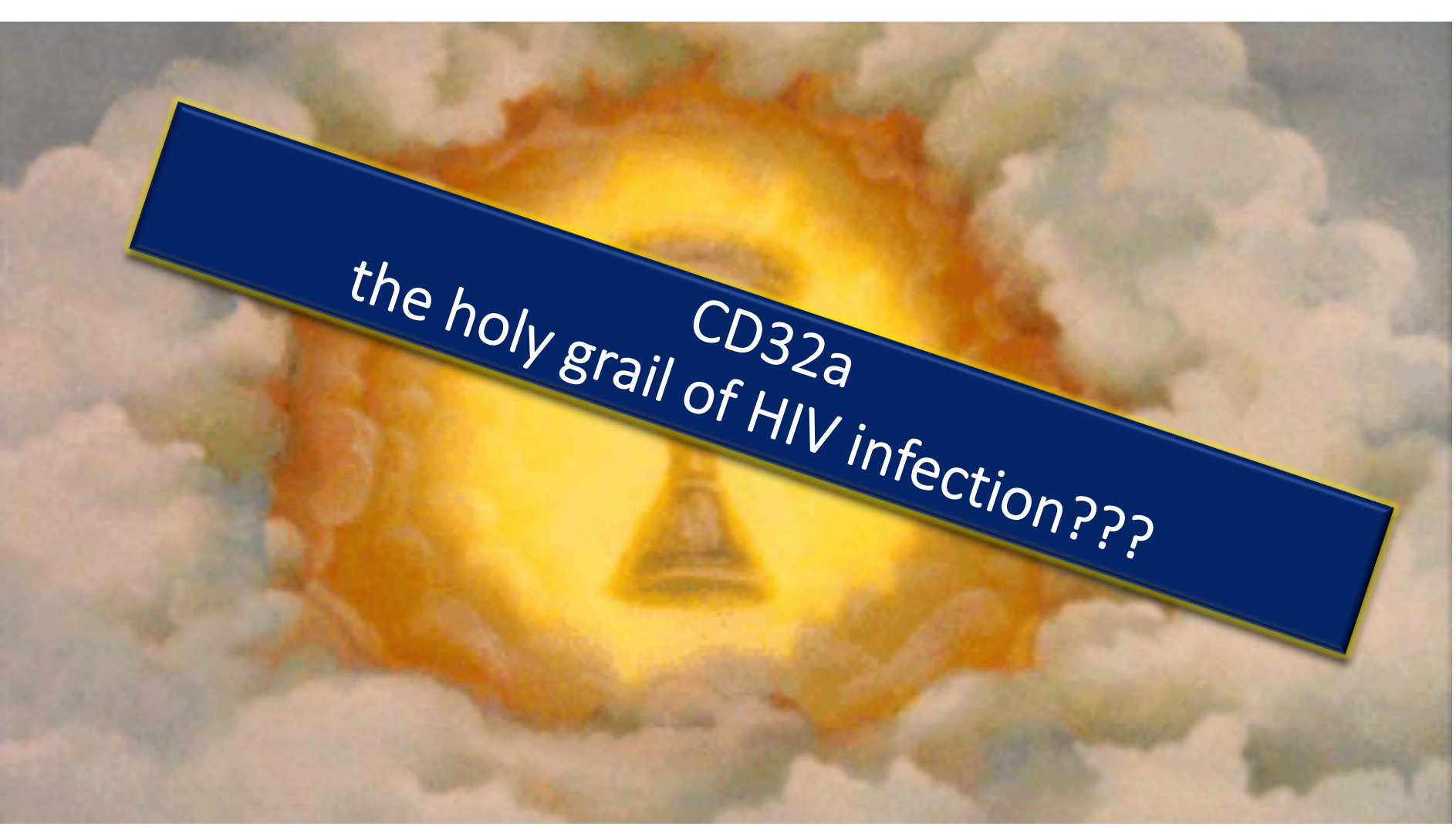
- Provide additional cytotoxic functions (the kill) to the immune responses
- Blinatumomab (CD19xCD3 BiTE) was recently approved for the treatment of acute lymphoblastic leukemia

DARTS

- Dual-Affinity Re-Targeting (DART) molecules
  - Bind CD3 – pulling in T cells
  - Bind antigen
    - eg anti-Env IgG antibodies



- Increase target killing
- Improve on natural immunity
- **Phase 1 trials in AML, lymphoma and colorectal cancer**


CD32a
the holy grail of HIV infection???

March 2017

“Marker 1” revealed:

CD32a – another new target……

LETTER

doi:10.1038/nature21710

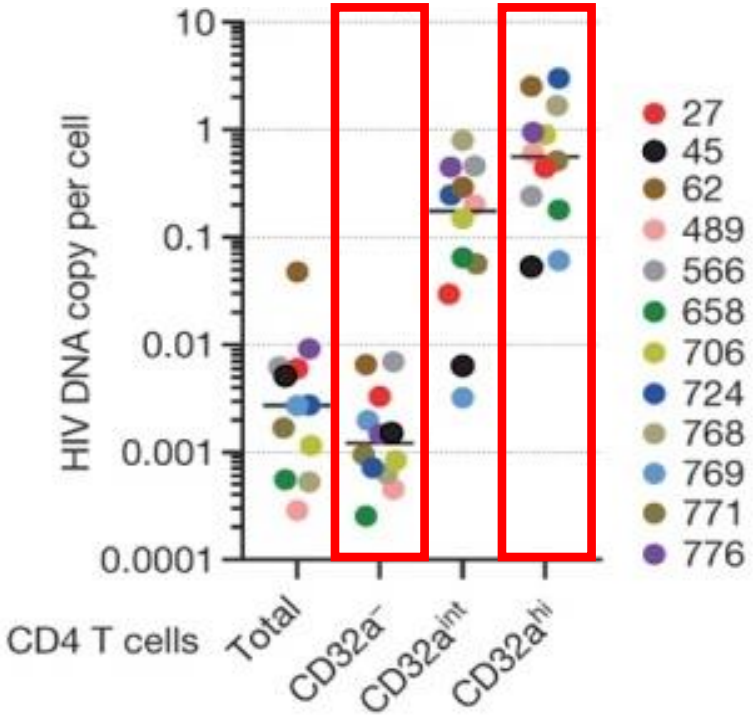
CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses

Benjamin Descours[1]*, Gaël Petitjean[1]*, José-Luis López-Zaragoza[2,3,4], Timothée Bruel[2,5], Raoul Raffel[1], Christina Psomas[6], Jacques Reynes[6], Christine Lacabaratz[2,3,4], Yves Levy[2,3,4], Olivier Schwartz[2,5], Jean Daniel Lelievre[2,3,4] & Monsef Benkirane[1]

The persistence of the HIV reservoir in infected individuals is a major obstacle to the development of a cure for HIV[1–3]. Here, using an *in vitro* model of HIV-infected quiescent CD4 T cells, we reveal a gene expression signature of 103 upregulated genes that are specific enriched in inducible replication-competent proviruses and can be predominant in some participants. Our discovery that CD32a$^+$ lymphocytes represent the elusive HIV-1 reservoir may lead to insights that will facilitate the specific targeting and elimination

March 15, 2017; Nature

CD32a identifies the CD4 T-cell HIV reservoir



B Descours *et al. Nature* 1–4 (2017) doi:10.1038/nature21710

nature

If CD32a is a marker for the reservoir…..

- A biomarker for latent infection
- An opportunity to understand latency much better.
- A new lab test to assess cure-based interventions
- …..or even a new therapy: “anti-CD32a monoclonal antibody therapy”

Closing remarks

- The field of HIV has a track record translating basic science into clinical practice
- ART changes the questions faced by scientists and clinicians
- Work in HIV translates across to other fields (e.g. cancer) – and vice versa
- Time for a drink – look out for the black swans!

THANK YOU