

Highlights from the Keystone Symposium on HIV Persistence 2015 26–30 April 2015, Boston, Massachusetts, USA

Sulggi A Lee

University of California San Francisco, CA, USA

The Keystone Symposium on HIV Persistence was held at the Boston Park Plaza Hotel in Boston, Massachusetts from April 26–30, 2015. The conference was organised by **Olivier Lambotte** (University of Paris South, France), **Steven Deeks** (University of California, San Francisco, USA) and **Guido Silvestri** (Emory University, USA).

Despite long-term control of HIV replication with combination antiretroviral therapy, HIV persists as quiescent integrated DNA in memory CD4+ T cells, and possibly in other cellular reservoirs, including naïve cells and macrophages. Low-level replenishment of the reservoir via limited cycles of replication, may also contribute to persistence, at least in some patients. Curing HIV infection will only occur if these barriers are reversed, or if host capacity to control HIV indefinitely is improved.

The meeting was organised into several sessions encompassing: (1) the biology of HIV latency, including discussions on HIV-specific integration sites and HIV-specific cytotoxic lymphocyte responses; (2) the detection and quantification of HIV reservoirs; (3) CD4+ T cells as reservoirs, including discussion on the role of T follicular helper (Tfh) cells in maintaining HIV latency; (4) non-CD4+ T cell reservoirs, including the central nervous system and myeloid cells; (5) functional eradication of HIV reservoirs including latent reactivating agents and targeted nucleases; (6) the role of immune exhaustion in maintaining HIV persistence; (7) non-human primate models of HIV latency; and (8) limitations of antiretroviral therapy, including early treatment during hyperacute infection.

Keynote address

The keynote address was given by **Françoise Barré-Sinoussi** (Institut Pasteur, France), and was entitled 'HIV cure as an aspirational goal'. The talk emphasised the need to accelerate HIV cure research. Professor Barré-Sinoussi highlighted six major priorities in the current field of HIV research. These are to better understand: (1) post-transcriptional repression mechanisms (e.g. blockage of nuclear viral RNA export, HIV translational repression); (2) HIV reservoir sites in cell subsets and tissues (e.g. naïve T cells, memory stem T cells, Tfh cells, myeloid cells, astrocytes, haematopoietic progenitor cells, B cell follicles); (3) drivers of chronic immune activation (e.g. the role of inflammation, monocyte activation, T cell activation, natural killer cells and pro-inflammatory cytokines such as interferon alpha); (4) host and immune mechanisms of control of HIV/SIV infection (host restriction factors, virus-specific and broad cytotoxic CD8+ T cell responses); (5) assays to study and measure persistent infection (beyond current PCR, culture-based methods); and (6) the use of latency-reversing agents to cure HIV (mostly histone deacetylase inhibitors and protein kinase C agonists that have been tested and shown to reverse latency but have not significantly decreased the size of the reservoir, newer immune-based therapies such as the use of broadly neutralising

antibodies and T cell vaccines, and methods to reduce T cell activation or proliferation such as cytokines or anti-cytokines and anti-inflammatory drugs). Professor Barré-Sinoussi concluded her keynote address urging continued support for HIV research and announced the publication and launch of the revised IAS Global Scientific Strategy for HIV Cure at the AIDS 2016 conference in Durban, South Africa.

Recent advances in the area of HIV cure research

Of the four main aims for the meeting, the first objective was to present and discuss recent advances in the broad area of HIV cure research.

Matthieu Perreau (University Hospital of Lausanne, Switzerland) [1] demonstrated that lymph node PD-1+ Tfh cells may serve as a major CD4+ T cell compartment for the production of replication-competent and infectious HIV in long-term antiretroviral therapy (ART)-suppressed participants. These cells may present an obstacle for HIV functional cure strategies given that these cells are sequestered away from cytotoxic CD8+ T lymphocyte surveillance. These data are supported by recently published work demonstrating that replication competent SIV in elite controllers is restricted to this Tfh population [2].

Richard Koup (NIAID, National Institutes of Health) then presented data suggesting a novel strategy to target HIV in these Tfh cells using broadly neutralising antibodies (bNAbs) to detect and kill HIV-expressing cells before they can produce infectious virus [3]. He discussed using bNAbs to isolate individual HIV-expressing CD4+ T cells with a high degree of certainty *ex vivo*. Thus, using bi-specific antibodies (VRC07 bound to anti-CD3 antibody), HIV-infected CD4+ T cells might be targeted by redirecting bulk CD8+ T cells to lymph node germinal centres.

Joseph Wong (University of California, San Francisco) reviewed prior data demonstrating that the HIV reservoir is greatest in tissue (e.g. lymph nodes, gut-associated lymphoid tissue) compared to blood (e.g. resting CD4+ T cells) [4]. He described a novel collaboration with the San Francisco Medical Examiner's Office to obtain post mortem samples. Samples from individuals who had experienced 'sudden death' without a long period of illness provide a unique opportunity to perform a comprehensive survey of tissue reservoirs. Preliminary data suggest that low levels of HIV RNA and DNA are detectable in many tissues including frontal brain, paratracheal, subclavian, hilar, periaortic, mesenteric, inguinal, distal ileum and sigmoid colon lymph nodes.

Robert Siliciano (Johns Hopkins University) reviewed the current challenges in accurately measuring the HIV reservoir – including gross underestimation of the inducible latent reservoir size with the 'gold standard' viral outgrowth assay and overestimation using total HIV-1 DNA levels by quantitative PCR [5]. Using analytic treatment interruptions to determine time to viral rebound as a surrogate for HIV reservoir size carries clinical risks and may be an insensitive measure of the HIV reservoir. Based on estimations by Hill *et al.* [6], there are enormous variations in time to rebound for reasons of inter-individual variability and the stochastic nature of viral rebound. He showed data demonstrating that defective proviruses accumulate rapidly even in acute HIV

*Corresponding author: Sulggi A Lee, Division of HIV/AIDS

University of California, San Francisco
995 Potrero Avenue Building 80, Box 0874, San Francisco, CA 94110
Email: sulggi.lee@ucsf.edu

infection. Therefore, although DNA PCR assays are widely used for reservoir analyses, they may not be ideal for monitoring HIV cure interventions since defective viruses may not produce infectious virus.

Young investigators

The second objective was to encourage students and junior scientists within or outside the HIV community to become involved in HIV cure research. During each session, young investigators had the opportunity to present original research in short talks and poster presentations that allowed them to interact with senior-level investigators. There were several presentations by young investigators that highlighted key topics during the meeting.

Gregory Del Prete (AIDS and Cancer Virus Program, Frederick National Laboratory) presented several studies demonstrating the challenges of non-human primate (NHP) models of HIV latency [8]. These challenges include differences in drug availability, pharmacokinetics, toxicity, drug delivery, drug availability, virus and sustainability of ART suppression in monkeys. His group's work demonstrated that combination ART is feasible, effective (even with a simple, co-formulated triple-therapy regimen of tenofovir, emtricitabine, and dolutegravir), and sustained (as long as 2 years in one cohort studied). Experiments using the HDACis vorinostat and romidepsin produced similar increases in acetylated histone levels and viral transcriptional ratios as seen in human clinical trials.

New knowledge from other fields

The third objective was to bring new knowledge in topics such as lymphocyte homeostasis, macrophage biology and metabolic imaging to the HIV cure research community, thus opening new axes of research in HIV persistence.

Rafick Sékaly (Case Western Reserve University) presented a systems biology approach to identify latently infected cells such as PD-1+, LAG-3+ and TIGIT+ expressing CD4+ T cells enriched for integrated HIV DNA [9]. These cells appear to be regulated by the transforming growth factor-beta signalling pathway, regulating cell cycle, metabolic, inflammatory and nuclear receptor pathways involved in controlling the latent HIV reservoir size.

Jason Brenchley (NIAID, National Institutes of Health) and **Serge Benichou** (Cochin Institute, France) presented opposing data regarding whether macrophages represent a site of HIV latency or simply represent phagocytosis of HIV-infected T cells. Dr Benichou presented *in vitro* data suggesting that macrophages may be directly infected by cell-to-cell transfer from infected CD4+ T cells [10], while Dr Brenchley demonstrated that myeloid cells found to contain viral DNA in an NHP model were only attributed to phagocytosis of SIV-infected T cells, not direct infection of macrophages [11].

Ronald Germain (NIAID, National Institutes of Health) described novel imaging techniques for visualising immune cells *in vivo* [12]. Using multiphoton intravital microscopy he demonstrated the microanatomy of CD4+ and CD8+ T cell interactions in a mouse lymph node, and using 'histo-cytometry', or flow cytometry gating applied to data derived from immunofluorescent analysis of stained tissue sections, demonstrated specific myeloid cell localisation patterns. He also discussed the further application of this method to 3D analysis of human surgical or biopsy specimens.

Next-generation drugs and strategies

The fourth aim was to gather scientists, physicians and pharmaceutical firms together in order to create new collaborations and discuss the next generation of drugs and therapeutic strategies that could be used in HIV cure.

Thomas Rasmussen (Aarhus University Hospital, Denmark) summarised several latency reactivating agent (LRA) studies, performed in collaboration with pharmaceutical firms, evaluating HDACis [13]. Despite latency reversal, vorinostat, panobinostat or romidepsin demonstrated no durable effect on reducing the size of the HIV reservoir, and recent data suggest that HDACis may impair T cell immunity [14]. However, Dr Rasmussen presented clinical data demonstrating no adverse effect on T cell responses and referenced previously presented *in vitro* data suggesting that the combination of HDACis and PKC agonists may prove more effective in reducing the reservoir size [15], although safety is a concern. Finally, he concluded his talk emphasising the great need for well-designed studies combining the strengths of academic research and industry.

Paula Cannon (University of Southern California) demonstrated several experiments performed in collaboration with Sangamo Incorporated using targeted nuclease to create sequence-specific DNA breaks to control or eliminate HIV [16].

References

Unless other details are given, all talks and abstracts were presented at the Keystone Symposium on HIV Persistence 2015. April 2015. Boston, MA, USA. The abstract book is available from www.keystonesymposium.org. All speakers and authors have given their permission for their report to be published.

1. Perreau M. Short talk: lymph node PD-1+/Tfh memory CD4 T cells are the major source of HIV-1 replication competent and infectious virus in long-term treated aviremic HIV-infected subjects.
2. Fukazawa Y, Lum R, Okoye AA *et al*. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. *Nat Med* 2015; **21**: 132–139.
3. Koup R. Tfh cells are a site of active HIV replication.
4. Wong J. Characteristics of tissue HIV reservoirs *in vivo*.
5. Siliciano RF. HIV reservoirs: what we know and what we don't know.
6. Hill AL, Rosenbloom DI, Fu F *et al*. Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1. *Proc Natl Acad Sci U S A* 2014; **111**: 13475–13480.
7. Euler Z. Eradication of HIV reservoirs with antibody-mediated killing.
8. Del Prete G. Development and utilization of non-human primate models of combination antiretroviral therapy for studies of HIV functional cure/eradication.
9. Sékaly R. Promoting CD4 differentiation.
10. Benichou S. Short talk: productive HIV-1 infection of macrophages by viral cell-to-cell transfer from infected CD4+ T lymphocytes.
11. Brenchley J. Phagocytosis of SIV-infected T cells can explain viral DNA within myeloid cells *in vivo*.
12. Germain R. Imaging immunity: creating a spatiotemporal understanding of host defense.
13. Rasmussen T. Reversal of HIV-1 latency by HDAC inhibitors: part of a curative strategy?
14. Jones RB, O'Connor R, Mueller S *et al*. Histone deacetylase inhibitors impair the elimination of HIV-infected cells by cytotoxic T-lymphocytes. *PLoS Pathog* 2014; **A**: e1004287.
15. Laird G, Bullen CK, Martin A *et al*. *Ex vivo* identification of highly effective latency-reversing drug combinations. *Conference on Retroviruses and Opportunistic Infections*. February 2015. Seattle, WA, USA. Abstract 419.
16. Cannon P. Targeted nuclease approaches for a functional cure.