



HAL
open science

Highlights from the Inaugural HIV Reservoirs and Immune Control Conference, October 1st–4th 2023, Malahide Ireland

Una O’Doherty, Javier Martinez Picado, Asier Saez-Cirion

► **To cite this version:**

Una O’Doherty, Javier Martinez Picado, Asier Saez-Cirion. Highlights from the Inaugural HIV Reservoirs and Immune Control Conference, October 1st–4th 2023, Malahide Ireland. Highlights from the Inaugural HIV Reservoirs and Immune Control Conference, October 1st–4th 2023, Malahide Ireland, Pathogens and Immunity, 8 (1), pp.161-169, 2023, 10.20411/pai.v8i1.653 . pasteur-04599418

HAL Id: pasteur-04599418

<https://pasteur.hal.science/pasteur-04599418v1>

Submitted on 3 Jun 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Highlights from the Inaugural HIV Reservoirs and Immune Control Conference, October 1st- 4th 2023, Malahide Ireland.

Una O'Doherty¹, Javier Martinez Picado², Asier Saez Cirion³

¹ Department of Pathology and Laboratory Medicine, University of Pennsylvania

² Retrovirología y Estudios Clínicos, IrsiCaixa

³ Viral Reservoirs and Immune Control Unit, Institut Pasteur

Abstract: The inaugural FASEB HIV Reservoirs and Immune Control Conference brought researchers together from across the globe to discuss reservoir dynamics in clinical cohorts. The conference extended over four days in the seaside town of Malahide Ireland. The eight scientific sessions covered a broad range of topics including HIV pathogenesis and control, reservoirs and viral expression, pediatric reservoirs, innate immunity and B cell responses, environmental factors of pathogenesis, loss of virologic control, and HIV-2. The following article provides a brief summary of the meeting proceedings.

Guido Silvestri of Emory University opened the meeting with the Keynote. He gave a historical perspective of pioneering work that uncovered CD8 T cells can have suppressive role on HIV transcription in addition to their well described killing activity. One of the first clues that CD8s were suppressing viremia came from CD8 depletion studies, which showed reactivation of virus production in SIV-infected ART-treated rhesus macaques. Importantly CD4 depletion did not result in viremia suggesting this CD8 effect was not simply due to cellular proliferation following lymphodepletion. This interpretation was bolstered by evidence that latency reversal by agents such as N803 and AZD5582 is more frequently detected when CD8s are depleted in rhesus macaque studies. Overall, this data suggests that CD8 suppression could play a role in latency establishment and maintenance which they are studying using an in vitro model. A long-term objective is to block CD8 T cell suppression while stimulating CTL clearance. (doi: 10.1038/s41586-020-1946-0; 10.1038/s41586-020-1951-3; [10.1038/s41591-023-02570-7](https://doi.org/10.1038/s41591-023-02570-7); 10.1016/j.immuni.2023.03.010.

The first two sessions of the meeting focused on **HIV Pathogenesis and Control** and covered a wide range of approaches including monitoring of Post-Treatment Controllers over time, probing mechanisms of CD4 decline, and identifying correlates of immune control.

Stephen Migueles, National Institutes of Health, discussed lessons learned from studying natural control of HIV that could be applied to vaccine design. He provided experimental evidence that degranulation is the best predictor of control. In vitro analysis of the Ad5/HIV vaccine trial demonstrated that this vaccine failed to induce high-level CD8+ T Cell responses despite cytotoxic protein expression. Moreover, the effector CD8+ T cells degranulated poorly due to low functional avidity. He concluded that future vaccine strategies should include assays to measure degranulation efficiency

as a surrogate of strong CD8 T cell responses. He also advocated for vaccines with a persistent vector or low MHC ligands to select for high-avidity responses.

Laurent Hocquelox, Chu d'Orléans, provided an update on his longstanding cohort of Post-Treatment Controllers (PTC). He discussed the first 30 cases that were enrolled in the VISCONTI cohort which have now been followed for 20 years. He proposed the reservoir in post-treatment controllers resides in T cells with shorter half-lives. Intriguingly, these individuals lack strong CD8s and are associated with HLA-B35. While HLA-B35 is associated with rapid progression, it is also associated with KIR education of NK cells.

Bonnie Howell, Merck, presented approaches that her team at Merck are taking to reduce the reservoir. A major focus of her team is to exploit Targeted Activators of Cell Kill (TACK). These molecules are designed to induce cell death of infected cells. Currently, the team are keen to exploit the pathway where nonnucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz and rilpivirine can prematurely activate intracellular protease and cleave the Caspase Recruitment Domains- Containing Protein 8 (CARD8) inflammasome leading to cell death. A compound screen for TACK activity revealed a pyrimidone compound (Pyr01) with a strong ability to kill HIV infected cells without decreasing overall cell viability. She also covered the use of dipeptidyl peptidase 9 (DPP9) inhibitors to activate CARD8 and shared that In vitro assays have shown efavirenz works synergistically with DPP9 inhibitors to kill infected cells.

Liang Shan, Washington University, elaborated on his discovery that CARD8 inflammasome activation plays a central role in HIV pathogenesis. This work may explain the longstanding paradox: there is a massive loss of CD4 T cells in the setting of rare infected T cells. The CARD8 inflammasome is activated immediately after HIV fusion and occurs even when reverse transcription is inhibited. Activation of this pathway may underlie accelerated CD4 loss. Mechanistically, CARD8 is activated immediately after viral fusion by viral protease leading to rapid pyroptosis. Moreover, loss-of-function mutations are found in Sooty mangabeys which may explain why these infections are nonpathogenic in these natural hosts.

Mirko Paiardini, Emory University, described a new SIV-specific lymphoid tissue CD8 T cell subset, expressing both TCF-1 and CD39, that exhibits effector and stem-like profiles. He showed the frequency of this population in rhesus macaques increases after SIV infection. While this population phenotypically carries exhaustion markers and expresses low levels of granzyme B, it degranulates and produces interferon- γ when exposed to SIV peptides. The frequency of these cells correlates inversely with viral load in viremic animals and reservoir size in ART-treated rhesus macaques.

The third scientific session on **Reservoirs and Viral Expression** highlighted emerging evidence that the reservoir is neither silent nor stable.

Una O'Doherty, University of Pennsylvania, presented evidence from her group that the level of naïve infection predicts the size and diversity of the HIV reservoir. She compared a group of elite controllers and chronic progressors and found elite controllers were unique for the low level of naïve infection and the high levels of clonal sequences. She then compared proviral turnover in elite controllers and chronic progressors using Venn diagrams to capture clonal persistence over time. This analysis revealed that proviral turnover appeared slower in elite controllers. Overall, individuals with higher levels of naïve T cell infection have higher proviral turnover. This result initially appears to be counterintuitive since naïve T cells have a longer intermitotic half-life. A model invoking reservoir expression by memory T cells was proposed to resolve this conundrum: infected naïve T cells differentiate into memory T cells that express HIV and therefore should turnover more rapidly than their uninfected memory counterparts. This model is consistent with continual selection over time (doi: 10.1172/jci.insight.150794).

Mathias Lichterfeld, Harvard University, reviewed a body of work from his group that the reservoir is often transcriptionally active which provided additional evidence of a highly active and dynamic reservoir. Using sophisticated molecular techniques, his team characterizes proviruses over time on ART. His major evidence includes that proviruses that are detected after years of ART are often localized in gene deserts and are less transcriptionally active. This selection is more apparent in EC, but it is also apparent in some individuals that have been on ART for more than two decades.

Expanding on the prior talks, **Alexander Pasternak**, Amserdam UMC, provided complementary evidence that the reservoir is more actively expressed than previously appreciated. He compared HIV cell-associated RNA expression of HIV-infected cells in 3 cohorts: elite controllers off ART, ART-naïve, and individuals on ART. Unique to this study, Dr. Pasternak carefully determined the efficiency for every step of the process of measuring HIV RNA. Using limiting dilution RT-PCR, he then quantified the frequency of infected cells expressing HIV RNA and a per-cell HIV expression level. With this approach, he revealed a remarkably high frequency of HIV RNA-expressing cells among total infected cells in ART-treated individuals.

Alberto Bosque, George Washington University, discussed work utilizing his recently developed TRACER assay for Translational CompEtent Reservoir assay. This technology is based on a planar array ultrasensitive p24 Gag ELISA. Using this technology, the Bosque team identified a strong negative correlation between CD8 T cell count and IL-15 reactivation and a positive correlation between CD4/CD8 ratio and IL-15 reactivation in ART suppressed patients (n=12). Overall, the TRACER assay looks to be a promising tool for reservoir assay.

The session culminated with an animated talk by **Daniel Reeves** from Fred Hutchinson Research Center. Specifically, Dr. Reeves presented an exciting model to capture the contribution of CD4 T cell subsets to HIV reservoir persistence using a beautiful animation. His model revealed a near equilibrium state of differentiation and death with T_{CM} , T_{TM} , T_{EM} contributing 10-100 fold more DNA to the reservoir than T_N or T_{SCM} . The best models for predicting HIV integrated DNA levels were based on cellular

proliferation to a greater extent than differentiation. Taken together this work suggests that inhibiting cellular proliferation and cellular differentiation may lead to significant reductions in reservoir size. His paper on the topic was recently published in Nature Communications (DOI: [10.1038/s41591-023-02570-7](https://doi.org/10.1038/s41591-023-02570-7)).

The oral abstract session on **HIV Reservoirs and Latency** focused on new cells that contribute to the reservoir, the role of BRD4 and lncRNA Cytos in perturbing HIV expression.

Later that evening **Riddhima Banga**, Lausanne University Hospital, presented evidence that the reservoir extends to lymph node DCs. These DCs contain intact replication competent HIV. Her data suggest this was not due to DC-T cell conjugates since T cell receptor excision circles (TRECS) were not detected. This work was recently published in Cell Reports (DOI: [10.1016/j.chom.2023.08.020](https://doi.org/10.1016/j.chom.2023.08.020)).

Ran Taube, Ben Gurion University, reported on Cytos a lncRNA that activates HIV expression by binding to the HIV promoter and associating with positive transcription elongation factor B (PTEFb). In Jurkat T cells, activation profoundly increases Cytos expression while depletion of Cytos induced global changes in cellular RNA expression including actin pathways.

Two drugs that perturb the reservoir were discussed by **Eline Pellaers** from Zeger Debyser's group in KU Leuven. She compared the effects of two drugs that perturb Bromodomain-containing protein 4 (BRD4, JQ1 versus ZL0580). ZL580 increased the localization of BRD4 with acetylated histones and was associated with decreased transcription. It has additive effects with Lens epithelium-derived growth factor inhibitors (LEDGINs) suggesting there may be potential to target this pathway for block and lock approaches.

The second day started with a focus on **Pediatric Infection**. All speakers emphasized the need to extend reservoir studies to children who are not "little adults".

Philip Goulder, Oxford University, reported an exciting observation in South African mother-child pairs observing that male fetuses are less susceptible to *in utero* transmission than females. They found that the virus transmitted to male fetuses was more IFN-I sensitive and of higher viral replication capacity (VRC) than those transmitted to females. In this context he proposed that some very early cART-treated children – especially males - infected by significantly low replication capacity viruses could achieve post-treatment control without additional interventions. Overall, he suggested that early-life innate immune sex differences modulate vertical transmission, which may be critical to [future interventions designed to](#) optimize cure potential in children born with HIV.

Ann Chahroudi, Emory University, made the case for studying reservoirs that form during the neonatal period given the important differences between the adult and neonatal immune system. She emphasized the dominance of naïve T cell infection especially in children who become infected from maternal fetal transmission as a critical

distinction. Her team is using Positron Emission Tomography (PET) scans to track HIV reservoir expression. She highlighted several examples where outcomes in pediatric studies differ from adults, reinforcing the importance of studying reservoirs after neonatal infection.

The role of inhibitory receptors was explored in children infected with HIV by **Hugo Soudeyans**, Centre de recherche du CHU Sainte-Justine. While inhibitory receptors are known to correlate with reservoir size in adults this has not been studied in vertical transmission cases. He described a unique cohort of individuals infected vertically that were enrolled in Canada, termed EPIC for Early Pediatric Initiation-Canada Child Cure Cohort Study. Markers of exhaustion on CD4s and CD8s were found to correlate with reservoir size. However, this correlation was particularly strong for exhaustion markers especially PD1 on CD8+ T cells.

The fifth session took a break from T cell biology to explore the impact of **Innate Immunity and B cell responses** on the HIV reservoir.

Paula Cannon, University of Southern California, provided an overview of her work on engineering B cells to express custom antibodies based on heavy chain only antibodies (HCAb) found in camelids. She emphasized the flexibility of the platform including the ability to accommodate non-antibody molecules and modify the Fc region. She welcomes collaborators with new ideas.

Compelling evidence that NK cells play a role in reservoir size was presented by **Maria Buzon** from Vall d'Hebron Research Institute, . She first showed that a designer antibody capable of binding envelop activates NK cells and enhances antibody-dependent cell-mediated cytotoxicity (ADCC) killing of infected cells after latency reversal. This antibody consists of an Fc portion capable of binding Fc receptors on NK cells and a binding domain capable of binding the HIV envelope. However, in her mouse model the treatment failed yet provided new insights. Specifically, she demonstrated this antibody expanded memory like NK cells, but reduced NK cells overall, resulting in larger reservoirs. Thus, the experiments provided a proof of concept that NK cells likely have a role in reservoir size and represent an important target.

The oral abstract session **New Approaches to Studying HIV Reservoirs** included a barcoded virus and imaging strategies to study nuclear import.

Alfonso Oceguera, University of Pennsylvania, shared exciting evidence that HIV infected cells die and divide faster than uninfected cells. Infected resting CD4+ T cells were infected with a barcoded virus and monitored for the frequency of clonal proviruses over time while simultaneously tracking cell division by eFluor. Using three complimentary approaches, he showed that the division rate of the uninfected cells was much slower than that of the infected cells.

The role of several cellular proteins in nuclear import was explored by **Wout Hannes**, KU Leuven, from Zeger Debyser's group. These factors include Transportin SR2, Transportin 1 and Cleavage and polyadenylation specificity factor 6. After depleting

these proteins in cell lines, he infected cells with fluorescent virions prior to imaging. He thereby demonstrated there was a delay in nuclear import. Moreover, there was a change in integration sites selection as demonstrated by sequencing. He concluded that these three proteins have a role in nuclear import and integration site selection by HIV.

Session six **Environmental Factors of Pathogenesis and Control** covered correlates of HIV rebound include glycans (**Mohamed Abdel Mohsen**), cell free DNA as a marker of immune control (**Satish Pillai**) and the role of inflammation in pathogenesis.

Jean-Pierre Routy, McGill University Health Centre, discussed evidence that acyl coA binding protein is a checkpoint inhibitor for autophagy and may provide a new target for increasing autophagy. His team found elevated acyl coA binding protein in people living with HIV treated with ART is associated with inflammation and T cell dysfunction. Given that activated autophagy and low glycolysis is associated with strong CD8+ T cell responses, this could lead to new therapies to increase these responses.

Priscilla Hsue, University of California San Francisco, builds the case that chronic inflammation is a major contributor to cardiovascular disease in chronic viral infection. This hypothesis is supported by the correlation between the level of HIV transcription and reservoir size with atherosclerosis (McLaughlin et al 2020). Moreover, elevated levels of IL-6 also play a role as they are associated with adverse cardiac outcomes in individuals with HIV or long-COVID syndrome. The increased risk of cardiovascular disease has historically been attributed to toxicity of antiretrovirals; however, there is evidence for HIV effects including elevated levels of IL-6 and a thicker intima-media of the carotid arteries. There are also parallels to individuals with long-COVID since elevated IL-6 is associated with reduced exercise capacity and chronotropic incompetence. Thus, anti-inflammatory therapies should be investigated to treat chronic inflammation in the setting of viral illness (doi:10.1001/jamanetworkopen.2020.19099).

The day ended with another captivating math lecture by **Ryan Zurakowski** from the University of Delaware, on lymph node reservoir dynamics. He **presented** the virtual lymph node model that his team developed to study antiretroviral kinetics. Using fine and coarse meshes, the team model drug concentrations throughout the lymph node. His team then test the utility of their model using perfused ex vivo human lymph nodes.

The last day started with a series of lectures on **Immune Control and loss of control in HIV-1 and HIV-2**.

Ana Espada de Sousa, Instituto de Medicina Molecular, captured the attention of her audience by sharing her enthusiasm for the study of HIV-2 infection. She compared the pathogenesis of HIV-2 to HIV-1, pointing out the challenges of treating HIV-2 infected individuals with undetectable or near undetectable HIV-2. Lisbon, Portugal is a center for HIV-2 research due to its history of immigration from West Africa in the late 1990s. There are similarities between individuals infected with HIV-2 and elite controllers as they have better virologic control and very low rates of disease progression. She

presented work that the GI tract mucosa is maintained in HIV-2 compared to HIV-1 but that there is disruption of lymph node architecture. She emphasized the distinct role of Vpx which is preserved in HIV-2 only, resulting in lower levels of SAMHD1 in HIV-2 infected cells. Notably, HIV-2 infected individuals have stronger CD8 responses possibly related to IFN- α stimulation of the cyclic GMP-AMP synthase stimulator of interferon gene (cGAS STING) signal transduction which is supported by data from Victor Appay.

Using an in vitro model, **Victor Appay**, University of Bordeaux, probed induction of antigen specific responses to HIV-1 vs HIV-2. HIV-2 produced antiviral CD8 T cells more effectively than HIV-1. Overall, T cell responses were dependent on type I interferon which activated the cGMP-AMP synthase cGAS/STING pathway.

Additional evidence that actin pathways play important roles in HIV was illuminated by **Robert Furler-O'Brien**, Weill Cornell Medicine, using time lapse microscopy. Among HIV infected cells, he showed two morphologies that resemble cytoskeletal disease involving ARP2/3. Intriguingly, these pathways are partially Nef dependent and less apparent in a subset of elite controllers.

The conference ended with a session on **the role of ART, early ART and new drugs in HIV therapy**. This session discussed the role of ART in elite controllers, the ability of early ART to preserve naïve T cell phenotype and function, and the potential for tyrosine kinase inhibitor intended to treat CML to reduce the HIV reservoir.

The difficult decision “to treat or not to treat” elite controllers was explored by **Nicolas Noel** from Paris-Saclay University. He discussed the heterogenous spectrum of controllers, spanning those with complete viral control and preserved immune systems to those who show evidence of losing control. Overall, those individuals with evidence of losing control will clearly benefit from antiviral therapy; however, the need for antiretroviral therapy in those with undetectable viral loads and small reservoirs is less clear.

The effect of early antiretrovirals on the naïve compartment was studied by **Robert Badura at the** Universidade de Lisboa. When individuals were treated very early after infection their naïve T cells were indistinguishable from healthy controls. Specifically, in typical HIV-1 and HIV-2 infection, CXCR3 naïve and stem cell memory are expanded. This expansion is not detected in individuals who are treated early after infection. It was also noted that HIV-2 infected individuals have an expanded CXCR3 regulatory naïve population with elevated CD95, CD23, and FOXP3 expression.

Mayte Coiras, Instituto de Saude Carlos III, reviewed a clinical case of a patient with HIV who was diagnosed with CML and treated with the tyrosine kinase inhibitor dasatinib. The patient experienced a remarkable 2 log reduction in HIV DNA. Moreover, lower HIV DNA persisted after dasatinib discontinuation for ~3 months at which point the patient had CML relapse and was restarted on dasatinib. Clinical trials are planned to evaluate more individuals with HIV on dasatinib therapy.

All summarized presentations and abstracts included in this article were done so with the express permission of the author.