

## Invited Review

# CROI 2017: Basic Science Review

Mario Stevenson, PhD

*The Conference on Retroviruses and Opportunistic Infections (CROI) continues to be the preeminent gathering for HIV/AIDS researchers. It provides a forum in which investigators involved in all aspects of research on HIV/AIDS and related conditions can present and be updated on the latest scientific developments in the field. In 2017, basic research continued to be strongly represented at the conference. Most of the presentations focused on mechanisms of viral persistence in the face of effective antiretroviral therapy as well as approaches to measuring viral reservoirs and intervening in those reservoirs. The intermix of basic and clinical research presentations provided a dynamic forum for the sharing of knowledge that is crucial to moving the field forward.*

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### Viral Reservoirs and Mechanisms of Antiretroviral Therapy Persistence Under Antiretroviral Therapy

HIV/AIDS researchers are exploring various approaches aimed at achieving a cure to HIV infection. Cure can be defined as a functional cure in which the virus is still present but is being contained in such a way that control of the virus does not require antiretroviral therapy and that the infected individual is unable to transmit the virus. A sterilizing cure is one in which all vestiges of replication-competent virus have been eliminated. Under the definition of a sterilizing cure, some residual proviral DNA may remain. However, that DNA may be defective and, as such, unable to drive production of infectious virus. Development of approaches that generate a functional or a sterilizing cure will require a better understanding of

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the mechanisms of HIV-1 persistence under effective antiretroviral therapy. Although there has been great progress in defining the processes that underscore HIV-1 persistence and the cellular reservoirs that sustain this persistence, there is still much to be learned.

In terms of the cells supporting HIV-1 persistence, much of the focus has been on peripheral blood-derived CD4+ T cells where numerous studies have demonstrated the presence of viral genomes in a latent state (one in which there is no virus production unless the cell is stimulated with agents that induce host cell activation). The latent viral reservoir in long-lived memory CD4+ T cells may provide lifelong persistence and is the single biggest obstacle to viral eradication.

As important to the identification of the cellular reservoirs sustaining HIV-1 persistence under effective antiretroviral therapy are the methods employed to measure those reservoirs. This aspect of cure research has been particularly challenging. Methods used to measure the latent reservoir are labor intensive, lack precision, and require a large number of cells.

The limitations to assays currently being used to track reservoir size will complicate attempts to monitor the effectiveness of strategies to eliminate those viral reservoirs. Kaufman and colleagues (Abstract 46) described a highly sensitive approach to simultaneously detect HIV-1 Gag protein and RNA in clinical samples. The method is a variation on RNA flow-fluorescence in situ hybridization (Flow FISH).<sup>1</sup> CD4+ T cells from leukapheresis samples are negatively selected using magnetic beads and then allowed to remain quiescent overnight or stimulated with latency reactivating agents. The cells are subsequently intracellularly stained for HIV-1 Gag and incubated with oligonucleotide pairs that recognize conserved sequences in the HIV-1 genome. The next steps involve amplification (branching) and labeling that amplifies the signal several thousand fold. The dual RNA and protein staining achieved by this approach brings substantial specificity to the detection of reservoir cells and allows detection of as few as 1 infected cell per million CD4+ T cells.

The assay was used to compare the frequency of infected cells in HIV-1 infected, untreated subjects, with subjects on antiretroviral therapy before and after stimulation *ex vivo* with phorbol<sup>12</sup> myristate<sup>13</sup> acetate (PMA) and ionomycin. For individuals with chronic infection, RNA/Gag-positive cells, in the region of 1 to several hundred or million CD4+ T cells, could be detected. The number increased 3-fold with PMA/ionomycin stimulation. Surprisingly, for individuals on suppressive antiretroviral therapy, in half of the individuals, low numbers of RNA/Gag-positive cells (1-10/million CD4+ T cells) were detectable prior to PMA/ionomycin stimulation. With stimulation with PMA/ionomycin, there was a 10-fold increase in the frequency of RNA/Gag-positive cells from

Dr Stevenson is Professor of Medicine and Chief of the Division of Infectious Diseases at the University of Miami Medical School in Miami, Florida. Send correspondence to Mario Stevenson, PhD, University of Miami Leonard M. Miller School of Medicine, Life Science Technology Park, 1951 NW 7th Avenue, Rm 2331B, Ste 200, Miami, Florida 33136.

aviremic participants. Comparison of RNA/Gag Flow FISH assay with measures of proviral and total DNA indicated that DNA-positive cells were 2 to 3  $\log_{10}$  more abundant than RNA/Gag-positive cells. This is most likely explained by the high frequency of defective proviruses present in infected individuals. Although reservoir measurements of viral DNA are technically straightforward, the fact that most proviruses are defective limits the ability of DNA measurements to inform on the size of the biologically active reservoir, that is, those cells harboring replication-competent virus. For individuals on antiretroviral therapy, there was, as expected, a correlation between the frequency of RNA/Gag-positive cells and integrated DNA copies. The RNA/Gag Flow Fish assay is an important advance in the technologies that can be applied to viral reservoir measurement.

Cells harboring viral RNA and *gag* are more likely to represent cells harboring functional proviruses as opposed to cells identified on the basis of viral DNA positivity. The high sensitivity and specificity of the approach will allow small changes in viral reservoir size to be tracked, which will be crucial for gauging the efficacy of cure strategies. Still remaining is the limitation that the RNA/Gag Flow FISH assay requires large numbers of cells such that leukapheresis is necessary. In addition, there was a surprisingly poor correlation between the RNA/Gag Flow FISH assay and the quantitative viral outgrowth assay (qVOA) that is considered the “gold standard” approach for measurement of the biologically active viral reservoir. Similarly, there was a poor correlation with the *Tat/Rev*-induced limiting dilution assay (TILDA), in which the post-treatment changes in viral RNA levels are gauged with latency-reversing agents (LRAs). Until reasons for the differences are understood, parallel use of several viral reservoir assays will likely be needed to gauge reservoir size. Nevertheless, the ability to track the frequency of RNA- and *gag*-positive cells before and after use of LRAs provides a powerful addition to the cure researcher’s toolbox.

Kearney (Abstract 47) provided in-depth insight into the role of expanded clones in viral persistence. The study by Chomont, Sekaly, and colleagues indicated that HIV-1-infected cells may persist through homeostatic proliferation

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that would allow duplication of proviruses between daughter cells produced at mitosis.<sup>2</sup> Several groups followed up these initial observations by demonstrating the existence of clonally expanded proviruses that arose as a result of numerous proviral duplication events from the proliferation of infected cells. A central question is the extent to which proviral expansion through homeostatic proliferation contributes to the maintenance of a biologically active viral reservoir.

Two factors would work against the ability of homeostatic proliferation to maintain the viral reservoir. First, the vast

majority of proviruses in infected individuals contain APO-BEC3G-mediated hypermutations as well as premature stop codons and deletions that render the provirus nonfunctional. Second, the process of cell division may drive proviral gene expression, which would lead to destruction of the host cell by viral cytopathicity or host-mediated immune clearance. Kearney examined whether expanded proviruses were replication competent and whether cells harboring those proviruses also harbored viral RNA transcripts. The study focused on previously described individuals in whom expanded clones represented the major population of proviruses. Previous studies from the group of Maldarelli and Hughes identified a clonally expanded provirus (AMBI1) that was highly represented among clonal proviral populations in an infected individual.<sup>3</sup> Viral outgrowth assay (VOA) results indicated that AMBI1 produced replication-competent viruses. Among the cells that harbored clonally expanded proviruses, approximately 4% also expressed viral RNA that was similar to the frequency of RNA-expressing cells harboring defective proviruses. This further indicated that more than 90% of replication-competent proviruses within cells were in a latent state.

The authors next determined whether there were any differences between blood and lymph nodes in the frequency of cells harboring replication-competent, expanded proviruses or in the frequencies of those cells expressing viral RNA frequency. The same expanded clones were found in CD4+ T cells from blood and lymph nodes. Furthermore, a larger proportion of expanded proviruses in lymph node CD4+ T cells expressed viral RNA than in blood CD4+ T cells. These results beg the question as to how cells harboring expressed, clonally expanded proviruses survive over time. Kearney offered the hypothesis that such clonal populations persist because the majority of cells harboring expanded proviruses are in a latent state. Thus, although the subpopulation of RNA-expressing cells are likely to be cleared by viral cytopathicity and immune surveillance, there is a steady state maintained by the continuous expansion of clones through homeostatic proliferation.

There are important implications to these findings. Therapies that promote killing and clearance of RNA-expressing cells would limit the maintenance of the clonally expanded reservoir and, perhaps, of the cells harboring unique proviruses (if the frequency of RNA-expressing cells is a finite property of infected cells). It remains to be determined what dictates the frequency of expanded clones that express viral RNA or what triggers promote proviral expression in infected cells. Those triggers can be thought of as natural latency reactivation processes because they achieve the desired effect of promoting clearance of the infected cell. It is possible that intervals of RNA expression is a consistent feature of all infected cells. As such, the reservoir may be more dynamic and subject to greater degrees of turnover than previously suspected. This, in turn, bodes well for viral cure efforts.

Picker (Abstract 49) discussed the role of therapeutic vaccination in viral cure strategies. Kearney’s earlier talk in the same session raised the possibility that continuous maintenance of a fraction of infected cells that express viral

RNA renders the viral reservoirs susceptible to immune surveillance and cytopathicity. This provides the rationale for the use of therapeutic vaccines that could potentially promote the elimination of reservoir cells actively expressing viral antigens. Picker's group previously demonstrated that

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when macaques were vaccinated with Rhesus cytomegalovirus (RhCMV) vectors expressing simian immunodeficiency virus (SIV) antigens and then infected with SIV, the engendered immune responses not only arrested viral spread, but also led to elimination of the viral reservoirs. This surprising result suggests that the RhCMV vector-elicited immune responses were effective in clearing a stable SIV reservoir, or alternatively, that the viral reservoir was unstable and arrest of viral spread by RhCMV vector-driven immune responses led to its decay and clearance.

To distinguish between these possibilities, animals were infected with SIV and placed on early antiretroviral therapy. Animals were then challenged with RhCMV SIV vectors. On discontinuation of antiretroviral therapy, time to rebound correlated with the timing of antiretroviral therapy initiation, with the greatest delay in viral rebound occurring in the animals receiving earliest antiretroviral therapy. In addition, some animals receiving early antiretroviral therapy did not rebound. This was due to elimination of the viral reservoir in those animals as opposed to immune-mediated elite control. Although adoptive transfer from cleared animals to uninfected monkeys failed to transmit infection, low levels of residual viral DNA and RNA could be detected in tissues of monkeys that failed to rebound following discontinuation of antiretroviral therapy. In one monkey receiving antiretroviral therapy 6 days postinfection that had apparently cleared the viral reservoirs (no infection after adoptive transfer), there was a rebound in viral replication 8 months after the antiretroviral therapy. The results indicate that the reservoir established early (within the first 4-5 days) is short lived and if virus spread is blocked by RhCMV SIV immune responses, clearance is achieved within 6 months. However, if the initiation of antiretroviral therapy is delayed by as little as 1 day, the reservoir is more permanent and is not cleared. Taken together, the data argue that immune responses engendered by RhCMV SIV do not enhance reservoir clearance, but rather arrest viral spread prior to establishment of a long-lived reservoir. The results highlight the differences in the control of primary infection versus post-antiretroviral therapy reactivation.

The determinants that dictate viral rebound kinetics after treatment interruption are not well understood. It has been suggested that the time to rebound is an indicator of viral reservoir size. For example, studies from Li and colleagues have demonstrated a direct relationship between time to rebound and levels of cell-associated viral RNA.<sup>4</sup> In one

study, Ananworanich and colleagues (Abstract 124) examined whether individuals initiating antiretroviral therapy in Fiebig stage I exhibited longer intervals to rebound following treatment interruption.<sup>4</sup> The rationale was that reservoir size would be much smaller in individuals initiating very early antiretroviral therapy and, as a result, exhibit delayed rebound after treatment interruption or perhaps virologic control. The cohort comprised 7 male and 1 female participants who at Fiebig stage I initiated antiretroviral therapy and were on effective antiretroviral therapy for a median of 2.8 years. Unfortunately, viral rebound after treatment interruption was similar for individuals in Fiebig stage I initiating antiretroviral therapy and for those initiating antiretroviral therapy during chronic infection. Thus, despite early antiretroviral therapy limiting viral reservoir size, it is unable to promote viral remission or control of the viral reservoirs that drive viral recrudescence after treatment interruption. One possibility is that time to rebound is not an accurate gauge of viral reservoir size and that rebound timing is reflected by the magnitude of immune responses levied against the viral reservoir. In such a scenario, the intermittent release of infectious virus from the viral reservoirs would result in rebound intervals that poorly correlate with the size of the reservoir.

Mitchell and colleagues (Abstract 125) examined myeloid and dendritic cell subsets in individuals who initiated antiretroviral therapy in Fiebig stage III or IV (Western blot negative) and underwent treatment interruption. Plasmacytoid (p) dendritic cells (DCs) and inflammatory monocytes sense viral RNA and serve as an early response to acute infection. The authors reported that there was a significant increase in the percentages of pDCs during treatment interruption that preceded appearance of plasma viremia by 1 week. There was also an increase in the frequency of nonclassical monocytes during treatment interruption and before viral recrudescence. No changes were observed in the myeloid DC population. These results are important in that they indicate that viruses are being sensed before the appearance of viremia and, as such, provide a biomarker of posttreatment interruption rebound. It remains to be determined whether the magnitude

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of the changes in pDC or nonclassical monocytes correlate with viral reservoir size or the interval to viral rebound. The availability of cellular biomarkers will be invaluable in studies aimed at deciphering determinants of posttreatment interruption rebound kinetics.

Kearney and colleagues (Abstract 120) addressed the controversial topic of whether ongoing viral replication might contribute to maintenance of the viral reservoirs. Ongoing viral replication, as defined by numerous cycles of viral replication, would be expected to lead to evolution in viral sequences. The authors examined rates of sequence evolution over a period

of 7 years for 10 children on effective antiretroviral therapy. Viral diversity for 8 of the children whose virus was fully suppressed did not change between samples obtained early and those obtained late in the treatment period. In contrast, there was sequence evolution in 2 children who had partial virus suppression. The authors concluded that there is no evidence of ongoing viral replication in individuals on suppressive antiretroviral therapy, and that there is no role for ongoing virus replication in the maintenance of the reservoir. Unfortunately, the study as conducted cannot exclude contribution of new rounds of infection to reservoir maintenance. Two studies using raltegravir intensification demonstrated de novo infection in the face of suppressive antiretroviral therapy.<sup>5,6</sup> One way to reconcile the conflicting reports is that de novo infection under effective antiretroviral therapy is single round rather than numerous rounds. In such a scenario of single-round replication, reservoir cells maintain HIV-1 production, and those viruses are able to initiate single-round infection of new cells. However, those newly infected cells do not efficiently drive a new round of infection. Under these conditions, de novo infection would not lead to sequence evolution. The debate on whether reservoir cells can be established by new rounds of infection is not settled.

Murry (Abstract 118) presented an update on studies with the investigational TLR7 agonist GS-9620. At previous CROIs, the group demonstrated that GS-9620 induced transient blips of viremia in SIV-infected macaques and that, remarkably, led to sustained remission in 2 of 9 animals following antiretroviral therapy interruption and no viral rebound even after CD8+ T-cell depletion. The investigational TLR7 agonist also potentiated the activity of an Ad26/MVA vaccine regimen with delayed rebound posttreatment interruption and viral control in 3 of 9 animals. The group further investigated potential mechanisms through which GS-9620 exerted biological activity. GS-9620 was found to induce virus production from patient peripheral blood mononuclear cells (PBMCs). In addition, GS-9620 increased HIV-specific CD8+ T cell activation and proliferation and SIV-specific cytolytic activity. GS-9620 was also found to enhance antibody-mediated targeting of HIV-1–infected cells. These effects were found to require type I interferons. These results indicate that GS-9620 enhances immunity to HIV-1 through production of type I interferons from pDCs. The resulting responses include an increase in HIV-1 production followed by subsequent increases in cell-mediated immunity to those cells. GS-9620 holds promise in cure strategies aimed at combating viral reservoir persistence.

## Basic Research

Despite substantial progress in research on the viral etiology of AIDS, aspects of the virus replication cycle remain relatively obscure. Arguably, the processes that regulate how HIV-1 translocates through the cell to integrate within cellular DNA is one of the most intriguing, yet poorly understood, steps of the viral replication cycle. Once the virus binds to, and fuses with, the target cell, it must liberate its core (which contains the viral genome and reverse transcriptase and integrase

enzymes that catalyze the complementary [c] DNA synthesis and integration steps) into the cell cytoplasm. The core itself must then disassemble to liberate the viral genome and enzymes inside the cytoplasm so that the process of reverse transcription can proceed. Once synthesized, the viral cDNA

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must then somehow find its way to the nucleus of the cell so that it can contact and integrate into cellular DNA. The uncoating of the viral capsid is a mysterious event that must be coordinated with the timing of reverse transcription. A better understanding of the individual steps involved could reveal new targets for intervention.

Mamede and colleagues (Abstract 15) presented findings using live-cell fluorescent imaging to visualize the events that occur when the capsid core disassembles shortly after fusion between viral and cell membranes. In this approach, the HIV-1 Gag protein is fluorescently labeled and incorporated into virion particles as a polyprotein. During virion assembly and maturation, the labeled Gag protein is liberated and some labeled Gag molecules end up inside the viral core and some on the outside of the virus core yet still within the virus particle. This then allows the step of fusion of the viral membrane with the cell membrane (which releases the labeled Gag outside the core) and the breakdown of the capsid core (which releases the labeled gag protein inside the core) to be visualized separately. The authors then challenged cells with a low multiplicity of labeled virions, essentially 1 virion per cell. This is important because at higher multiplicities of infection, many virions enter a nonproductive pathway of infection that complicates interpretation of the data. The authors observed that most labeled gag was released into the cytoplasm within 30 minutes of infection. This suggests that capsid disassembly is rapid and occurs in the cytoplasm as opposed to results from others indicating that capsid disassembly is delayed and occurs closer to the nuclear envelope.

Lentiviruses in general have the remarkable ability to infect nondividing cells and the use of lentivirus-based vectors allows transduction of nondividing targets such as neurons, microglia, and muscle cells. This ability is all the more enig-

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matic given the constraints placed on the virus to cross the nuclear membrane. During initial infection, the fusogenic nature of the viral envelope permits fusion of viral and cell membranes that are required to allow entry of the viral core into the cytoplasm. Similar events must occur at the nuclear envelope. In this case, a subviral particle known as the reverse transcription complex or preintegration complex,

which comprises nascent viral cDNA and viral proteins, such as integrase, must traverse the nuclear envelope. As the complex approximates the size of a ribosome, it is unlikely that it simply diffuses across the nuclear envelope. There are also no virion proteins that would allow it to fuse with the nuclear

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envelope. Therefore, facilitated transport is required but the process used by lentiviruses to traverse the nuclear envelope remains a mystery.

Burdick and colleagues (Abstract 17) presented data on live cell imaging of the events that occur during translocation of the virus across the nuclear envelope. In this case, the authors labeled viral preintegration complexes through a fluorescently tagged APOBEC3F protein that is incorporated in viral cores during virus replication, or with a fluorescently tagged integrase that must remain in association with preintegration complexes to catalyze the integration of viral with cellular DNA. The majority of contacts of preintegration complexes with the nuclear envelope were short lived (in the order of a few seconds) and only a minor population of complexes maintained longer contacts (20 minutes or more). The ability of the preintegration complex to form a stable association with the nuclear envelope was compromised by capsid mutations that destabilize the viral core or by knockdown of the nuclear envelope protein Nup358. More detailed analysis of individual preintegration complexes indicated long intervals of association with the nuclear envelope that extended to approximately 1.5 hours. Once the nuclear envelope was crossed, complexes rapidly moved away from the point of nuclear entry. Given the rather rapid intervals for nuclear envelope contact and movement away from it, the long contact time of preintegration complexes with the nuclear envelope is intriguing and perhaps reflects orchestrated rearrangement of preintegration complex components that are required to allow the complex to physically pass through the nuclear envelope.

In the same session, Xue and colleagues (Abstract 18) examined viral and cellular factors involved in passage of the viral preintegration complex across the nuclear envelope. A number of studies have highlighted the role of the capsid in translocation of the viral reverse transcription complex through the cytoplasm. The capsid has interfaces for CPSF6 and cyclophilin A that have important roles in reverse transcription, interaction of the nuclear pore complex, and association with chromatin. How the capsid might be involved in docking of the complex with the nuclear envelope is not clear. The authors conducted a small interfering RNA (siRNA) screen of all known nucleoporins and identified novel cofactors (Nup35 and POM121) as being required for HIV-1 infection. Intriguingly, Nup35 and POM121 are FG-nucleoporins that maintain the nuclear diffusion barrier and serve as docking sites for nuclear transport receptors. The requirement

for FG nucleoporins in HIV-1 infection was dependent on the interaction of the capsid with cyclophilin A, and disruption of this interaction reversed the impairment of infectivity created by Nup35 knockdown. The authors proposed that HIV-1 uses cyclophilin A to restrict access to a domain in the capsid until the preintegration complex docks at the nuclear pore complex. This suggests that the HIV-1 core acts as a nuclear transport receptor and achieves nuclear entry through successive FG interactions. This design of small molecules that inhibit capsid binding to CPSF6 could represent a novel class of antivirals that limit nuclear import of viral DNA.

Treatment of permissive cells with interferon induces the expression of genes that potentially antagonize HIV-1 infection. Although some of these interferon-induced restrictions have been identified, novel interferon-induced antiviral factors were discussed by Ohainle and colleagues (Abstract 19). The authors used clustered regularly interspaced short palindromic repeats (CRISPR) technology to knockout genes in T-helper type 1 (THP-1) cells that normally become nonpermissive to HIV-1 infection upon treatment with interferon. The screen identified known restriction factors including MxB and interferon-stimulating gene (ISG)15 as well as previously unknown restrictions such as the nucleolar Nedd4-binding protein 1 that is incorporated in promonocytic leukemia (PML) bodies. The next step will be to identify how these antiviral factors antagonize viral replication.

### Zika Virus Research

For the first time, CROI featured presentations on Zika virus (ZIKV). Of particular interest is the nature of ZIKV persistence in body fluids. Given the tragic consequences of pregnancy during active ZIKV infection and the fact that ZIKV can be sexually transmitted, a full understanding of the duration of ZIKV persistence is necessary to guide reproductive health. The Centers for Disease Control and Prevention (CDC) recommends that women with confirmed ZIKV infection or symptoms of infection should wait at least 8 weeks before trying

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to get pregnant, and men should wait at least 6 months after the onset of symptoms before engaging in unprotected sex. The true interval of ZIKV RNA persistence is, however, not known. In order to minimize the occurrence of ZIKV infection during pregnancy, it is essential to devise guidelines based on the actual duration of ZIKV persistence as well as assays best designed to reveal persistent ZIKV.

Most information regarding the persistence of viral RNA has been derived from case studies and, as a result, the window for detection in various body fluids is uncertain. Studies from the outbreak in French Polynesia indicated that viremia is of low intensity and is short lived, in the order of several days after onset of symptoms. However, viral RNA could

be detected up to 60 and 81 days in whole blood, perhaps because of adherence of ZIKV virions to erythrocytes. The time frame over which viral RNA can be detected in saliva did not appear to be much different to that in serum. However, in 2 case reports of individuals infected with ZIKV while traveling to Haiti, ZIKV RNA was detectable in saliva by RT-PCR 47 and at 91 days after resolution of symptoms. Because of the potential for male to female transmission, several reports have examined the duration of ZIKV persistence in semen and reported detectable ZIKV RNA between 60 and 188 days following resolution of symptoms. The most comprehensive analysis on duration of ZIKV RNA in different compartments was conducted in cynomolgus and rhesus macaques infected subcutaneously with ZIKV (Abstract 1055LB). Viremia was resolved by day 10, but viral RNA remained detectable in saliva and semen until the end of the study, 3 weeks after resolution of viremia. Unfortunately, the limited duration of the study did not allow assessment of the full duration of viral RNA in saliva or semen.

Paz-Bailey and colleagues (Abstract 1055LB) presented a detailed evaluation of the persistence of ZIKV in body fluids. A total of 150 study participants with acute infection were

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enrolled on the basis of ZIKV RNA in urine or blood. Serum, saliva, urine, and semen or vaginal secretions were obtained weekly over the first month, then at 2, 4, and 6 months. ZIKV RNA in body fluids was assessed by RT-PCR. The 50th and 95th percentiles, respectively, for time to loss of ZIKV RNA detectability was 14 and 54 days for serum, 8 and 39 days for urine, and 34 and 81 days for semen. Few subjects had detectable ZIKV RNA in saliva or vaginal secretions. These data suggest revision of guidelines for ZIKV-exposed women

planning pregnancy. Unfortunately, whole blood was not monitored in this study and case reports suggest extended persistence of ZIKV RNA for 81 days in whole blood.<sup>7,8</sup> Therefore, additional studies are required to define the full extent of ZIKV persistence to more effectively guide reproductive decisions for couples in whom there has been infection with ZIKV. 

**All cited abstracts appear in the CROI 2017 Abstracts eBook, available online at [www.CROIconference.org](http://www.CROIconference.org).**

*Financial affiliations in the past 12 months: Dr Stevenson has no relevant financial affiliations to disclose.*

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