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CROI 2019: Advances in Basic Science Understanding of HIV

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Running Head: CROI 2019: Basic Science

Abstract: *The annual Conference on Retroviruses and Opportunistic Infections remains the preeminent venue for the sharing and dissemination of research advances in the field of HIV and AIDS research. The 26th conference in Seattle featured highlights including news of additional individuals who experienced long-term virologic remission following a bone marrow transplant. The factors driving reservoir persistence gathered a lot of interest, as well as data presented on new factors involved in regulating HIV-1 latency. The effectiveness of the conference in disseminating new findings is further enhanced through themed discussions that focus the attention of participants on abstracts with a common theme. In addition, the Program Committee workshops provide an outstanding venue, directed to new investigators, fellows, and students, to receive updates on different aspects of HIV and AIDS research. These sessions add to the information-sharing environment provided by the conference.*

Keywords: CROI, 2019, virology, reservoirs, HIV, cure

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Virology

The basic virology presentations at the 2019 Conference on Retroviruses and Opportunistic Infections (CROI) each year continue to challenge our notions that we know everything there is to know about HIV-1. During the replication of HIV-1, the integrated provirus usurps cellular factors that interact with the long terminal repeat (LTR) to promote transcription of viral RNA that are then used as templates for production of viral proteins. A full understanding of the transcriptional regulation of the virus is key to the design of strategies to eliminate viral reservoirs through, for example, “shock and kill” or “block and lock” (terms coined for strategies that reactivate viral latency or that lock the virus in a latent state, respectively). More than 30 years of research has shed considerable insight into the cellular factors that regulate HIV-1 transcription. Goff (Abstract 110) presented findings on a cellular complex that blocks HIV-1 transcription. More than 40 years ago, researchers observed that the transcription of many retroviral genomes such as murine leukemia virus (MLV), was inhibited in developmentally primitive cells such as embryonic and hematopoietic stem cells. The phenomenon was also noted by gene therapy researchers showing that vectors introduced into human hematopoietic stem cells were silenced. Silencing has also been shown to occur with unintegrated viral genomes. The extent of transcriptional silencing is profound, essentially affecting 100% of the genomes and possibly occurring in all cell types, not just undifferentiated cells. Tripartite motif-containing 28 (TRIM28) is the major factor involved in silencing of retroviral DNA in developmentally primitive cells. TRIM28 is a ubiquitin and E3 ligase and serves as a scaffold to link transcriptional silencing proteins to a DNA target. Unintegrated NDA is also potently silenced. This is evident when a cell is infected with a viral variant that has an inactivating mutation or deletion in the viral integrase. The degree of silencing is particularly striking with unintegrated viral DNA and is also cell-type independent. The silencing can almost completely be reversed with the addition of histone deacetylase inhibitors such as trichostatin, demonstrating that the silencing involves histones. Histones are loaded onto viral DNA rapidly upon entering the nucleus and the loaded histones or nucleosomes are retained after integration of the viral genome. These histones rapidly acquire acetylation modifications that drive silencing of the viral genome including H3K9 trimethylation. A whole-genome CRISPR (clustered regularly interspaced short palindromic repeats) knockout screen was then used to silence 20,000 genes in HeLa cells that were subsequently infected with an integrase minus MLV. Cells in which factors involved in silencing of viral DNA would then allow expression from the unintegrated DNA (as evidenced by expression of a green fluorescent protein [GFP]

transgene in the viral vector). The screen revealed 3 proteins, periphilin, transgene activation suppressor (TASOR), and M-phase phosphoprotein 8 (MPP8) that comprise the human silencing hub (HUSH) complex. The recent demonstrations that simian immunodeficiency virus (SIV) Vpr and Vpx proteins have evolved to inactivate the HUSH complex[1,2] suggests that they hinder viral replication. Silencing of components of the HUSH complex resulted in gene expression from unintegrated MLV DNA. The authors further demonstrated that the DNA binding protein NP220 is required for silencing of unintegrated DNA by tethering the HUSH complex to unintegrated DNA. These proteins disappear from integrated DNA and the process through which that occurs, is as yet, not understood. In conclusion, HUSH and NP220 are the major proteins directing silencing of unintegrated DNA. Whether these protein mediate HIV-1 silencing in the memory CD4+ T cells has yet to be determined. This research sheds light on a long standing question in the field, namely, why unintegrated retroviral DNA serves as a very poor transcriptional template. This information could help in the design of next generation retroviral vectors that do not integrate. This would alleviate the hazards associated with integration such as insertional activation of cellular genes. Future studies will may also help define whether agents that inactivate the HUSH complex could be employed for reactivation of HIV-1 latency.

Abstract 168 provided a twist on the conventional wisdom surrounding mechanisms of HIV-1 drug resistance. Virologic failure in individuals on regimens containing the integrase strand transfer inhibitor dolutegravir can occur without mutations in integrase. The authors attempted to define what governs dolutegravir resistance in this instance. Dolutegravir-resistant virus was derived in vitro and resistant viruses were found to have mutations in the gp41 region of the viral envelope (Env) gene. Two Env mutants, Env-A556T and Env-A539V, were studied in order to define how they underscore dolutegravir resistance. Each mutant was found to increase the efficiency of cell-to-cell transmission relative to a wild-type virus. In the presence of 1.5 nM dolutegravir, cell-to-cell fusion of wild-type virus was blocked. However, the 2 Env mutant viruses remained competent for cell-to -ell fusion in the presence of dolutegravir. This was reflected by accelerated replication kinetics of the Env mutants relative to wild-type virus. When cells were infected with a GFP reporter virus, the geometric mean fluorescence of cells infected with the Env mutants was far higher than that of cells infected with wild-type virus, suggesting that there may be more infection events per cell following infection by the Env mutants. The authors propose a model in which concentrations of dolutegravir that efficiently inhibit cell-free transmission are insufficient to block cell-to-cell transmission because of the numerous integration events occurring in those cells. Therefore, Env mutations that increase the efficiency

of cell-to-cell infection, may further promote insensitivity to dolutegravir inhibition. This study provides insight into the mechanism by which mutations outside the target can confer resistance in vitro. It remains to be determined whether envelope mutations are involved in drug resistance in individuals on dolutegravir-containing regimens.

Viral Reservoirs and Cure

Timothy Brown, formerly known as the Berlin Patient, is the only individual to have been cured of his infection. In 2006, Mr Brown received 2 bone marrow transplants to treat his leukemia. His physician, Gero Hütter, used bone marrow from an individual with a chemokine receptor 5 (CCR5)-Δ 32 mutation. This mutation renders the CCR5 receptor incapable of acting as a cell receptor for HIV-1 infection. In addition, Mr Brown underwent intensive chemotherapy and whole-body irradiation to kill the resident leukemic cells. He is now considered to be cured of his HIV infection. The most sensitive approaches have failed to reveal any remnants of the virus and in addition, Timothy no longer has antibodies to the virus. Although this cure galvanized researchers who are trying to develop a safe and scalable strategy to cure HIV-1 infection, it is a single case. Not surprisingly, presentations on potential additional cures generated considerable conference and media interest at the conference. In Abstract 29, Gupta described HIV-1 remission in an individual who underwent an allogeneic CCR5-Δ 32 stem cell transplant. Gupta described an individual who was diagnosed with HIV-1 infection in 2003. In 2013, the man (whose identity remains undisclosed) was diagnosed with Stage IVb Hodgkin's lymphoma. The Numerous lines of chemotherapy failed as did an autologous stem cell transplant. At that point he was a candidate for an allogeneic hematopoietic stem cell transplant, and fortunately, a human leukocyte antigen match with a CCR5 delta 32 mutation was found. Antiretroviral therapy (ART) was maintained throughout the transplant procedure. One-hundred percent chimerism was achieved by day 30 posttransplant. At 16 months after the transplant, the clinical care team received approval to interrupt ART. As of February 2019, the patient has been off ART for 18 months with no viral rebound. During this time, cellular viral DNA levels have been below the limit of detection and several viral outgrowth assays using 25 million CD4+ T cells were negative. Perhaps most telling is the demonstration that this patient exhibited diminished antibody responses to HIV-1. In addition, although there were T-cell responses to HIV-1 gag pretransplant, those responses are no longer evident. There are important parallels as well as differences between this subject (now referred to as the London patient) and Mr Brown. Although both individuals had transplants with CCR5-Δ 32 stem cells and exhibited mild graft

versus host disease and 100% chimerism, the London patient received a single transplant with reduced intensity conditioning and no whole-body irradiation (as opposed to 2, high intensity conditioning stem cell transplants and whole-body irradiation in Mr Brown). It remains to be conclusively shown whether the London patient is another cure. Although, to date, there has been 18 months of virologic remission, cases involving infants who despite being treated very early, rebounded after extended virologic remission,[3] show the need for caution in calling this a cure yet. The issue surrounding reductions in HIV-specific antibodies declining following the stem cell transplant in the London patient was further expanded upon in Abstract 386. Salgado, talking on behalf of the ICISTEM (International Collaboration to Guide and Investigate the Potential for HIV Cure by Stem Cell Transplantation) consortium of which the London patient was a participant, reported results from 13 individuals who underwent allogeneic hematopoietic stem cell transplant under ART. Longitudinal plasma samples were examined using qualitative and low sensitivity HIV-1 antibody assays. Anti p24 (*gag*) and p31 (integrase) antibodies disappeared within several months in 9 of 13 patients whereas anti-envelope antibodies persisted in most individuals. However, antibody levels in 2 individuals declined to below detectable levels. As these subjects remain on ART, the authors propose to use HIV-specific antibody levels as a tool to prioritize individuals who could be considered for a treatment interruption. These studies, although pointing to important markers that could be used to inform on the status of a cure trial, need to be taken in context. The use of stem cell transplant for hematologic malignancies in individuals with HIV-1-infection offers an opportunity to identify correlates of a cure. However, it is important that researchers transmit these findings to the lay community in a way that does not convey false hope for individuals living with HIV-1.

Studies investigating the mechanism of persistence of the viral reservoir under effective ART, as well as approaches to measure the reservoir, continue to dominate the basic science sessions. Understanding how HIV-1 is able to persist under effective ART is key to the design of strategies that eliminate the reservoirs. Most of the attention has focused on the role of memory CD4+ T cells and their ability to sustain lifelong viral persistence. The persistence of HIV-1 is dependent on its ability to integrate into the genome of the host cell. The integrated provirus can then enter a latent state for extended intervals where it is invisible to immunologic clearance mechanisms of the host. In the past few years, several groups demonstrated that, during the process of cell division, the latent provirus can be duplicated by mitosis to result in 2 daughter cells harboring identical proviruses with identical integration sites. This can apparently occur without cytopathic effects of the virus on the host cell undergoing mitosis. The integrated provirus contains transcriptional regulatory elements in the LTR and as such, can influence the

activity of neighboring cellular genes. If that cellular gene is involved in cell cycle regulation, the provirus can promote dysregulated expression of the cellular, which would result in an increased rate of host cell division. For that reason, expanded proviruses can represent a substantial proportion of the proviral population in memory CD4+ T cells. Duplicated proviruses are also competent for virus production and that viruses in plasma can originate from the duplicated provirus pool.[4] Since viruses originating from expanded proviruses are derived from a chronic source, they would be expected to be unaffected by ART. Abstract 22 presented data that might explain persistent, low-level viremia during effective ART. Peripheral blood mononuclear cells (PBMCs) were obtained at to time points from 9 individuals on effective ART who exhibited residual plasma viremia of greater than 20 HIV RNA copies/mL for more than 6 months. Proviruses were characterized by single-genome sequencing and integration site analysis. In 6 of 9 subjects, viral sequences in plasma matched proviral sequences in PBMCs. Plasma viral RNA and proviral sequences were identical to viral sequences in viral outgrowth assays in 4 subjects. Intact proviruses comprised 4% to 15% of all proviruses in PBMCs. This study demonstrates that expanded proviruses can generate measurable levels of plasma viremia in the face of suppressive ART. It remains to be determined as to the relative contribution of the expanded proviral pool to viral rebound if treatment is interrupted.

The relationship between intact proviruses in PBMCs and plasma rebound viruses was discussed in Abstract 340. The results were derived from a clinical trial involving 15 patients that examined the impact of 3 administrations of a broadly neutralizing antibody (bNAb) combination (3BNC117 and 10-1074) during an analytic treatment interruption.[5] In that study, it was shown that antibody administration significantly delayed (by approximately 6 months) time to viral rebound upon treatment interruption. The authors sought to understand the relationship between the viruses in the latent reservoir that existed before bNAb administration and rebounding viruses after analytical treatment interruption. PBMCs were obtained 2 weeks before antibody administration, viral outgrowth assay was performed to assess the latent proviruses, and near full length polymerase chain reaction (PCR) was performed to identify the intact latent proviruses. Single genome envelope PCR was performed on the rebounding plasma viruses. There was an approximately 40% overlap between envelopes from intact proviral sequences and envelopes from inducible proviruses. However, there was no overlap between latent proviruses and envelopes in rebounding plasma. Instead, approximately 50% of the viruses in the rebounding viruses were recombinants of sequences present in the latent proviruses. Since recombination requires 2 viral genomes in an individual infected cell, this is surprising. The frequency of memory CD4 T-cells harboring a provirus is in the order of several hundred per

million. The majority of those are defective and as such, the chances of a functional recombinant emerging is very small. It is also unclear whether the recombinants were present prior to antibody administration and treatment interruption.[6] Regardless of the mechanism behind the frequent presence of recombinants in the rebounding plasma in this study, an equally important issue is the poor correlation between rebounding viral sequences and proviral sequences in PBMCs. If rebounding plasma reflects the nature of the viral reservoirs that persist under ART, it is possible that the PBMC provides a very limited or perhaps distorted window into that reservoir. Additional studies are required to determine the relationship between proviruses in PBMCs and the viral reservoir that fuels viral rebound upon treatment interruption.

The role of central nervous system (CNS) reservoirs in viral persistence is poorly understood. Although neuropathogenic manifestations of HIV-1 infection appear to persist in a substantial proportion of individuals on effective ART, it is unclear whether viral activity is responsible. The composition of the viremia that rebounds following treatment interruption can provide insight into the nature of the reservoir from which the viremia originated. Abstract 391 examined the composition of viral RNA in cerebrospinal fluid (CSF) and in blood following treatment interruption. The study involved 9 individuals. Six were treatment failures and 3 were suppressed at the time treatment was interrupted. The viral envelopes were characterized by deep sequencing, and additionally, envelope clones were generated by single genome amplication and assessed from tropism and fusogenicity on Affinofile cells expressing various CD4 densities. Rebound virus in the CSF was found to be comprised predominantly with viruses found in blood. Selected sequences were selected for tropism analysis and all CSF rebound envelopes were found to be CCR5 tropic but poorly fusogenic for low CD4 expressing cells. This is a characteristic of T-tropic viruses and contrasts with the phenotype of macrophage-tropic viruses that efficiently fuse with low CD4-expressing cells. The authors concluded that CSF rebound is most likely driven by virus that originated from infected CD4+ T-cells trafficking in the CNS. However, the authors acknowledged that more rapid expansion of viruses from trafficking CD4+ T-cells could be obscuring more slowly replicating viruses emerging from a true CNS reservoir. This theme was extended by Swanstrom in a plenary presentation (Abstract 121). He presented an overview of research investigating the nature of the viruses that populate the CNS. Compartmentalization of viruses in CNS versus blood has been reported using either brain tissue obtained at autopsy or the CSF. However, whether viruses in the CNS and CSF can originate from macrophages, especially under ART, is not known. The picture is further clouded by misunderstandings around the receptor usage of viruses that can or cannot infect macrophages. Although macrophage-tropic viruses use CCR5 as the principle co-receptor,

CCR5 usage does not distinguish a macrophage tropic virus from a T-tropic virus. Instead, it is the ability to use low levels of CD4 on the macrophage that is the principle determinant of macrophage tropism. The density of CD4 on the macrophage is 25-times lower than that on a T-cell. To use such CD4 levels, the envelopes of macrophage tropic viruses have a high CD4 affinity. This higher CD4 affinity confers the ability to efficiently fuse with macrophages. The laboratory of Clapham has demonstrated the presence of macrophage tropic envelopes in post-mortem CNS tissue of individuals with and without neuropathology such as pleiocytosis.[7] Similarly, Swanstrom presented evidence that more than half of individuals with HIV-associated dementia, who started therapy at low CD4+ cell counts and who exhibited phylogenetic compartmentalization between blood and CNS, had macrophage tropic viruses in the CSF. Some individuals on ART exhibit CSF escape, which is characterized by episodes of transient or more prolonged CSF viremia. Viral envelopes obtained from individuals with transient CSF viremia were T–tropic. However, envelopes obtained from individuals with prolonged CSF viremia under ART were macrophage tropic. This provides a clearer picture of the nature of CSF escape in individuals on effective ART. Transient CSF viremia reflects a genetically homogeneous and drug-sensitive virus. In contrast, the persistence of HIV in the CSF reflects can occur in either the presence or absence of CNS symptoms. These viruses are genetically heterogeneous and contain drug-resistant mutations suggesting that they can replicate during ART suppression in the circulation. However, during treatment interruption, viral rebound in blood occurs earlier than in CSF, and as a result, viral rebound in CSF is obscured by blood viruses in trafficking T-cells. Therefore, there may be a CNS reservoir that is hidden from detection due to predominance more rapidly emerging blood viruses. As such, the presence of a CNS reservoir comprising macrophages remains unanswered. The “last gift” cohort of Smith comprises terminally ill, individuals with HIV-1 infection who have consented to donate their body for AIDS research postmortem (Abstract 327). The material provided by these exceptional individuals could provide answers to long-standing scientific puzzles around the nature of the reservoirs that persist under ART.

Abstract 392 assessed the composition of rebounding virus in blood and seminal plasma following treatment interruption in individuals enrolled in a therapeutic vaccine trial. Viral rebound in semen was significantly delayed and of lower magnitude than that in blood. Gag, pol and envelope sequences in paired blood and semen for five individuals rebounding virus was determined using an Illumina miSeq platform. Viral diversity was higher in semen for all subjects. In addition, unique viral populations were found in semen that were not present in blood. Whether these results reflect distinct reservoirs in these separate anatomic

compartments is unclear. However, the study illustrates the challenges involved in characterizing the viral reservoirs that persist during of ART.

Analytic treatment interruption may inform not only on the nature of the viral reservoir fueling viral rebound, but also on the size of the reservoir. However, use of treatment interruption to gauge viral reservoirs has caused concern since treatment interruption may reset the viral reservoirs. For example, individuals with a small reservoir may reseed the reservoir during a treatment interruption. Abstract 389 examined subjects in the ISALA (Analytical Treatment Interruption in HIV Positive Patients) trial, which was a multicenter, nonrandomized prospective study to determine post-treatment control in individuals with a smaller reservoir size. Inclusion criteria included low levels of cell-associated RNA and unspliced viral RNA in PBMCs. Fourteen individuals underwent a treatment interruption. All participants had rebound within 8 weeks of treatment interruption. Virologic control was achieved within 12 weeks of reinitiating ART. In conclusion, no parameters were found to be predictive of the dynamics of viral rebound. However, treatment interruption was found to have a relatively minor impact on the size of the reservoir after treatment was reinitiated. This study indicates that, based on measures of cell-associated viral DNA and RNA, reservoir size returns to pre-treatment interruption levels upon re-initiation of treatment and alleviates concerns surrounding use of treatment interruptions to gauge the viral reservoirs.

Although the HIV research field is attempting to define strategies to eliminate viral reservoirs, there remains the challenge of how best to measure the reservoir so that the efficacy of those cure strategies can be followed. As outlined above, PBMC sampling may offer a limited window into the viral reservoirs. It has been known for more than a decade that the blood accounts for perhaps less than 2% of the infected cells in an individual. However, tissue access in living subjects is a challenge especially when large cell numbers are required. Therefore, researchers have looked to in situ approaches to visualizing and quantifying the reservoir. Estes discussed progress in developing tissue imaging approaches for examining viral reservoirs (Abstract 4). The greatest progress has been made in development of RNA and DNA Scope in situ hybridization tools to image the viral reservoir. These methods are now being used to define the total virus burden, including active (viral RNA positive) and total (viral DNA positive) reservoirs, before and during ART. These approaches also allow visualization and quantitation of virion-associated genomic viral RNA. Application of these methods to the non-human primate model has provided detailed insight into the size, distribution, and dynamics of the viral reservoir on and off ART. One important observation from those studies was that ART had a relatively modest impact on the frequency of viral RNA-positive cells.[8] When these methods were applied

to individuals with HIV-infection on long-term ART, there was a 2 $\log_{10}$ decline in viral DNA-positive cells in the lymph nodes but surprisingly, almost no reduction in the frequency of viral DNA-positive cells in the rectum even after 2 years of ART. Furthermore, there was no difference in the overall frequency of DNA-positive cells between the lymph node and rectum after 2 years of ART. This effectively meant that even after 2 years of suppressive ART, there were between 2 x 10^7 and 10^8 infected cells in these compartments. Estes described a new tool, multiplexed ion beam imaging, developed by Nolan,[9] which can define the spatial arrangement of numerous targets in a tissue section that can be reproduced in a manner similar to confocal images. This approach provides a platform to examine the nature of the infected cell in the context of the environment in which it resides. This powerful approach can identify signals that may reside on infected cells, and perhaps, on latently infected cells.

All cited abstracts appear in the CROI 2019 Abstracts eBook, available online at www.CROIconference.org.

Financial affiliations for the past 12 months: Dr Stevenson has no financial conflicts to report for the past 12 months.

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