Challenges inherent in detecting HIV persistence during potentially curative interventions.

SA Yukl[1], T Chun[2], MC Strain[3], J Siliciano[4], E Eisele[4], R Buckheit[4], YC Ho[4], JK Wong[1], MP Busch[5], G Hütter[6], DD Richman[3], RF Siliciano[4], SG Deeks[1]

[1]University of California, San Francisco, San Francisco, CA

[2]National Institute of Health, Bethesda, MD

[3]Unversity of California, San Diego, San Diego, CA

[4]Johns Hopkins University, Baltimore, MD

[5]Blood Systems Research Institute, San Francisco, CA

[6]Institute of Transfusion Medicine and Immunology, University Heidelberg, Mannheim, Germany

Background. The size of the HIV reservoir during long-term effective antiretroviral therapy and in "elite" controllers is close to the limit of detecting using standard assays. This imposes challenges for the design and assessment of potentially curative interventions. We applied a series of measurements of HIV persistence to the study of the "Berlin Patient", who underwent a hematopoietic stem cell transplant from a donor who was homozygous for the CCR5 delta-32 deletion and who had exhibited clinical evidence of being cured. Our objectives were to (1) determine if HIV had been fully eradicated as a consequence of the transplant and (2) define the potential role of various reservoir measurements in cure research.
Methods. The subject underwent a series of intensive virologic and immunologic studies beginning approximately five years after this transplantation. Replication-competent virus was measured in two laboratories, and HIV DNA and RNA levels (from blood and rectal mucosa) were measured in several laboratories using different approaches
Results. A large volume apheresis was performed and 9 billion PBMCs evaluated for the presence of replication-competent virus. All wells were negative for HIV p24, indicating that the frequency of replication-competent HIV was therefore estimated to less than one infected cell per 1.4 billion CD4+ T cells. A repeat experiment in a second laboratory confirmed these findings. Using a variety of assays and approaches, very low levels of HIV RNA were intermittently detected in plasma, although sequence analysis of these variants were different from each other and different from those present before the transplant. Digital PCR for HIV DNA was negative for 1 copy per 2 million cells with a 95% confidence limit of less than 1.9 copies per million cells. Collagenase-digested rectal biopsy-derived cells were positive for very low levels of HIV DNA but not RNA; no sequence for confirmatory studies could be obtained. HIV antibodies levels were low and declined over the course of approximately 18 months.
Conclusion. Although the subject has had intermittent evidence for HIV persistence in some assays in some laboratories, the extremely low levels of virus which were detected, while

pushing the limits of sensitivity and specificity, and the inability to match sequence with the subject's pre-therapy virus make it impossible to conclude that the subject remains HIV infected.