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Defining and targeting residual virus on combined antiretroviral therapy

Christopher James Kevin Ward*

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ABSTRACT: Therapeutic targeting of residual HIV following successful antiretroviral suppression represents a potential mechanism of HIV cure. This meeting report describes the findings of two eminent investigators speaking at a session of the 20th International Conference of the International AIDS Society. This conference attracted approximately 14,000 delegates comprising patients, scientists, clinicians and other key stakeholders. Herein, two putative targets of novel therapy are described: the kick and kill approach offered by HDAC inhibitors and the use of bispecific antibodies to direct CD8 T-cell killing in germinal centers.

HIV-1 infection, suppressed by combination antiretroviral therapy, is now a chronic illness. However, the existence of latent reservoirs wherein HIV remains dormant, particularly in resting immune cells, has long been identified as a major challenge to HIV cure [1]. Multiple novel techniques to remove otherwise 'protected' HIV genomes in resting cells or otherwise induce targeted enhancement of the human immune system have been reported over the past several years and remain under investigation [2]. These concepts were further developed in the oral abstract session *Defining and Targeting Residual Virus on cART* chaired by Sarah Palmer (University of Sydney, Australia) and the eminent Christine Rouzioux (Necker Hospital & University Paris Descartes, France).

Kicking & killing HIV-1

Therapeutic induction of HIV transcription within resting immune cells in a patient suppressed on HAART, followed by virion release, could enable the immune system to kill the latently infected cells. Histone deacetylase (HDAC) inhibitors are well established in their ability to induce HIV mRNA transcription in latently infected resting CD4 T cells, although as ME Sogaard (University of Denmark, Denmark) explained, their ability to effect this varies between candidate compounds [3].

Sogaard detailed a small clinical trial with an HDAC inhibitor called romidepsin. Romidepsin, an HDAC inhibitor licenced for peripheral and cutaneous T-cell lymphomas, is known to increase HIV transcription from memory and resting T cells, as studied *ex vivo* in patients on antiretroviral therapy [4]. In a small (n = 6) nonrandomized interventional trial, Sogaard's group administered three doses of intravenous romidepsin over 14 days. Following initial infusion, CD4 T-cell, CD8 T-cell and neutrophil count were measured. All three cell subsets showed initial decline around 10 days, followed by a return to baseline levels at day 21.

Sogaard and colleagues determined HIV transcription by monitoring the cell-associated unspliced HIV RNA. The most pronounced increases in cell associated unspliced HIV RNA occurred following

*Innate Immune Sensor Group, Institute of Infection & Immunity, Cardiff University School of Medicine, Cardiff, Wales, UK; chrisjward@gmail.com

the second and third romidepsin doses. Sogaard then asked whether romidepsin was able to induce increases in HIV plasma RNA: using two different assays (Taqman HIV-1 Test v.2.0; and the transcription-mediated assay Quantitative NAT Screening System). Curiously, for most participants ($n = 5/6$), the most pronounced increase in HIV plasma RNA occurred at day 10, 3 days following the second romidepsin infusion. Following the third romidepsin infusion, only half of the participants had HIV RNA levels above the limit of detection. By day 21, all participants HIV plasma RNA levels were below the limit of quantification (<20 copies/ml).

Concerning the immunological impact of romidepsin, Sogaard observed a shift towards larger proportion of naive CD4 T cells and a smaller proportion of effector and memory CD4 T cells at day 1, which reverted to baseline distribution by day 10. As a marker of memory CD4 T-cell activation, CD69 approximately doubled following first infusion, reverting back to baseline around day 10. As a marker of the latent reservoir, total HIV-1 DNA in CD4 T cells was measured: on a global level there was no significant change over 21 days, although in one participant there was an 80% reduction in total HIV-1 DNA.

In his concluding remarks, Sogaard noted that T-cell phenotypic changes were an area of future investigation. While the total latent reservoir remained unchanged at 21 days overall, Sogaard noted that changes following first and second dose in terms of HIV-1 transcription and CD4 T-cell phenotype make romidepsin a promising candidate for further study. Sogaard explained that romidepsin is now being investigated in an ongoing study in combination with a therapeutic vaccine (anti-p24 Vacc-4x) [5].

Responding to questions from the floor, Sogaard explained that specific observations in the study could be the result of an additive effect of the second dose, as romidepsin levels had not reached baseline following the first dose by day 7. Sogaard also explained that his group did not monitor for HIV protein levels or potential boosting of HIV antibody levels. Sogaard hypothesized that increases in T-cell activation was not a virus-specific effect, and rather a consequence of the experimental HDAC inhibitor. Would a more persistent effect of romidepsin on plasma HIV RNA have been anticipated? It was also postulated there remains a question mark over whether one would be clinically concerned

if plasma HIV RNA continued to increase despite ongoing romidepsin doses.

• Two-faced antibodies in germinal centers

Richard Koup (Vaccine Research Centre, MD, USA) outlined his research performed in collaboration with researchers at the HGU Gregorio Maranon (Madrid, Spain) and the HU Virgen de la Rocio/IBiS/US (Sevilla, Spain). This collaboration identified a population of CD8 T cells, located in germinal centers, thought to be functionally capable of effecting bispecific antibody mediated killing of HIV-infected T cells. Koup explained that bispecific antibodies are antibody-like molecules possessing a CD4 targeting antibody recognizing HIV-expressing CD4 T cells, the other half targeting CD3 and thus able to engage CD8 T cells that are not necessarily HIV specific, leading to redirected lysis and killing of HIV infected CD4 T cell.

Koup first confirmed that this type of bispecific antibody can lead to lysis of both constitutively expressing and inducible HIV-infected CD4 T-cell lines. Lymph nodes are complex structures, Koup explained, in which CD4 T cells reside in the T-cell zones and T follicular helper cells reside within B-cell follicles, characterized by the expression of CXCR5. T-follicular helper cells have the highest copy number of HIV DNA in CD4 T-cell populations and are also a major source of inducible HIV replication in long-term nonprogressors. Koup noted a potential challenge posed by the finding that CD8 T cells are generally excluded from B-cell follicles, so CD8 T cells may not be present to allow bispecific antibodies to engage [6].

The objectives of Koup's study were to evaluate the distribution of CD8 T cells in T- and B-cell zones in lymph nodes or tonsils, assessing frequency, phenotype and to investigate changes during HIV infection. Koup also aimed to determine the ability of B-cell zone CD8 T cells to mediate bispecific antibody-mediated killing of HIV-infected CD4 T cells and compare that to CD8 T cells from other regions of the lymph node.

Koup explained how CD8 T cells are expanded in HIV-infected lymph nodes. Fluorescent imaging of HIV-uninfected B-cell follicles revealed expression of a few CD4 T cells, almost no CD8 T cells; and almost no CD8 T cells in an HIV-infected lymph node. Imaging of CD8 T cells in HIV-infected lymph nodes displayed massive infiltration of CD8 T cells in the B-cell follicle. Expansion of CD8 T cells was mostly within

the central memory (CD27^{high}, CD45RO^{high}) population, which Koup explained is the population of CD8 T cells thought to be able to access B-cell germinal centers.

Koup described how in uninfected donors, the majority of T cells in germinal centers are CD4 T cells. However, in HIV-infected lymph nodes, there were more CD8 T cells than CD4 T cells in B-cell germinal centers. In HIV-infected lymph nodes, the CD8 T cells showed the greatest co-expression of granzyme and perforin. Over 5 h, co-expression of newly formed CD107A (a degranulation marker) and perforin was observed, with HIV-infected lymph nodes showing highest co-expression of CD107A and perforin.

Koup then described the observation that HIV-infected lymph nodes show the highest expression of CD107A and perforin, that is, they have killing potential. Koup then asked: do these CD8 T cells effect bispecific antibody-mediated killing? HIV-infected cell lines were treated with the anti-HIV Env/anti-CD3 bispecific antibody, and CD8 T cells from multiple samples. Killing was monitored by probing for expression of annexin 5 and other markers, and appeared to be HIV specific and caspase dependent. The greatest CD4 T-cell killing was shown to be mediated by the germinal center-localized CD8 T-cell population.

Koup concluded that there is recruitment of CD8 T cells into B-cell germinal centers during HIV infection, defined by high expression of CXCR5 and low expression of CCL7 by flow cytometry (and supported by confocal imaging). Increased cytolytic potential of CD8 T cells in B-cell follicles compared with extrafollicular CD8 T cells, especially in HIV-infected lymph nodes, was confirmed by probing for CD107, perforin and granzyme.

Koup explained that his group are collaborating with other groups to investigate HIV specific

T-cell clonality. Almost all HIV-infected donors were not on antiretroviral treatment, nor were they classified as 'elite controllers', and Koup now seeks to obtain lymph nodes from HIV+ donors suppressed on antiretroviral therapy.

Conclusion

This report has described two potential strategies intended to target HIV, otherwise unachievable by conventional combination antiretroviral therapy: the broad 'kick and kill' approach offered by HDAC inhibitors, and the specific action of bispecific antibodies in germinal centers. While these strategies remain in the early phases of development and continue to expand into larger models of investigation, initial findings are highly intriguing. Current plans to test romidepsin in combination with a vaccine candidate in a clinical trial [5] indicate once again that HIV cure will only come from a multifaceted approach to the numerous biological obstacles posed by this complex infection.

Financial & competing interests disclosure

CJK Ward is a PhD student at the Institute of Infection & Immunity, Cardiff University School of Medicine. CJK Ward is the Hepatitis Coordinator for the European AIDS Treatment Group, through which he has previously been a community representative in the Forum for Collaborative HIV Research 'Cure Working Group 1'. CJK Ward received an International Scholarship from the International AIDS Society covering transport to the conference; and support for accommodation and expenses throughout the conference from the European AIDS Treatment Group. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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