IAS Corporate Partnership Programme Towards an HIV Cure: Industry Collaboration Group

Cure research: Non-invasive measurement of HIV reservoirs

Workshop, 19 October 2022

Report, 26 October 2022

Background

Several therapeutic strategies are being tested to develop an HIV cure. Stable reservoirs of persistently HIV-infected cells in blood and different anatomical sites remain the main challenge to an effective cure. These reservoirs may persist despite cure interventions.

Reducing the size of the HIV reservoirs to a point where virologic control can be achieved without treatment is a goal of many cure strategies. Therefore, measuring the size of these reservoirs is an essential component of cure research and has been identified as a research priority in the 2021 IAS HIV Cure Global Scientific Strategy.

Two strategic recommendations concern the development of modalities to quantify the size, distribution and activity of the reservoir in cells and tissues and the harmonization of assays across laboratories.

Several approaches have been developed to measure HIV reservoirs. This workshop focused on the much-needed non-invasive techniques and surrogate markers that could detect and quantify HIV reservoirs in a range of cells and tissues beyond blood and that can be used in resource-limited settings where HIV cure research is increasingly being conducted.

The identification of such markers could also accelerate the development of an HIV cure by providing mechanistic insights, potentially leading to the development of new therapeutic approaches, and providing an alternative to, or complementing, analytic treatment interruption (ATI) and viral load measurements commonly used in cure research.

This workshop brought together Industry Collaboration Group members, representatives from the industry, clinicians and trial participants to discuss innovative non-invasive technologies for the measurement of HIV reservoirs in low-and middle-income countries.

Agenda

15:00-15:05	**Welcome** *Tuuli Reissaar IAS, Switzerland – Bonnie Howell, Merck, USA*	
15:05-15:15	**HIV reservoir measurement today** *Nicolas Chomont, Université de Montréal, Canada*	
15:15-15:25	**Predictors of viral rebound** *Jonathan Li, Harvard University, USA*	
15:25-15:35	**Setting the stage:** Moving forward with non-invasive assays for HIV reservoir measurement *Mohamed Abdel-Mohsen, The Wistar Institute, USA*	
15:35-15:50	**Talk 1: Single-cell analysis technologies and assays** *Mathias Lichterfeld, Ragon Institute of MGH, MIT and Harvard, USA*	
15:50-16:00	**Talk 2: Viral Sequencing** *Sarah Palmer, The Westmead Institute, Australia*	
16:00-16:20	**Questions**	
16:20-16:30	**Break**	
16:30-16:45	**Flash Talks: Whole body imaging** • *Tim Henrich, University of California San Francisco, USA* • *James McMahon, The Alfred Hospital and Monash Medical Centre, Australia*	
16:45-17:00	**Flash Talks: Imaging** • *Zaza Ndhlovu, University of KwaZulu-Natal, South Africa* • *Claire Deleage, Frederick National Laboratory for Cancer Research, USA*	
17:00-17:15	**Questions**	
	Breakout room discussion	
17:15-17:50	**Blue Room** **Lead: Bonnie Howell**	**Red Room** **Lead: Tim Henrich**
17:50-18:00	**Lead report**	**Lead report**
18:00-18:25	**Group discussion**	
18:25-18:30	**Closing and what's next**	

Key points from the presentations

HIV reservoir measurement today 00:36:45 – *Nicolas Chomont, University of Montreal, Canada*

Nicolas Chomont presented a selection of assays for measuring HIV reservoirs. Main points included:

- Robust assays to measure reservoirs using VL off ART are available.
- While on ART, assays should be chosen to answer a particular question.
- Challenges of HIV reservoirs measurement include the rarity of cells in which HIV persists and the persistence of HIV in many deep tissues.
- Several markers can be measured in different infection settings (for example, latent versus productive infection).
- The Quantitative Viral Growth Assay (QVGA) has been the gold standard for many years. This cumbersome assay is the only tool that can measure the frequency of cells harbouring replication-competent virus. However, there is a 2-log difference between the level of HIV DNA and the frequency of cells harbouring replication-competent viruses. This is because on ART, many viruses are defective and some are difficult to induce. Therefore, assays have been developed to sequence the near full-length HIV genome to better measure the reservoir.
- The Intact Proviral DNA Assay is a digital droplet PCR assay that targets frequently deleted regions in defective HIV genomes. It is easy to run and relatively inexpensive and can give an idea of the frequency of supposedly intact HIV genomes in blood and tissues. Detection is not perfect and may not work for all HIV clades.
- Assessing the genetic integrity of HIV genomes is critical, but their inducibility is also important. Several reservoir induction assays have therefore been developed.
- The research question should determine the assays to be used. Other important factors in the choice of an assay are HIV clades, number of cells available, number of samples and equipment needed, and budget.
- The chosen assay should be sensitive, non-invasive and able to measure what is targeted.

Chomont concluded with recommendation on how to discuss assays to measure the HIV reservoir. For example, Chomont reiterated there is no assay that is the "gold standard"; it depends on what question you are seeking to answer. (Editor's note: Slide available at 00:48:32)

Predictors of viral rebound after ATI 00:49:45 – *Jonathan Li, Harvard University, USA*

Jonathan Li started by providing the background for evaluating the success of cure interventions, which will have to demonstrate clinically meaningful results during ATI.

Li summarized some of the downsides of ATI and emphasized the need to identify biomarkers that can predict time to viral rebound, as well as the need to evaluate promising treatment strategies.

He summarized a range of assays used in various cure studies and their ability to predict rebound. He noted that DNA and low levels of cell-associated RNA (CA-RNA) can predict the timing of viral rebound.

Li then summarized some of the findings of the A5345 study:

- Modern ART did not appear to delay viral rebound.
- Multiple assays were used to measure the reservoirs.
- Some measurements were predictors of rebound and it was possible to distinguish predictors of rebound in function of the stage of the infection.
- There are non-reservoir predictors of HIV faster/slower rebound (T-cell and soluble factors) in early and chronically treated people.

Li presented some of the findings of a study with extreme reservoir depletion (Henrich, Annals IM 2014).

Moving forward with non-invasive assays for HIV reservoir measurement

01:01:54 – *Mohamed Abdel-Mohsen, The Wistar Institute, USA*

Mohamed Abdel-Mohsen started by explaining the need to identify non-invasive surrogate markers to fulfil the requirement to measure the total body burden of the inducible replication competent reservoirs and the immunological pressure of the reservoirs in tissues. There are major barriers to measuring reservoirs in these tissues. Among points made:

- Quantitative and qualitative characterization of the reservoir is important because of the difference between tissues with small and large inducible or non-inducible reservoirs.
- These reservoirs may release molecules in the blood (cells, virus, Ab, metabolites, proteins/cytokines/chemokines, lipids, glycans, exosomes, cell-free nucleic acids) that can become biomarkers.
- Some of these molecules are biologically active molecules and can alter other cell functions and can also be used as biomarkers.
- Glycomic and metabolic markers can distinguish between post-treatment controllers and non-controllers.
- These markers are not randomly distributed. Some markers are associated with delayed or accelerated viral rebound and can reflect immunological

or inflammatory pressure associated with viral rebound. They may directly or indirectly impact rebound (for example, L-glutamic acid).

- There are several challenges in identifying potential markers:
    - Small number of participants, window period to viral rebound
    - Inconsistent definition of viral rebound
    - Inconsistent measurements
    - Confounders

Abdel-Mohsen concluded that it is unlikely that a single or small number of markers can be predictive of viral rebound.

Profiling individual HIV-1 reservoir cells: four generations of technology 1:13:50 – *Mathias Lichterfeld, Harvard Medical School, USA*

Mathias Lichterfeld described four single-cell assays to measure reservoirs *ex vivo*:

- **FLIP-Seq**, based on the analysis of NFL sequencing, FLIP-Seq provides multiple information from sequencing data from which you can infer intactness and clonality of proviruses.
- **MIP-Seq**, designed to analyse proviral sequences and corresponding chromosome integration sites, can reveal important differences related to the site of integration and provide qualitative features of the reservoir cells. The integration site may also be used as a biomarker for selection of reservoir cells.
- **PRIP-Seq** assay, based on NFL sequencing of viral DNA and RNA from single infected cells, can evaluate the "depth" of latency. Chromosomal location is very much correlated to viral transcriptional activity.
- **PheP-Seq**, an analysis of the proviral sequence in combination with cell phenotyping, allows learning about the immune susceptibility of reservoir cells. Phenotypic markers can be associated with chromosomal integration sites, genomic integrity and susceptibility and vulnerabilities or reservoir cells to immune interventions.

HIV viral sequencing 01:30:45 – *Sarah Palmer, The Westmead Institute, Australia*

Sarah Palmer discussed the application of full-length HIV DNA sequencing. Among points made:

- Full-length HIV DNA sequencing was performed on Peripheral Blood Mononuclear Cells (PBMCs) obtained from a cohort of untreated participants who were either HIV-positive/HBV-positive or HIV-positive.

- A high level of genetically intact proviruses was found in the PBMCs of these untreated individuals (30 to 3,000 per 10^6 CD4+ versus 0 to 26 in people on ART).
- Other studies showed that most proviruses are genetically defective in people on ART.
- Genetically intact proviruses are unequally distributed in distinct CD4+ T-cell subsets.
- Effector memory (EM) cells and EM cells that express HLA-DR contain significantly higher levels of genetically intact proviruses than other cell subsets.
- This was confirmed in other studies, which also showed differences in genetically defective proviruses in EM cells according to the location of the deletion.
- Further sequencing revealed that intact Nef sequences were concentrated in EM cells. The expression of Nef downregulates major histocompatibility complex (MHC), making HIV-infected cells invisible to CD8+ T-cells.
- Intact gag and nef sequences were also concentrated in EM cells, indicating that an intact Nef ORF can protect other viral genes.
- A plasma-derived HIV RNA long-range sequencing (PRLS) assay (8.3kb and 4.7kb) was developed to assess how plasma virions contribute to persistent HIV. When used with samples from untreated participants, 65% of plasma-derived genomes were genetically intact. The most common defects are frameshifts. The proportion of intact viral sequences was higher, although not statistically significant, during ATI.
- There are some indications that the reservoir developed during pre-therapy can contribute to a rebound during ATI.
- Several factors hamper the use of this technology around the world:
    - Cost
    - Technological know-how, which may be lacking in different parts of the world and will require significant investment in training and capacity building
    - Intellectual property rights protection, which could slow the rollout in countries where protection is weak

Q&A part 1 (1:41:15)

➢ *Nicolas Chomont asked Sarah Palmer whether the assay was used with people on suppressive therapy and whether it is possible to sequence the few viral particles still circulating in the blood. If so, is it a better predictor of the virus that will rebound?*

Palmer responded that plasma viral DNA is a limitation. The 4.7kb sequencing region was applied, but 8.3kb is more challenging. Sequencing

circulating viruses during suppressive therapy, especially long-range sequencing, would be useful to identify which virus is contributing to the rebound.

- *Victor Garcia asked Sarah Palmer whether the circulating Nef protein had the downmodulating function of MHC class I.*

Palmer said this was confirmed and the work had been published in JCI.

Garcia queried whether the activity was in the context of the provirus or the protein. Palmer said this had not been looked at.

- *Mathias Lichterfeld asked for confirmation that the viruses sequenced from people on ATI were more likely to be intact.*

Palmer confirmed that full-length sequencing showed that 87% of the viruses from these participants – who have been through three ATIs – were genetically intact and that there were much more defective viruses prior to ATI. This may be because these cells have replication-competent viruses.

- *Alan Landay noted that many great pathogenesis studies were done and asked Jonathan Li how these assays would be integrated into clinical trials. The Industry Cure Collaboration Group is interested in moving the field forward and applying these tools in clinical trials, using them effectively to understand the role of cure interventions and their outcomes.*

Li commented this is still a discovery phase with different assays looking at different things and that a focus on one assay could lead to missing the big picture. The newer assays are tremendously exciting (single-cell assays, sequencing) and the technology is catching up with the questions the field wants to answer.

- *Alan Landay asked how to integrate some assays in clinical trials.*

Li said the reservoir measurement assays are not perfect, but there may be surrogate markers that could be even better than measuring the reservoir, or there could be downstream events that could be a better reflection of the whole-body reservoir and provide a view of what is happening in the tissues.

- *Timothy Henrich said that the technology is evolving rapidly and more and more tools are available. Regardless of the study done, it is important to ensure samples are carefully collected, including tissues, in sufficient quantity for future studies.*

Flash talks

Whole body tissue imaging of the HIV reservoir and immune responses

02:02:40 – *Timothy J. Henrich, University of California San Francisco, USA*

Tim Henrich presented on the potential application of PET imaging of HIV persistent reservoirs using monoclonal antibodies directed against gp120. PET imaging can answer several questions, for example, where HIV persists, what the dynamic is of HIV decay on ART, and what tissues are responsible for viral rebound. Henrich summarized studies performed with ^{89}Zr-VRC01 immunoPET in viraemic, ART-suppressed and HIV-negative participants. Points included:

- The EXPLORER high-sensitivity PET scanner allows for imaging with high resolution while increasing the ratio signal/noise and the sensitivity of detecting tracer throughout the body. This scanner is FDA approved for clinical diagnosis and will be at the forefront in the next 10 years.
- PET imaging has also been used to understand HIV rebound dynamics across time and body sections. Uptake was observed in bone marrow, spleen, lymph nodes, NALT and colon in patchy areas.
- This technique can be used in cure trials to look at differences in HIV activity before and after blocking TGFβ signalling (which reactivates the HIV-1 reservoir) and shows an increase in gut and lymph node uptake of tracers.
- The technique could be used beyond HIV to look at other markers and immune responses (for example, trafficking T-cells and what they are producing).
- Upcoming applications could include looking at HIV persistence in elite/exceptional controller cohorts to determine the early site of viral responses and be applied to a variety of cure-related clinical studies and ATI protocols.

PET-based imaging of HIV *in vivo* 02:12:45 – *James McMahon, Monash University, Australia*

James McMahon presented the results of the ^{64}Cu-3BNC117 studies in macaques and humans, as well as future research directions. Points included:

- The study builds on previous work and enrolled people living with HIV and people who were HIV negative or people with HIV off or on ART. QVGA and PhenoSense mAb were used to screen participants for sensitivity to 3BCN117. Whole-body imaging was performed.
- The intervention was safe, and ^{64}Cu-3BNC117 could bind and neutralize the HIV envelope with an efficiency similar to that of 3BCN117. No

difference was seen between the three groups. Multiple differences between macaques and humans could explain the difference between studies.
- Future studies will include labels with longer half-lives to assess HIV biodistribution over a longer period, increasing available targets by combining nAbs and using latency reversal agents that increase the expression of HIV proteins.

Imaging the HIV reservoir in human lymph nodes 02:20:35 – *Zuzu Ndhlovu, University of KwaZulu-Natal, South Africa*

Zuzu Ndhlovu presented work on understanding the mechanisms of HIV persistence in tissues during ART and the CD8-mediated mechanisms of HIV reservoir control/elimination. Points made:
- The FRESH cohort provided the necessary samples that are very important when characterizing HIV reservoirs.
- Research was performed using TissueFax microscopy, Omics approaches and spatial transcriptomics using a GeoMX DSP, allowing for the precise localization of persistent HIV in tissues, for example, lymph nodes.
- HIV-infected CD4+ T-cells are predominantly localized within the germinal centres in lymph nodes. Ongoing viral transcription was confirmed by other methods.
- Heterogeneity within the germinal centre was observed and spatial genomics showed that many genes were downregulated but also upregulated during infection, suggesting TREC infiltration.
- In conclusion, HIV infection persists in lymph node germinal centres for years despite early ART initiation and the persistent HIV burden can induce immune-regulatory changes in the microenvironment.

Imaging tissues with non-invasive techniques 02:30:53 – *Claire Deleage, Frederick National Laboratory for Cancer Research, USA*

Claire Deleage shared her perspective on non-invasive measurements of HIV reservoirs using imaging techniques. She shared the following points:
- Currently, the best way to localize, identify and quantify HIV reservoirs is to work with tissues and look for:
    a) Latent cells only harbouring DNA
    b) Cells where there is active transcription
    c) Cells where there is active translation
- It is now possible to perform highly multiplexed tissue phenotyping of those cells. One "high plex" approach using phenocycler fusion, developed by Akoya (CODEX platform), uses barcoded antibodies to test for up to 100 markers using a single tissue section.

- Special analysis capability can help identify new populations of cells and elicit information about the macroenvironment in the tissues. Biopsies are still required.
- In contrast, MRI can be used to evaluate pathology and the impact of the HIV infection that leads to inflammation in lymphoid tissues, altering its integrity and impairing immune and vaccine response.
- MRI was adapted to evaluate functional changes by following impaired dendrimer uptake in a monkey model.
- CD169 identified the recruitment of macrophages around the subcapsular areas, perturbing the draining functions of the lymph node and explaining the MRI observations. ART did not repair these damages.

Deleage concluded that a non-invasive approach could help in understanding the pathology and be useful in cure research.

Q&A part 2 (02:43:22)

➢ *Bonnie Howell asked Tim Henrich how robust the reactivation needs to be to detect a signal using the PET imaging given what we know with current LRA.*

Henrich said that efforts are ongoing to increase the sensitivity, but at this stage, we don't know. This is what we need to look at next.

➢ *Deborah Persaud noted that knowing baseline susceptibility to bnAbs will be critical for their use in imaging studies. For example, in paediatric cure research, 27% of children in sub-Saharan Africa are resistant to VRC01; she asked whether pre-screening will be required or whether there are ways to use combinations of antibodies for the labelling studies.*

Henrich said that a mixture of antibodies can be used, although it is not possible to tell which antibody will work. Henrich noted that full neutralization is not needed; rather, sufficient binding to perform the imaging is needed. The ability of the Ab to bind is more important than neutralization. There are also challenges with pre-screening in participants suppressed for a long time.

➢ *Lynda Dee asked Tim Henrich about the availability of the PET imaging technology at research sites and added that it would be important to try to make the technology broadly available.*

Henrich commented that this is very important. Only a PET CT scanner is required for the PET imaging. Antibodies can be made locally and shipped to centres that have the scanner.

- *Deborah Persaud asked Tim Henrich whether there will be challenges using labelled bnAbs for imaging in cure interventions that use bnAbs.*

Henrich said that it will be challenging as microdoses of labelled bnAbs are used for the imaging and there will be competition. Different bnAbs could be used for the imaging.

- *Bonnie Howell asked Claire Deleage how to extract data from hundreds of markers in a meaningful way and how long it takes.*

Deleage said that any marker can be conjugated to an antibody using a barcode. It takes less than two days to run the assay and the analysis can be done using software that identifies clusters and populations of cells. It can become more complicated depending how markers are used. It also depends on the question asked as the analysis is time consuming; it could take months without a precise question.

Breakout room summaries

Bonnie Howell – Blue Room

Discussed:

- The non-invasiveness of the various assays and the need to collect samples (blood, urine)
- Whether to worry on not about defective genomes, recognizing that defective genomes can contribute to some protein production, which can influence immune function and dysfunction, and inflammation. But the consensus in the room was that with a focus on cure, intact genomes are probably the most relevant measure. However, when working with interventions that are targeting viral proteins, it is important to look at the transcriptional activity of the reservoirs; this would allow a better assessment of the intervention.
- How can industry help bring assays to scale?
- Use of imaging applications, especially PET. There is interest in the data, but it will take time to leverage these in research settings.
- Assay sensitivity and their ability to visualize reservoirs in different organs
- Importance of figuring out the use of biomarkers for those who are not going to rebound and distinguishing responders and non-responders retrospectively
- Enrolling individuals in clinical studies

Tim Hendrich – Red room

Discussed:

- How to start integrating lower technology that could be scalable to reach the relevant clients and populations in different settings
- The need for markers that can be reachable across platform validation
- Looking at the macro environment and type of markers in ATI and suppressive environments
- Tissue and prioritization in terms of what studies we want to validate across platforms in these clinical trials. This is to identify clinically relevant markers to measure success, in addition to better understanding the pathogenesis.
- How to work with the industry to motivate, through foundation-type structures, support for the development of these types of assays. They may not be commercially viable for diagnostics and there is a need to understand how to build partnerships to get these platforms funded in all regions.

Discussion

Bonnie Howell commented that there is an opportunity for academia to partner with industry that is committed to leveraging some of these technologies and work out how to build partnerships. Ways to engage with a range of industry partners should be explored. The cancer research field is learning how to detect rare events. HIV cure research should leverage these technologies and approaches.

Lynda Dee asked whether a large meeting has ever been held to discuss partnership and collaboration. Bonnie Howell reported interactions through the Martin Delaney Collaboratory and added that a meeting is a great idea to bring more partners and stakeholders to the table.

Omolara Baiyegunhi asked about the acceptability of these assays to those most vulnerable. Howell said that it depends on the request and the invasiveness of the assay. A better perspective from the community is needed. Howell reported on the work done by Merck with the "at-home" collection device.

Lynda Dee said that it is important to describe how to involve participants in these studies. These require more time compared with standard methods and some compensation may be required while avoiding incentivization. It may be time to look at reimbursement levels. Jeff Taylor agreed, adding that this is hugely important and more research is needed.

Alan Landay reported on previous talks with diagnostics companies and the challenges for developers. Landay also noted that discussions are siloed

and that the IAS could help identify all the groups involved and bring them together with the community.

Roger Tatoud reported on the work done by the IAS Industry Liaison Forum. Tatoud asked how to move forward with the development of these assays, drawing attention to assay harmonization and the need to ensure that industry is on board.

Tim Henrich commented that there are many different stakeholders and groups discussing these issues and how to engage and work together. It is difficult to approach a company or funder unless there is a unified consensus on what we want to do. This is essential to have more leverage with industry and funders.

Lynda Dee wondered if the industry partnership at the IAS could shepherd the organization of the discussion as currently there is no coordination. Bonnie Howell agreed that it would be an interesting initiative and that ultimately these assays would have to be built at scale. It is important to understand the needs and the ask and what the different partners can contribute, all in support of the eradication effort.

Tim Henrich responded that this does not have to be HIV specific. These platforms are adapted from general research and if we are looking at scale and want to attract industry's interest, we should emphasize that this work is not to develop expensive platforms for a single niche market, but that they can be used for multiple diseases, for example, cancer research. If platforms can be integrated and standardized, they can be made available to general laboratories. Further these non-viral markers may also be relevant to non-HIV pathologies.

Alan Landay said that when we are thinking globally, we are thinking about non-communicable diseases (NCDs); the intersection between infection and NCDs is huge and the research can also contribute to disease preparedness. Heather Ann Brauer commented on her experience in oncology where similar discussions are taking place. There is a lot of thinking about how to leverage assays in development in the non-viral space.

Lynda Dee suggested that the first question a meeting could address could be about the intersection of these things, discussing cancer and other conditions. Bonnie Howell said that this is a great idea and concluded the meeting, thanking all for joining and participating.

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