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HIV Reservoir Characterization Symposium 19 September 2016, Ghent, Belgium

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Abstract

The HIV Cure Research Center (HCRC) in Ghent organised the first HIV Reservoir Characterization Symposium, and brought together virologists, molecular biologists, immunologists and clinicians to discuss the most recent developments in HIV reservoir characterisation with a view to achieving an HIV cure. The one-day symposium covered new developments in the field of HIV reservoir and HIV cure research, with the latest news on the European HIV cure trials. This report summarises the major themes discussed during the symposium.

Keywords: HIV reservoir, digital PCR, HIV cure, symposium

Introduction

The symposium, organised by Linos Vandekerckhove and Ward De Spiegelaere, brought together over 100 participants from 17 countries to Ghent, Belgium to discuss the latest developments in the field of HIV reservoir and HIV cure research. New methods to characterise and quantify HIV reservoirs were discussed and the latest discoveries in HIV cure studies were presented creating a stimulating environment for all participants. The symposium participants benefited from presentations by experts in the field: Maria Buzon, Christine Rouzioux, Manfred Schmidt and Annemarie Wensing, who were invited guest speakers (Table 1). The scientific gathering further included a selection of oral and poster abstracts presenting the most recent HIV reservoir characterisation method strategies within the HIV cure efforts. This symposium was directly followed by a three-day HIV Reservoir Characterization Workshop, which was aimed at providing the participants with thorough training on HIV reservoir quantification tools with an emphasis on digital PCR, including hands-on and data-analysis training.

Session 1

In the opening remarks, organiser Linos Vandekerckhove addressed the critical issues in HIV reservoir characterisation within the search for an HIV cure. Professor Vandekerckhove outlined the recent history of HIV reservoir research and highlighted the current HIV cure approaches giving an overview of clinical HIV cure studies currently being conducted in Belgium. ISALA [1] is an analytical treatment interruption (TI) study that is addressing the research question of whether some patients who harbour a low reservoir (as measured by total HIV-1 DNA and cell-associated (CA) HIV-1 RNA) have the potential to control the virus in the absence of antiretroviral therapy (ART). Another ongoing study, the STAR study involving in-depth tissue sampling [2], aims to locate the anatomical origin of the rebounding virus after TI. Lastly, the ABIVAX study aims to explore the potential of a nuclear export inhibitor (ABX464) to suppress HIV viral load (VL) in the absence of ART [3].

Following the introduction, co-organiser Ward De Spiegelaere opened the morning session and provided a comprehensive overview on the use of digital PCR in HIV reservoir quantification. According to Professor De Spiegelaere the advantages of digital PCR are higher accuracy and precision, it is less refractory to inhibition and has an increased reproducibility when compared to qPCR (quantitative PCR). Professor De Spiegelaere demonstrated that droplet digital PCR (ddPCR) is less prone to inhibition caused by high genetic diversity within, and among, HIV-1-infected patients compared to qPCR, which is especially important for measuring reservoir in HIV-1-infected patients. However, he also mentioned that current ddPCR platforms do not have a higher sensitivity compared to qPCR due to the occurrence of false positives in ddPCR. The cause of the false positives is being explored extensively and has been shown not to be due to contamination. This may hamper the use of ddPCR as a diagnostic tool to assess whether HIV is present in a sample. It is, however, widely applicable to quantify HIV DNA or HIV RNA copies in situations where the status of the patients is known, such as in HIV cure studies. Professor De Spiegelaere highlighted digital PCR as an optimal tool to quantify 'needles in a haystack' with diverse applications in HIV research; however, some issues related to the technology and data analysis need to be overcome to further enhance precision and accuracy of this novel technique. Consequently ddPCRquant was discussed as the first data-driven tool with a valid statistical basis for ddPCR data analysis [4].

Virginie Mortier presented HIV-1 DNA load data from 249 patients on ART that demonstrated links with the pre-treatment CD4+ T cell count nadir and peak VL, suggesting an added value of HIV-1 DNA load analysis in routine patient monitoring. They determined HIV-1 DNA load with an in-house qPCR and classified the patients into two groups based on the total HIV-1 DNA measured. Members of the cohort with total HIV-1 DNA lower than 150 copies/million cells were more prone to have a CD4 cell count nadir below 200 cells/mm^3. Furthermore, in a longitudinal follow up of nine patients, there was a slower reduction of total HIV-1 DNA after ART initiation with a smaller decline compared to VL.

Sofie Rutsaert presented a study on cross-validation of HIV-1 DNA assays to quantify different HIV-1 subtypes by ddPCR. The study analysed the accuracy of numerous described assays designed to quantify total HIV-1 DNA from clade B viruses and to detect other HIV-1 subtypes, namely A, B, C, D, CRF01_AE and CRF02_AG. Based on *in silico* analyses of 71 assays, resulting from a systematic literature search using a bioinformatics pipeline developed in house, 16 assays were selected for *in vitro* testing. All selected assays were able to capture subtype B samples, in contrast to the circulating recombinant forms CRF01_AE and CRF02_AG, which were challenging to quantify. The two best performing assays captured more than 90% of HIV-1 DNA in more than 80% of the samples, thus suggesting that subtype-specific assays or the use of multiple, complementary assays is recommended to accurately measure HIV-1 DNA from different clades.

Session 2

Maria Buzon opened the second session of the symposium with an overview of existing *ex vivo* assays to measure viral reservoir

Table 1. Speakers and their presentations at the 2016 HIV Reservoir Characterization Symposium

Speakers	Title
Linos Vandekerckhove	Introduction
Ward De Spiegelaere	Digital PCR in HIV reservoir quantification
Virginie Mortier	Added value of HIV-1 DNA load analysis in routine patient monitoring
Sofie Rutsaert	Cross-validation of HIV DNA assays to quantify different HIV-1 subtypes by ddPCR
Maria Buzon	*Ex vivo* assays to measure viral reservoirs
Pieter Pannus	Quantifying and characterizing the HIV reservoir in virally suppressed patients
Virginie Fievez	Spatial Distribution of HIV in antiretroviral-treated humanized mice
Christine Rouzioux	Total HIV1-DNA a marker of viral reservoir dynamics
Ulrike Lange	CRISPR/Cas9-derived activation systems as new tools for induction of latent Human
Sophie Bouchat	Reactivation capacity by latency reversing agents ex *vivo* correlates with the size of the HIV-1 reservoir
Manfred Schmidt	Integration site analysis in HIV infected patients
Roxane Verdikt	Implication of DNA methylation in HIV-1 post-integration latency
Alexander Pasternak	Cell-associated HIV-1 unspliced RNA level predicts both time to virological suppression and duration of post-treatment virological control in patients treated with temporary early ART
Annemarie Wensing	EPISTEM: European Project to guide and investigate the potential for HIV cure by Stem Cell Transplantation
Linos Vandekerckhove	Closing remarks

size. Dr Buzon presented both PCR-based methods as well as cell culture-based methods, focusing on advantages and disadvantages of the assays as well as providing technical details of presented approaches. Her talk included the latest updates on methods such as flow cytometry-based RNA assays and murine quantitative viral outgrowth assays for the quantification of the HIV reservoir. She closed her talk by suggesting that analytical TI was the 'ultimate' assay and is the only way to validate whether HIV-infected patients are cured.

Pieter Pannus described a positive correlation between HIV-1 DNA and unspliced (US) mRNA, as well as a negative correlation between both total HIV-1 DNA and US mRNA with a pre-treatment nadir CD4+ T cell count in 53 ART-suppressed subjects. The associations between these clinical parameters will be of importance in future TI studies aimed at achieving post-treatment control of infection. Virginie Fievez presented results of spatial distribution of HIV-1 in ART-treated humanised mice, demonstrating the presence of total HIV-1 DNA in organs such as spleen, bone marrow, lungs, lymph nodes and in blood, highlighting the very widespread distribution of the viral genome.

Session 3

Christine Rouzioux opened the afternoon session with a presentation on total HIV-1 DNA as a marker of viral reservoir dynamics. Professor Rouzioux pointed out the lack of consensus on the best markers for clinical trial endpoints. Total HIV-1 DNA quantification by qPCR is a commercial assay and used globally as a marker of HIV-1 reservoir. Total HIV-1 DNA has been shown to correlate with time to viral rebound after TI [5,6]. Moreover, total HIV-1 DNA levels are impacted by early ART [7]. Professor Rouzioux discussed the concept of remission and levels of total HIV-1 DNA in post-treatment controllers in the VISCONTI study [8,9]. In this context, quantifying HIV-1 DNA would select patients who could safely have cure interventions such as TI, this selection would be best made in combination with other markers. She also discussed a broader definition of HIV-1 reservoirs that includes all infected cells and tissues with all forms of HIV-1 persistence that can participate in HIV-1 pathogenesis [10], and not just the currently thought latent reservoir. This is because both the latent and activated reservoirs (HIV-1 particles and proteins) play a role in HIV-1 pathogenesis. Thus, we could estimate not only the HIV-1 reservoir size, but also its activity and capacity to produce further viruses. Furthermore, the use of total HIV-1 DNA in clinical practice was addressed so that it could help to adapt and guide treatment in patients with undetectable VL. As Professor Rouzioux said: 'there won't be a magic marker', but we will need a more integrative approach, including markers of inflammation, immunological markers and cellular markers.

Ulrike Lange discussed the use of a CRISPR/Cas-9-derived activation system to induce latent HIV-1 reservoir [11]. The data demonstrated transcriptional activation of a proviral genome in various cell culture latency models using CRISPR/Cas-9 technology, suggesting the usability of the technique in *in vitro* models aimed at reversing HIV latency, and its future potential use in patient-derived cells. Sophie Bouchat presented the capacity of combinational latency-reversing agents (LARs) *ex vivo* to diminish the size of the HIV-reservoir and demonstrated the positive correlation between the size of HIV-1 reservoir and HIV-1 recovery measurements in response to various LRAs.

Session 4

In the final session, Manfred Schmidt discussed viral integration site (IS) analysis in HIV-infected subjects. Professor Schmidt first reviewed gene therapy trials linked with emerged integration-mediated oncogene-activation. He went on to describe LAM-PCR methods [12] to identify integration sites, which is essential to assess the safety of such a treatment. Within HIV cure attempts, he postulated that viral IS analysis can aid in identifying high risk IS that can persist and amplify in the patient despite ART. It was further highlighted that novel methods allow us to investigate whether distinct HIV variants and IS patterns are expected to be associated with HIV persistence in different tissue reservoirs.

Roxane Verdikt presented work on DNA methylation inhibitors tested in several clones of the HIV-1 latently-infected J-Lat T cell lines in order to understand the processes of HIV-1 promoter demethylation, important for reversing HIV latency. Reactivation of HIV-1 expression was the highest on treatment with cytidine analogues but overall heterogeneity of methylation patterns was observed in different J-Lat clones, suggesting that different molecular mechanisms could be responsible for the establishment of varying methylation patterns in the HIV-1 promoter region.

Alexander Pasternak presented data from six biomarkers (plasma viraemia, total HIV-1 DNA, CA US HIV-1 RNA, multiply spliced HIV-1 RNA, CD4+ T cell count, CD4:CD8 ratio) analysed for the ability to predict the time to viral suppression upon treatment initiation in patients in primary HIV infection (PHI). US HIV-1 RNA at PHI was a significant predictor of time to viral suppression and predictor of time to viral rebound upon TI. It was therefore concluded that further exploration of the use of this biomarker should be undertaken in future large-scale HIV cure clinical trials. Furthermore, CA multiply spliced HIV-1 RNA level predicted disease progression (CD4+ T cell loss) after interruption of early ART.

Finally, Annemarie Wensing presented data from EpiStem: the European project to guide and investigate the potential for HIV cure by stem cell transplantation (SCT). Professor Wensing announced the change of consortium name from EpiStem to IciStem in response to the expanding international activities of the consortium. IciStem stands for International Collaboration to guide and investigate the potential for HIV cure by Stem Cell Transplantation. Professor Wensing explained the main role of the IciStem consortium, which is an observational study that investigates the potential of a cure in HIV-infected patients who receive allogeneic stem cell transplants due to life-threatening haematological conditions. The project aims to identify potential cases of HIV-1 eradication/remission and to understand the mechanisms leading to a reduction of viral reservoirs. So far 22 HIV-positive patients have received SCT under the IciStem umbrella and four cases were presented in detail. Out of three patients who had a successful transplant, two had undetectable HIV DNA by extensive measurements although the subjects remain on ART, and so definitive HIV cure status cannot be concluded. HIV-reservoir reduction in stem cell transplant recipients is speculated to be linked to immune responses associated with graft-versus-host responses establishing graft-versus-reservoir effects contributing to the killing of HIV-latently infected cells.

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