Highlights from the HIV Cure and Reservoir Symposium, 11–12 September 2017, Ghent, Belgium

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Abstract

For the second time, the HIV Cure Research Center (HCRC) at Ghent University organised the HIV Cure and Reservoir Symposium, in Ghent, Belgium, where in this two-day conference, virologists, molecular biologists, immunologists and clinicians presented the most recent achievements in the field of HIV cure, including data on therapeutic vaccines, HIV remission strategies such as 'shock and kill' or 'block and lock', benefits of early ART and potential of haematopoietic stem cell transplant in achieving cure. Furthermore, methods to characterise and quantify the HIV reservoir were discussed along with HIV reservoir characterisation in different body parts, including the central nervous system. An HIV activist and representative of a patients' agency also presented the patients' perspective on HIV cure. This report is a summary of all topics discussed during this symposium.

Keywords: HIV reservoir, cure

Introduction

Professor Linos Vandekerckhove and Dr Magdalena Sips organised the HIV Cure and Reservoir Symposium, 11–12 September 2017, in Ghent, Belgium. More than 100 participants from 15 countries were present to discuss the latest developments in HIV cure approaches, and the presence of many experts in the field facilitated high-level discussions: Guido Vanham (Institute of Tropical Medicine, Antwerp, Belgium); Beatriz Mothe (IrsiCaixa, Spain); Ole Søgaard (Aarhus University Hospital, Skejby, Denmark); Jintanat Ananworanich (US Military HIV Research Program, USA); Magnus Gisslén (University of Gothenburg, Sweden); Asier Sáez-Cirión (Institute Pasteur, France); Maria Buzon (Vall d'Hebron Institut de Recerca, Spain); Philip Goulder (University of Oxford, UK); Carine van Lint (ULB, University of Brussels, Belgium); Zeger Debyser (KU Leuven, Belgium); Sabine Kinloch (Royal Free London, UK); and Stephane De Wit (St Pierre University Hospital, Belgium). The scientific committee, comprising Linos Vandekerckhove, Magdalena Sips and Maria Buzon, selected the best submitted abstracts for oral and poster presentations. An overview of the programme, including invited and selected presentations at the symposium, is depicted in Table 1. This symposium was directly followed by a three-day HIV Reservoir Characterization Workshop, which aimed to provide the participants with a thorough understanding of HIV quantification tools, including PCR-based and cell-based functional assays, which are of great importance in therapy monitoring, for the development of HIV cure strategies. In addition to virological assays, the workshop presented an overview of immunological assays that are commonly used to assess the potency of both the cellular and humoral immune system. The workshops included theory sessions, hands-on practical sessions and data analysis training.

Session 1

Following the inauguration of the symposium by **Professor Linos Vandekerckhove**, where the critical issues in the search for an HIV cure were discussed, the symposium was opened by **Mr Fred Verdult**, a representative of a patients' communication agency, Volle Maan, who talked about the patient perspective on HIV cure, and presented intriguing differences between scientists' and patients' views of why an HIV cure is needed. According to HIV scientists questioned during a conference in 2011, the most important reasons to find an HIV cure are: to prolong life expectancy, to eliminate long-term side effects, to erase the pill burden and financial reasons. However, from 457 HIV-positive patients questioned, 94% considered finding a cure important, but prolonging life expectancy is only the ninth most important reason from the patient perspective. Side effects from HIV medication are the fifth most important reason to find a cure, and the pill burden ranks seventh, while financial considerations are not in the top-ten reasons at all. For patients, the most important reasons to find an HIV cure are long-term side effects from infection and treatment, which have an overall negative impact on general health. Importantly, patients consider infectiousness and stigmatisation as the third and fourth most important reasons why an HIV cure is needed. Social and emotional disadvantages, not important for scientists, appear to be very important from the patients' point of view, and the negative impact on relationships, and living with a secret, are the eighth and tenth most important drivers towards an HIV cure.

Following the opening talk, **Professor Guido Vanham** initiated the scientific sessions of the symposium, and provided an overview of state-of-the-art therapeutic vaccinations in HIV-infected patients on combination antiretroviral therapy (cART). The presentation reviewed different vaccination strategies based on properties of the vaccine antigen: (a) vaccines focusing on the most conserved parts of the HIV genome (Vacc4x of the Scandinavian consortium and HIV_{consv} of the University of Oxford); (b) vaccines combining the most representative 'potential T cell epitope' (PTE) variants (mosaic vaccines); and (c) vaccines with epitopes associated with evidence of *in vivo* protective T cell responses (HIVACAT-T cell immunogen, HTI, IrsiCaixa) [1-8]. He presented data showing that: Vacc4x induced a slight reduction of the viral set point after analytical treatment interruption (ATI), and that the vaccine in combination with romidepsin slightly reduced the viral reservoir; HIV_{consv} can induce CD8 T cell HIV suppressive capacity, and when combined with romidepsin, delays the viral rebound after ATI; mosaic vaccine in rAd26 vector, increases the breadth and depth of CD4+ and CD8+ T cell responses, and as adeno primed with Env protein boost, is considered a potent prophylactic vaccine when tested in monkeys; HTI therapeutic vaccination induces T cell responses associated with *in vivo* and *in vitro* CD8+ T cell-mediated HIV suppression. Lastly, Professor Vanham addressed novel concepts in vaccinology, such as mRNA vaccines, intranodal administration of vaccines and combinations with anti-latency treatment or checkpoint inhibitors.

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Table 1. HIV Cure and Reservoir Symposium 2017 programme

Speaker	Subject
Linos Vandekerckhove	Inauguration
Fred Verdult	Patient perspective on HIV cure
Guido Vanham	HIV cure efforts in Europe
Beatriz Mothe	Therapeutic vaccine in early treated cohorts
Ole Søgaard	Early lessons from shock and kill trials
Gerlinde Vansant	Towards a block-and-lock strategy: LEDGINs hamper the establishment of a reactivation competent reservoir
Sophie Bouchat	Identification of a new factor involved in DNA methylation-mediated repression of latent HIV-1
Michaela Madlenakova	Heme-arginate as a latency reversing agent for HIV-1 cure
Jintanat Ananworanich	Early ART and HIV remission
Alessandra Bandera	Early start of antiretroviral therapy (ART) during primary HIV infection (PHI) is associated with faster optimal immunological recovery: results of Italian Network of ACuTe HIV InfectiON (INACTION) retrospective study
Clarissa Van Hecke	The expression profile of host restriction factors in different cohorts of HIV-1-infected patients
Magnus Gisslén	Defining the CNS HIV reservoir by CSF and blood biomarkers
Marie-Angélique De Scheerder	CSF inflammatory profile in patients undergoing analytical treatment interruption
Asier Sáez-Cirión	Bone marrow transplantation in the IciStem consortium
Linos Vandekerckhove	ABX464 decreases total HIV DNA in PBMCs when administered during 28 days to HIV-infected patients who are virologically suppressed
Inge De Lepeleire	Single doses as low as 0.5 mg of the novel NRTTI MK-8591 suppress HIV for at least 7 days
Maria Buzon	New assays to measure the latent HIV reservoir
Pieter Pannus	Does viral suppressive capacity in cART-treated HIV-infected patients correlate with disease parameters, viral reservoir measures or cytotoxic T cell phenotype?
Basiel Cole	Integration site detection in 10 chronically infected patients
Sofie Rutsaert	Defining a total HIV DNA threshold as guidance for therapy simplification strategies
Philip Goulder	HIV cure/remission in paediatric infection
Jean-Christophe Beghin	Immunovirological outcome of HIV-infected children living in a resource-limited setting of South Africa
Linos Vandekerckhove	Closing remarks

Dr Beatriz Mothe reviewed the latest HIV therapeutic vaccine trials conducted in patients who started cART within 6 months after infection. In the BCN01 trial (NCT 01712425), 24 patients were vaccinated with two different vaccination schemes (12 vaccinated long arm, with 24 weeks between prime and boost, and 12 vaccinated short arm, with 8 weeks between prime and boost), and 24 patients received a placebo [3,9]. The vaccine regimen is a HIV_{consv} vaccine, which was constructed by assembling the 14 most conserved regions of the HIV proteome into one chimeric protein. The vaccine comprises a prime with an attenuated chimpanzee adenovirus serotype 63 (ChAd63) and a boost with a modified vaccinia virus Ankara (MVA), both encoding HIV_{cons}. After MVA boost, the subdominant responses to HIV_{consv} were effectively boosted in both vaccination schemes. Reactivation was not observed during peak immunogenicity, and the decay of proviral DNA was similar to early-treated but not vaccinated individuals. Some 15 out of 24 vaccinated patients from BCN01 were enrolled into a subsequent roll-over study (BCN02-Romi), in which the patients received a booster MVA vaccine followed by three doses of romidepsin [10]. Dr Mothe described the main findings about the immunogenicity and viral reservoir changes in these 15 patients. Finally, participants who showed positive immune response to the vaccine were invited to undergo an ATI. Fourteen patients underwent an ATI, and five of them showed a late viral rebound (>4 weeks, up to 8 months), while the other nine demonstrated a fast rebound.

Professor Ole Søgaard presented some of the emerging data using the shock-and-kill strategy, which aims to reduce the viral reservoir in individuals living with HIV. After introducing the concepts of sterilising and functional cure, Professor Søgaard presented data from trials using the following cure strategies: reactivate the latent HIV reservoir; enhance HIV-specific immunity; and enhance innate immunity. Different latency-reversing agents (LRA) (panobinostat, romidepsin, disulfiram, vorinostat) have been shown to be potent in reactivating part of the latent HIV reservoir; however, development of more potent LRAs has been suggested as an important step to move this strategy forward. Next, Dr Søgaard discussed immunotherapeutic approaches that could enhance the killing of the reservoir, showing results of: (a) immune checkpoint blockade with antibodies against PD1/PD-L1 or CTLA4; (b) HIV broadly neutralising antibodies (bNAb); (c) chimeric antigen receptor (CAR) T cells; and (d) TLR7/9-mediated immune activation. Finally, he presented data from the BCN02-Romi trial, which used combinations of LRAs with a therapeutic vaccine as a shock-and-kill strategy, previously discussed by Dr Mothe.

In the following oral abstract talk, **Ms Gerlinde Vansant** presented a completely different and opposite HIV cure strategy: block and lock. LEDGINs, novel allosteric HIV integrase (IN) inhibitors that target the lens epithelium-derived growth factor (LEDGF)/p75 binding pocket of IN, act as mediators of the block-and-lock strategy and are capable of blocking the establishment of a reactivation-competent reservoir by redirecting the integration of HIV into silent regions, resulting in inability of HIV to reactivate and therefore affording a functional cure. She demonstrated that LEDGINs are capable of inducing an integration shift out of the transcription units, and as a result, increase the latent fraction of the reservoir and hamper reactivation in cell lines.

Dr Sophie Bouchat showed the results of her research towards the identification of UHRF1, a new factor involved in DNA methylation-mediated repression of latent HIV. UHRF1 is involved in HIV latency via a DNA methylation-mediated silencing mechanism: it interacts with *de novo* methyl transferases and, as such, increases methylation and mediates epigenetic silencing of the HIV viral promoter region. These results suggest that UHRF1 might constitute a new potential molecular target for HIV cure therapeutic strategies.

The last speaker of the day, **Ms Michaela Madlenakova**, showed novel data implicating use of haem-arginate (HA) as a new latency-reversing agent for HIV cure. HA potentiates PMA-stimulated reactivation of HIV provirus in latently infected ACH-2

cells, and stimulates HIV expression in peripheral blood mononuclear cells (PBMCs) infected *ex vivo*. This reactivation is mediated by haem or iron-induced redox stress. In patients, HA Normosang induced reactivation of HIV, and subsequently a reduction in the HIV reservoir size.

Session 2

Professor Jintanat Ananworanich opened the second day of the symposium discussing the impact of early cART on HIV remission. She presented data from the acute RV254 study and related HIV remission trials. In the RV254 trial, 488 acutely infected patients (Fiebig stages I–V) from cohorts in Thailand were enrolled and placed immediately on cART. Such immediate cART initiation is expected to induce low viral reservoir establishment and effective immune restoration. Reservoir quantification was performed in blood, sigmoid colon and lymph nodes (LN), and revealed that the lowest total HIV DNA was detected in patients treated in Fiebig stage I, while no difference in reservoir size was detected after ART administration in Fiebig stages II–V. After Fiebig I, almost all LN samples during acute infection showed detectable HIV DNA. The median time to viral rebound during ATI, characterised by viral load (VL) >20 copies/mL, was 26 days (range 13–48 days) in Fiebig I treated individuals. Similar viral sequences between acute infection and post rebound suggest that VL rebound was due to clonal expansion of latently infected cells. Pre-ATI HIV DNA levels were not significantly associated with time to VL rebound. Owing to early treatment, effector CD8+ T cell immune responses expanded only after VL rebound.

Dr Alessandra Bandera evaluated the clinical and epidemiological characteristics of patients with acute HIV infection (Italian Network of ACuTe HIV InfectiON 'INACTION' retrospective study) and compared the effect of early versus late cART on virological suppression, discontinuation of the first cART regimen and immunological recovery (CD4 cell count, CD4% and CD4:CD8 ratio). She demonstrated that early cART initiation and a more preserved immunological status at presentation are predictors of optimal immunological recovery.

Ms Clarissa Van Hecke presented a study where the expression profile of host restriction factors was examined in different cohorts of HIV-infected patients: recent seroconverters, long-term non progressors (LTNP), cART-naïve non-controllers, early- and late-treated patients. Restriction factors APOBEC3G, SAMHD1, SLFN11 and BST2/tetherin were significantly upregulated in early- compared to late-treated HIV patients, suggesting that elevated expression of these restriction factors could be beneficial for maintaining host immunity. Unlike other restriction factors, SLFN11 was upregulated in LTNP and its expression was negatively correlated with integrated and total HIV DNA, suggesting that SLFN11 contributes to this non-progressing phenotype.

Professor Magnus Gisslén discussed the central nervous system (CNS) as a less well-known HIV reservoir of unknown relevance for cure efforts. CNS infection is generally well controlled by suppressive systemic cART, although sometimes HIV can be detected in the cerebrospinal fluid (CSF) despite undetectable plasma viral load. Professor Gisslén described in detail three possible types of CSF viral escape during ART: asymptomatic, symptomatic and secondary escape, and stressed the importance of paying attention to new-onset CNS symptoms during effective ART, especially during suboptimal drug adherence and with drugs with low CNS-penetrating ability. Some 10% of asymptomatic subjects exhibit CSF HIV RNA >50 copies/mL despite plasma RNA <50 copies/mL, and this CSF asymptomatic escape is associated with increased CNS inflammation. CSF blips are associated with CNS immune activation but not with increased markers of neuronal injury. Nevertheless, these CSF blips are not associated with progressive disease or treatment failure [11]. An important blood biomarker of active neuronal injury is plasma neurofilament light protein (NFL). NFL levels are 50–100 times lower in blood compared to CSF, although, it is still a good marker to distinguish new onset of neurological/neurocognitive symptoms, suboptimal penetration of ART regimens in CNS and potential harmful activation of CNS infection by LRAs [12].

On behalf of **Dr Marie-Angelique De Scheerder**, Professor Linos Vandekerckhove presented a treatment interruption study and its effects on neuro-inflammation. In the HIV STAR study (Sequencing after Treatment Interruption to identify the Anatomical Reservoir), where the main objective was to identify the anatomical compartment responsible for viral rebound during TI, extensive blood and tissue sampling of 12 individuals living with HIV was performed, including blood, CSF, colon, LN, bone marrow and bronchoalveolar lavage. Blood was collected at four time points, at baseline on cART, day 7 after ATI, at viral rebound and after cART restart (undetectable viral load), and CSF was sampled at baseline and during viral rebound. No significant increase of NFL or other inflammatory markers (nepterin/YKL-40) was detected, despite an increase in VL. These data suggested that it is unlikely that replication in CNS has started to a large extent early after treatment cessation. Moreover, the virus detected in CSF was similar to virus identified in blood; Env sequences identified in CSF after rebound were identical to the dominant strain of rebounding virus in plasma.

Session 3

Professor Asier Sáez-Cirión opened the final session of the symposium and presented new results from the IciStem project: an observational study to guide and investigate the potential of allogeneic stem cell transplantation (SCT) for HIV cure. Following a comprehensive overview of the study design and objectives, Professor Sáez-Cirión focused on results obtained from six specific patients who have successfully undergone SCT and are still in active medical follow-up. First, he showed that after SCT, five out of six patients have an undetectable HIV latent reservoir in different anatomical compartments (peripheral blood, ileum, CSF, LN, BM), as measured by different reservoir quantification techniques: HIV DNA PCR, quantitative viral outgrowth assay (qVOA) and cell-associated unspliced HIV RNA PCR. While SCT causes a tremendous reduction in the size of the viral reservoir, it is not sufficient to result in HIV remission/cure. He then showed that HIV-specific T cell responses could still be detected several years after SCT; however, these immune responses are weak in nature and have limited polyfunctionality. Therefore, therapies boosting immune responses could be of interest before advancing to treatment discontinuation. During the second part of the presentation, Professor Sáez-Cirión presented data on the use of natural simian immunodeficiency virus (SIV) controlling Mauritian cynomolgus macaques as a tool to study HIV specific T cell responses. Using this model, he demonstrated that the development of SIV-specific CD8+ T cell responses coincides with CD8 expansion in post-acute phase control of viraemia. Furthermore, he showed that CD8 T cells from SIV controllers show a strong capacity to suppress viral replication *ex vivo*; however, this response is weak during acute infection, suggesting that the development of the potent immune response occurs during later stages of infection.

Professor Linos Vandekerckhove presented results of a multi-centre, randomised, double-blind, placebo-controlled, Phase IIa clinical trial of ABX464, which is a first-in-class antiviral drug from Abivax. Regarding the safety assessment, only minor adverse events such as abdominal pain and headache were observed. From eight of fifteen participants who received the compound, a

significant decrease in HIV reservoir size was observed between the first day of drug administration and day 28, with a mean decrease of 186 HIV copies per million PBMCs. No responders were observed in the placebo group. These data illustrate that ABX464 might have a reservoir-reducing effect; however, this reduction did not correspond to a significant increase in the time to viral rebound upon TI. Professor Vandekerckhove concluded that follow-up studies involving more participants are needed as well as studies of mechanistic actions of ABX464.

Dr Inge De Lepeleire presented data showing that a single dose of a novel nucleoside reverse transcriptase translocation inhibitor (NRTTI), called MK-8591, is capable of suppressing HIV replication for at least 7 days. An open-label, single-dose study (ranging from 0.5 mg to 30 mg) was performed to evaluate the compound as monotherapy in ART-naïve, HIV-infected subjects. The primary study objectives were the assessment of safety and tolerability, and the degree of VL suppression. A robust VL decline was observed at all dose levels, even at 0.5 mg. MK-8591 was generally safe and well tolerated, with no evidence for dose-dependent adverse experiences. Only a limited number of mild-to-moderate adverse events were reported. The half-life of the compound in PBMCs was quantified at 79–128 hours, which makes the drug interesting as a once-weekly regimen.

Professor Philip Goulder provided a comprehensive overview of paediatric HIV infection and perspectives for HIV cure in children. He outlined several case studies of HIV-infected infants treated extremely early, and questioned whether HIV cure can be achieved in paediatric patients. Professor Goulder described a cohort of South African infants with slow or non-progressing phenotype among which elite controller (EC) phenotypes were observed with a frequency of 0.3–0.4%. However, unlike adults ECs, paediatric EC phenotype is not driven by protective HLA alleles and the viral load of paediatric ECs does not drop under the limit of detection. To explore the underlying mechanisms of viraemic control in infants, a second cohort of 63 HIV-infected infants was studied. Eleven of these infants were slow/non-progressors, characterised by a lower VL (1 $\log_{10}$ lower than progressors) and a higher breadth and magnitude of the CD8+ response. He showed that unlike in adult infection, during paediatric infection there is generation of cytotoxic T-lymphocytes (CTL) specific to escape variants. He postulated that HIV-specific CTLs are likely to be one component contributing to paediatric viraemic control, but most probably other factors, such as host genetic factors, also play a role.

Continuing on the topic of paediatric HIV infection, **Dr Jean-Christophe Beghin** discussed the immunological outcome of HIV-infected children living in South Africa upon treatment initiation. He compared a cohort of children who started cART within 1 year after birth (early starters cohort: ESC) with a cohort of children who started cART at age 2 years or later (late starters cohort: LSC). His study demonstrated that cART is highly effective regardless of age and CD4% at treatment initiation; however, ESC treated with a PI-based regimen achieved normal CD4% and sustained virological suppression more often.

Dr Maria Buzon presented a comprehensive overview of conventional and new *ex vivo* assays to measure the size of the HIV latent reservoir. The first part of her talk focused on conventional PCR-based and cell culture-based approaches, ranging from total HIV DNA quantification by PCR to quantitative viral outgrowth assay (qVOA). Advantages and disadvantages, as well as technical details, were provided for each of the described assays. In the second part of her talk, Dr Buzon discussed novel assays to measure the latent reservoir, going from full HIV genome sequencing to the murine viral outgrowth assay (mVOA). While some of these novel assays seem very promising in delivering an accurate measurement of the reservoir size, Dr Buzon concluded that ATI is eventually the only way to determine whether the patient is cured, making it the ultimate assay.

Mr Pieter Pannus presented correlates of viral suppressive capacity (VSC) in cART-treated HIV-infected patients focusing on CD8+ T cell responses. Using the viral inhibition assay (VIA), he quantified the viral suppressive capacity of CD8+ T cells obtained from a total of 36 successfully treated HIV-positive patients and showed that VSC can be enhanced *in vitro* by HIV peptide stimulation. While the magnitude of VSC did not correlate with measures of the reservoir size, it did correlate with IFN-γ production, PD1/CD160 co-expression and HLA-DR expression during co-culture.

Mr Basiel Cole presented analysis of virus integration site data obtained from 10n chronically infected, long-term-treated HIV-positive individuals. After elaborating on the importance of clonal expansion in the maintenance of the viral reservoir, Cole demonstrated that all but one participant showed the presence of clonally expanded HIV-infected cells, with a mean level of ~40% of clonality. Furthermore, he showed that integration was biased towards genes that play a role in histone modifications, DNA repair and DNA transcription, and that there is a clear preference for integration in the reverse orientation with respect to the gene.

In the final talk, **Ms Sofie Rutsaert** opened the discussion about whether total HIV DNA could serve as reliable marker in guidance for therapy simplification strategies. Total HIV DNA was measured by droplet digital PCR (ddPCR) in patient samples of two randomised clinical trials, PROTEA and DOMONO. In a substudy of PROTEA (NCT01448707), patients were switched to darunavir/r monotherapy and followed for 96 weeks, and in DOMONO (NCT02401828) patients were assigned to a dolutegravir monotherapy arm for 48 weeks. In both studies, patients with an undetectable viral load had a significantly lower level of total HIV DNA in comparison to patients for whom monotherapy failed virologically, which suggests that HIV DNA can aid in selecting patients eligible for therapy simplification strategies.

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