Abstracts of the HIV Cure and Reservoir Symposium 2017

11–12 September 2017
Ghent, Belgium

Stably integrated HIV inside the host cell genome is considered the final hurdle to cure one of the most important infectious diseases humans have ever faced. The latent HIV reservoir is able to refuel infection once the treatment is stopped and therefore a better understanding of both the composition and the size of the reservoir is important to aid the development of cure strategies as well as to monitor the efficacy of therapeutic interventions to reduce the HIV reservoir. Our symposium will comprehensively summarise the state of the art in HIV cure strategies and diagnostic tools to monitor therapeutic interventions and an international expert panel will guide the audience through the latest developments in the HIV cure field, focusing on reservoir characterisation and novel clinical strategies to achieve HIV cure.
Aims and objectives

The aim of this journal is to provide a specialist, open access forum and fast-track pathway to publish work in the rapidly developing field of virus eradication, particularly of HIV, HBV and HCV. The Journal has been set up especially for these and other viruses, including herpes and flu, in a context of new therapeutic strategies, as well as societal eradication of viral infections with preventive interventions.

Scope

The Journal not only publishes original research, but also provides an opportunity for opinions, reviews, case studies and comments on the published literature. It focuses on evidence-based medicine as the major thrust in the successful management of HIV and AIDS, HBV and HCV as well as includes relevant work for other viral infections. The Journal encompasses virological, immunological, epidemiological, modelling, pharmacological, pre-clinical and *in vitro*, as well as clinical, data including but not limited to drugs, immunotherapy and gene therapy. It will be an important source of information on the development of vaccine programmes and preventative measures aimed at virus eradication.

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Oral abstracts of the HIV Cure and Reservoir Symposium 2017

01
Towards a block-and-lock strategy: LEDGINs hamper the establishment of a reactivation competent reservoir
Gerlinde Vansant, Lenard S Vanrckx, Irena Zurnic, Suha Saleh, Anne Bruggemans, Frauke Christ, Zeger Debryser
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Background: Persistency of latent provirus is the main barrier towards a cure for HIV. We propose a novel strategy to reduce the HIV reservoir by drug-induced retargeting of HIV integration. A novel class of integration inhibitors, LEDGINs, inhibits the interaction between HIV integrase (IN) and the LEDGF/p75 cofactor, the main determinant of integration site selection. This results in an allosteric inhibition of HIV IN (early effect). Moreover, when present during production, progeny virus displays morphological and replication irregularities (late effect).

Methods: Integration sites were sequenced using 454 pyrosequencing. To evaluate reactivation potential, cell lines were infected with a double-reporter virus and LEDGIN was added during infection (early effect) or during production of the virus (late effect). Cells were reactivated and analyzed by FACS. In a multiple round experiment, primary activated CD4+ T cells were infected with wild type NL4.3 virus in the presence of LEDGIN or RAL. Cells were reactivated and virus production was measured by p24 ELISA.

Results and conclusions: LEDGIN-mediated inhibition of the LEDGF/p75-IN interaction blocks replication and relocates integration out of transcription units. This retargeting resulted in a residual reservoir that contains up to 95% of latent cells that are resistant to reactivation. In activated CD4+ T-cells, both LEDGIN and RAL reduced infection. The residual provirus established under LEDGIN treatment was hampered for reactivation, a phenotype not observed with RAL. Addition of LEDGINs early during acute infection does affect the formation of the latent reservoir. Bringing the majority of residual proviruses in a state of deep latency and defective for reactivation might represent an attractive approach to achieve an HIV remission.

02
Identification of a new factor involved in DNA methylation-mediated repression of latent HIV-1
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Background: Persistence of latent provirus is the main barrier towards a cure for HIV. We propose a novel strategy to reduce the HIV reservoir by drug-induced retargeting of HIV integration. A novel class of integration inhibitors, LEDGINs, inhibits the interaction between HIV integrase (IN) and the LEDGF/p75 cofactor, the main determinant of integration site selection. This results in an allosteric inhibition of HIV IN (early effect). Moreover, when present during production, progeny virus displays morphological and replication irregularities (late effect).

Methods: Integration sites were sequenced using 454 pyrosequencing. To evaluate reactivation potential, cell lines were infected with a double-reporter virus and LEDGIN was added during infection (early effect) or during production of the virus (late effect). Cells were reactivated and analyzed by FACS. In a multiple round experiment, primary activated CD4+ T cells were infected with wild type NL4.3 virus in the presence of LEDGIN or RAL. Cells were reactivated and virus production was measured by p24 ELISA.

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03
Heme-arginate as a latency-reversing agent for HIV-1 cure
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Background: Persistent latently infected HIV reservoirs resist effective therapy. Latent HIV-1 genomes are reactivated in vivo UHRF1 recruitment to the 5’LTR. UHRF1 knockdown using RNA interference and pharmacological approach showed increased levels of HIV-1 production in latently-infected cells and of HIV-1 transcription in ex vivo cell cultures from cART-treated aviremic HIV+ patients, respectively.

Methods: To explore this mechanism, we took advantage of two latently-infected cell lines studied. Interestingly, a site comprising one of this hotspot for decitabine-induced demethylation was shown to bind UHRF1, only in one of cell line. Treatment with decitabine caused a decreased in vivo UHRF1 recruitment to the 5’LTR. UHRF1 knockdown using RNA interference and pharmacological approach showed increased levels of HIV-1 production in latently-infected cells and of HIV-1 transcription in ex vivo cell cultures from cART-treated aviremic HIV+ patients, respectively.

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The expression profile of host restriction factors in different cohorts of HIV-1-infected patients

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After HIV-1 infection, several antiviral host genes are upregulated to suppress viral infectivity. These antiviral factors, defined as restriction factors, are part of a first line innate antiviral immune response. In this study, host restriction factor expression (APOBEC3G, SAMHD1, tetherin, TRIM5α, MX2, SLFN11 and PAF1) was determined in different cohorts of HIV-1 infected patients.

The expression profile of seven HIV-1 restriction factors was evaluated in 104 HIV-1 infected patients from six pre-defined cohorts: long-term-treated patients where ART is initiated during seroconversion (Early treated; N=24) or chronic infection (Late treated; N=32), long term non-progressors (LTNPs; N=17), recent ART-naïve seroconverters (Recent SRCV; N=19) and ART-naïve chronically infected patients (Chronic naïve; N=12). Patients were recruited in two clinical centers (Royal Free Hospital London and Ghent University Hospital) and an additional group of healthy individuals (N=14) was included as a control.

In general, elevated restriction factor levels for Recent SRCV, LTNP, early treated patients and chronically infected therapy naïve patients were determined. The restriction factor levels of healthy individuals and late treated patients remain low at similar levels. For example, APOBEC3G was significantly upregulated in ART-naïve recent SRCV and long-term-treated patients that started therapy early (Early treated), in comparison to healthy individuals (p < 0.001; p < 0.001). APOBEC3G was also elevated in comparison to long-term-treated patients started on ART during chronic HIV-1 infection (Late treated) (p < 0.01, p < 0.001). APOBEC3G levels were positively correlated with CD4 count at borderline significance level (r=0.44, p=0.076).

Host restriction factor expression levels are elevated in recent seroconverters as an early antiviral host defense mechanism. LTNP are able to maintain slightly upregulated restriction factor levels, suggesting a mechanism contributing to their non progressing phenotype. For HIV-1 patients on treatment, restriction factor levels are upregulated in the early treated in comparison to the late treated HIV-1 patients. HIV-1 patients that started treatment early are able to maintain higher restriction factor levels which could contribute to a better disease control.
**Methods**

parameters and the HIV reservoir.

**Results**

and a small latent viral reservoir is likely required to achieve post-treatment control of HIV infection. Here we investigated correlations between viral suppressive capacity, phenotype of CD8+ T-cells, clinical parameters and the HIV reservoir.

**Methods**: Thirty-six patients on cART with suppressed viremia and six healthy donors were recruited. Total HIV-1 DNA and unspliced mRNA (usRNA) levels were measured in PBMCs. Viral suppressive capacity of CD8+ T cells and their phenotype (cytokine production, cytotoxicity and immune checkpoint markers) were determined before and after peptide stimulation. In a sub-group of 21 patients infected with HIV subtype B, viral transcriptional activity was quantified with the TILDA assay. Linear regression and student t-test were used for correlation analyses.

**Results**: Total HIV DNA and usRNA levels (median: 187 and 2.2 cps/million PBMCs respectively) were as expected and correlated with each other (p < 0.05). TILDA values ranged from 0 to 313 cells [IQR: 1.4–55.8] with detectable HIV RNA transcripts per million CD4+ T cells after stimulation. CD8+ T-cell suppressive capacity was significantly (p < 0.01) increased with peptide stimulation.

**Conclusions**: In this group of virally suppressed patients, heterogeneity in terms of immune responses and reservoir size was observed, as expected. Interestingly, T-cell exhaustion markers CD160 and CD107a expression in CD8+ T cells and their phenotype (cytokine production, cytotoxicity and immune checkpoint markers) were determined before and after peptide stimulation. In a sub-group of 21 patients infected with HIV subtype B, viral transcriptional activity was quantified with the TILDA assay. Linear regression and student t-test were used for correlation analyses.

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P1

Contribution of IncRNAs in the establishment of HIV latency in central memory CD4 T Cells

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HIV cure research has been hampered by the existence of a latent viral reservoir that persists in infected individuals receiving antiretroviral therapy. To date, most of the cure research has focused on protein coding genes but recently the interest in the study of non-coding RNA has risen, as these molecules could provide insight in new therapeutic strategies.

Transcriptome profiling was performed (total RNA-Seq) in a primary HIV latency model of central memory CD4 T cells (TCM cells) to investigate changes in IncRNA expression. Subsequently, differentially expressed mRNAs and IncRNAs were identified and a guilt-by-association analysis was implemented to infer biological roles for the IncRNAs in HIV latency. In the primary HIV latency model, we identified 826 mRNAs (87.8%) and 115 IncRNAs (12.2%) that were significantly differentially expressed (FDR<0.05) between uninfected and latently infected TCM cells. Many of these IncRNAs were associated with pathways involved in cell cycle regulation and pathways with a link to HIV latency: IL-7, PTEN, CSK and CCR5. In addition, a cluster of 17 IncRNAs was associated with the p53 pathway and corroborate earlier findings in this TCM model that illustrated p53-dependent latency establishment. One of these upregulated p53-linked IncRNAs, 7SLRNA, has a characterized inhibitory role in the p53 pathway and would suit as a possible new therapeutic target.

Altogether, this study demonstrates that several IncRNAs play a role in HIV latency and can be linked to biological pathways with importance in HIV latency establishment and maintenance. Some of these IncRNAs, i.e. 7SLRNA, represent possible targets for reversing HIV latency and contribute to a HIV cure.

P2

Clinical evaluation of the next-generation sequencing (NGS) for detection of drug-resistance mutations in HIV-1

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Introduction: WHO urges action against HIV drug resistance threat. It is well known that the sensitivity of NGS is vastly superior to Sanger sequencing. The objective of this study was to evaluate the novel NGS HIV-1 drug resistance monitoring system, particularly those in low proportion.

Materials and methods: NGS analyses were performed on plasma samples from 41 HIV-1 infected patients with the Sentosa SQ HIV Genotyping Assay from Vela Dx. This kit was used on a semi-automated Ion Torrent-based platform. Sequences were compared to those obtained by Sanger method. Two samples were added inside and in separated runs. Quality control (QC) were added to control protease (PRO) and reverse transcriptase (RT) sequencing and one to control integrase (INT) sequencing.

Results: In the 41 patients analyzed, both methods detected 245 Drug Resistance Mutations (DRMs). The Sentosa SQ HIV Genotyping Assay detected 38 additional DRMs (mutation frequency ~7%). Sanger method detected 2 additional DRMs, 39A which is not included in the list of mutations by Vela Dx because of few clinical impact and the secondary mutation 63Q. The sequences were 98.2% homologous (counting variants as mismatch) and 99.9% homologous (not counting missed variants). Duplicate in a run were 95.7%(99.9%) homologous. Duplicate in two different runs were 98%(100%) homologous. QC results were manually assessed to a score of 340/340 for detection of DRM in PRO and RT and to 100% for INT sequencing.

Conclusions: To our knowledge, this is the first clinical evaluation in Belgium employing the Sentosa SQ HIV Genotyping Assay. The NGS appears as a promising tool for the detection of DRM in HIV-1. Results give a higher sensitivity compared to Sanger method. Studies assessing the clinical relevance of DRM in low frequency are needed.

P3

An insertion of seven amino acids in the envelope cytoplasmic tail of human immunodeficiency virus type 2 (HIV-2) selected during disease progression enhances viral replication

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Introduction: The cytoplasmic tail (CT) of the transmembrane envelope glycoprotein (gpTM) of HIV-2 includes amino acids (aa) sequences similar to lentiviral lytic peptides (LLP) described in other lentiviruses [1,2]. Within the putative LLP-2 region, we previously observed insertions of 3 or 7 aa in sequences deduced from plasma viral RNA of symptomatic HIV-2 infected individuals [3]. Based on these observations, we reproduced the insertions in a molecular clone to assess their impact on replicative fitness and cell death in vitro.

Methods: Using a molecular clone of the HIV-2 ROD reference strain, site-directed mutagenesis experiments allowed the generation of plasmids with the insertion L791TAI or L791QRAL TAI in Env protein. After transfection in HEK293T cells, the viral particles were used to infect H9 or Jurkat T cells. Viral release was quantified by RT-qPCR at three and six days post-infection. Cell viability was assessed with the percentage of living cells using a CASY cell counter. The viral infectivity was monitored using TZM-bl reporter cells.

Results: Compared to the control wild-type HIV-2 ROD virus (HIV-2 WT), the clone with a 7 aa insertion enhanced viral release thirteen times. Cell viability was 20% more impaired compared to the wild-type virus. The effect of the 3 aa insertion was milder, with a non-significant trend to enhance viral replication and cell death compared to the wild-type.

Interestingly, the insertions in the Env proteins did not induce a significant increase of viral infectivity, as revealed by the infectivity assay using TZM-bl cells.

Conclusion: A 7 aa insertion including positively charged aa in the putative LLP-2 enhances viral replication and cell death in vitro. The insertions in the Env CT observed in vivo from disease progressors may therefore be involved in the higher viral load observed in these individuals. This study may open the way to the development of laboratory diagnostic tools related to disease progression.

References
Long-term aviremia after discontinuation of antiretroviral treatment

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Background: Early ART initiation is associated with impact on HIV-1 reservoir establishment and decay with the potential for virological control post-treatment discontinuation. Underlying mechanisms of post-virological control remain unclear. We report on a clade C-infected female patient who has maintained undetectable viremia (VL) for 13 years after stopping a 6-year treatment period initiated at PHI with initial virological failure while on ART and describe her virological parameters and HIV-1 specific T cell responses.

Case report: A 23-year-old female seroconverted with a 3-week-long severe acute retroviral syndrome in October 1997. Blood parameters show a CD4<200 cells/mm³ on 3 occasions and VL >750,000 HIV-1 copies/mL (clade C) before ART initiation on 20.10.97 (AZT-3TC-indinavir 800 mg tds switched to ritonavir 600 mg bd 2 weeks later). Failure of this regimen over several weeks up to 94,000 c/mL prompted treatment intensification (double-boosted protease inhibitors). Aviremia was achieved in April 1999 on ART which was maintained until January 2004. Following treatment discontinuation aviremia persisted without viral blips for 13 years with preservation of CD4 T cells and CD4/CD8 ratio>1. HLA genotype was not one generally associated with a favourable outcome.

At 10 years of aviremia (2014), total HIV-1 DNA, integrated HIV-1 DNA and 2-LTR circles were 148.93 (95% CI: 76.99–229.64), 134.31 (95% CI: 56.47–304.39) and 3.89 (95% CI: 0–9.15) HIV-1 copies/million PBMCs, respectively. CD4 and CD8 HIV-1 specific T cell responses showed moderately potent CD8+ T cell inhibition of a clade-matched HIV-1 isolate equivalent to that which we have observed in ART-naïve chronically infected subjects with VL set-point <10,000 HIV-1 copies/mL. Unusually broad gag-specific IFN-γ CD4 responses were detected, targeting multiple regions of genetic vulnerability that are associated with virological control. Analysis of CSF (September 2015) showed an undetectable viral load. Her CD4 count in July 2017 was at 942 cells/mm³ with a ratio at 0.99

Conclusion: This is an unusual case of very prolonged aviremia after initial failure of treatment at the time of PHI. Intermediate levels of total and integrated HIV-1 DNA and broad HIV-1 gag-specific CD4 T cell responses, together with preserved CD8+ T cell viral inhibitory activity were noted during follow-up. Further insight should be gained into the potential role of CD4 T cells underlying virological control post-ART.