five African countries. Selected volunteers were further characterized with regards to early and persistent *in vivo* control of HIV-1 replication, in the absence of antiretroviral treatment. We utilised a propensity score matching approach to control for the influence of five factors (age, risk group, virus subtype, gender, and country) known to influence disease progression on causal observations. The concentrations of fifty-two soluble plasma proteins were assessed. This approach is unique because it focuses on the specific period of dynamic immunological control of viral replication for all volunteers in the absence of treatment.

Results: Among 603 volunteers, we identified three distinct groups of individuals (Low, Intermediate and High viral load volunteers) matched on all five factors and for whom samples were available at two timepoints within the dynamic phase of immunological control of *in vivo* replication following peak viraemia (i.e., within 0 to 365 days post-infection). We were able to confirm some of the factors related to, or already known to influence disease progression such as the possession of B*57 HLA Class I allele, and the infecting virus subtype. Our results also indicate possible roles for IL-17C and MIP-1α in the early and sustained control of infection.

Conclusions: Our results highlight the need to consider factors that could potentially introduce heterogeneity in datasets and mask valid differences when designing studies to define immune correlates.

OA14.04

Targeting sphingosine-1-phosphate signaling prevents cell-to-cell transmission of HIV-1

R. Resop[1]; R. Fromentin[2]; D. Newman[1]; H. Rigsby[2]; L. Dubrovsky[1]; M. Bukrinsky[1]; N. Chomont[2] and A. Bosque[1]

[1]George Washington University, Microbiology, Immunology and Tropical Medicine, Washington, D.C., United States, [2]CRCHUM and Université de Montréal, Microbiology, Infectious Diseases and Immunology, Mongtréal, Canada

Background: Sphingosine-1-phosphate (S1P) is a modulator of a myriad of cellular processes and therapeutic targeting of S1P signaling is under clinical investigation. We sought to determine the role of S1P in cell-to-cell transmission of HIV.

Methods: Using a primary cell model, we examined whether targeting S1P receptors altered HIV transmission in memory CD4+ T cells. We activated and expanded naïve human CD4+ T cells and infected them with HIV, in the presence of the S1PR functional antagonist FTY720 before or after infection. In addition, we examined transmission of HIV from infected macrophages to autologous CD4+ T cells in a coculture system in the presence of FTY720. We quantified productive infection by p24-Gag flow cytometry, measured frequency of cells harboring total and integrated HIV DNA by qPCR, and examined reactivation of latent virus following T cell receptor stimulation. Mechanistically, we investigated internalization of virions, cell cycle signaling, and innate HIV-1 restriction factor activity following FTY720 treatment.

Results: FTY720 treatment reduced HIV transmission between CD4+ T cells as well as between macrophages and CD4+ T cells in co-culture assays. Mechanistically, treatment with FTY720 targeted early stages of the HIV-1 life cycle, as evidenced by 1) Reduced internalization of virions by viral entry assay; 2) Relative increase in the active form of SAMHD1, an innate restriction factor; 3) Reduction in total and integrated HIV-1 DNA; and 4) Reduced frequency of productively infected cells. Moreover, FTY720 reduced susceptibility to infection by decreasing the percentage of transcriptionally active, cycling cells, and downregulated Cyclin D3. These alterations were consistent with reduction in cell-to-cell transmission, which directly corresponded to reduced latent virus in CD4+ T cells.

Conclusions: Our results demonstrate that targeting S1P signaling with FTY720 inhibits cell-to-cell transmission in multiple cell types. FTY720 targets early stages of the HIV-1 life cycle, including entry of virions and events prior to reverse transcription. Targeting S1P appears to modulate SAMHD1 phosphorylation as well as modify the cell cycle to promote a less infection-permissive state. Our research suggests that therapeutic targeting of this pathway early in infection may aid in the development of strategies to prevent establishment of initial infection and the latent reservoir.

OA14.05LB

Impact of HIV kick-and-kill therapy on host epigenetic and transcriptional programs in PBMC, and viral rebound after cART interruption

B. Oriol-Tordera[1,2]; A. Esteve-Codina[3]; M. Berdasco[4,5]; E. Goncalves[6]; M. Esteller[4,5,7]; T. Hanke[8,9]; J. Moltó[10]; B. Clotet[1,10,11]; M.L. Calle[11]; B. Combadiere[6]; A. Sanchez-Pla[12,13]; B. Mothe[1,10,11]; M. Ruiz-Riol[1] and C. Brander[1,11,14]

[1]IrsiCaixa AIDS Research Institute, Institute for Health Science Research Germans Trias i Pujol (IGTP), Badalona, Spain, [2]Autonomous University of Barcelona, Cerdanyola, Spain, [3]Centro Nacional de Análisis Genómico (CNAG), Parc Científic de Barcelona, Barcelona, Spain, [4]Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute, L'Hospitalet de Llobregat, Spain, [5]Josep Carreras Leukaemia Research Institute (IJC), Badalona, Spain, [6]Sorbonne Université, Centre d'Immunologie et des Maladies Infectieuses – Paris (Cimi-Paris), INSERM U1135, Paris, France, [7]Department of Physiological Sciences II, School of Medicine, University of Barcelona, Barcelona, Spain, [8]The Jenner Institute, University of Oxford, Oxford, United Kingdom, [9]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan, [10]Fundació Lluita contra la Sida, Infectious Diseases Department, Hospital Universitari Germans Trias i Pujol, Badalona, Spain, [11]Universitat de Vic-Central de Catalunya (UVic-UCC), Vic, Spain, [12]Statistics Department, Biology Faculty, University of Barcelona, Barcelona, Spain, [13]Statistics and Bioinformatics Unit Vall d'Hebron Institut de Recerca (VHIR), Barcelona, Spain, [14]Institució Catalana de Recerca i Estudis Avancats (ICREA), Barcelona, Spain

Background: BCN02 was a pilot kick-and-kill clinical trial that combined therapeutic vaccination (MVA.HIVconsv) with the latency reversing agent romidepsin (RMD) followed by a monitored antiretroviral pause (MAP) in 15 early-treated HIV+ individuals (NCT02616874). Out of 12 evaluable participants for an omics subanalysis, 8 participants showed early (pVL > 2000 copies/mL < 4 weeks) and 4 a more delayed viral rebound (pVL > 2000 copies/mL > 4 weeks) in MAP. Systems biology analyses identified epigenetic and molecular mechanisms associated with response to vaccination and RMD and viral rebound kinetics.

Methods: Genome-wide gene expression (Illumina HiSeq2500) and DNA Methylation (Infinium HumanMethylation450 BeadChip) were assessed in frozen PBMC-pellets at baseline, 1 week after vaccination and 1 week after the 3rd RMD infusion (post-RMD). After pre-processing and normalization steps we applied principal component analyses (PCA) and differential expression/methylation analyses (limma-R/Biocondcutor). GSEA was used for functional analysis, and sPLS-DA (MixOmics-R/Bioconductor), to identify pathways explicative of differential viral rebound kinetics.

Results: The largest impact on host transcriptional and DNA methylation (DNAm) profiles was observed after the combined effect of vaccination and RMD, with 733 differentially expressed genes and 5695 differentially methylated positions being detected between baseline and post-RMD (adjusted $p < 0.1$). Modulated pathways at gene expression and/or DNAm level, were mainly associated with processes including cell cycle, DNA repair or metabolism and HIV, innate and adaptive immunity (GSEA q-value < 0.15). Of note, PCA revealed that only DNAm levels after the combined intervention segregated participants by their early or late viral rebound kinetics in MAP. We

summarized the therapy-modulated pathways with eigenvectors of DNAm at post-RMD, and identified HIV, innate immunity and T-cell pathways among the most relevant to discriminate the two viral rebound profiles in MAP. Interestingly, 3 CpG positions in the HIV category, 37 in the innate immunity group and 10 in the T cell category were differentially methylated between individuals with different viral rebound profile (adjusted $p < 0.1$).

Conclusions: Host transcription and epigenetic programs provide a deeper understanding of the molecular mechanisms induced during HIV cure therapies. While DNAm after a kick-and-kill strategy could be used to predict viral rebound kinetics after ART interruption, further study is warranted in future controlled studies.

OA15.01

Heterologous vaccination with DNA and two different poxvirus vectors, expressing HIV-1 envelope on the surface of Gag virus-like particles, elicit autologous Tier 2 neutralising antibodies

R. Chapman[1]; M. van Diepen[1]; N. Douglass[1]; S. Galant[1]; E. Margolin[1]; P. Ximba[1]; T. Hermanus[2]; P. Moore[2]; E. Rybicki[3] and A.-L. Williamson[1]

[1]University of Cape Town, Pathology, South Africa, [2]National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa, [3]University of Cape Town, Moelcular & Cell Biology, South Africa

Background: The only HIV vaccine trial (RV144) to show any efficacy used a canary poxvirus vector and recombinant protein for immunisation; leading to the further exploration of poxviruses as HIV vaccine vectors. Our group has shown that heterologous prime boost immunisations of mice and macaques with a novel capripoxvirus vector, lumpy skin disease virus (LSDV) and modified vaccinia Ankara (MVA) induced high magnitude, broad cellular immune responses. Here, we present data on further modifications to the vaccines, which were tested in different regimens, for immunogenicity in rabbits.

Methods: DNA (D), MVA (M) and LSDV (L) vaccines expressing HIV-1 CAP256SU gp150 and subtype C mosaic Gag (Gag^M) were constructed. The expression of Env and Gag by all three vaccine was characterised *in vitro*. Groups of five rabbits were inoculated with four different vaccine regimens: DDMMLL; DDMLML; DDLMLM and DDLLMM at 0, 4, 8, 12, 16 and 20 weeks. The titres of binding and neutralising antibodies in the rabbit sera were determined.

Results: The expression and secretion of HIV-1 CAP256SU gp150 and Gag^M by all three vaccine vectors was verified *in vitro*. In addition, the formation of Gag virus-like particles containing gp150 was confirmed using negative stain electron microscopy and western blotting following density gradient centrifugation. The quaternary structures of gp150 on the cell surface was assessed by immunostaining with human monoclonal binding antibodies (MAbs) to HIV-1 Env, and fluorescent microscopy or FACS analysis. Binding of MAbs, PG16, PGT145 and CAP256 VRC26_08, confirmed that all three vaccines expressed some native, trimeric Env. All four vaccination regimens elicited high titres of neutralising antibodies (NAbs) to Tier 1A pseudovirus MW965.26 two weeks after the final immunisation. However, 5/5 rabbits in the DDLMLM, 3/5 in the DDLLMM, 2/5 in the DDMLML and none in the DDMMLL group developed NAbs to Tier 1B pseudovirus 6644. Two of the rabbits from the DDLMLM group also developed low levels of autologous Tier 2 NAbs.

Conclusions: Low levels of autologous Tier 2 NAbs were induced in 2/5 rabbits inoculated with the DDLMLM vaccination regimen. Further studies are planned to determine if these vaccines elicit good cellular immune responses.

OA15.02

In vitro and *in vivo* analyses of HIV-1 clade C Envelope trimers highlight optimal antigenic profiles of novel HIV-1 Env-based vaccine candidates

A. Hauser[1]; D. Peterhoff[1]; T. Hartinger[1]; M. Schachtner[1]; N. Friedrich[2]; A. Trkola[2]; C. Moog[3]; W. Weissenhorn[4]; Q. Sattentau[5]; R. Sanders[6] and R. Wagner[1]

[1]University of Regensburg, Institute of Medical Microbiology and Hygiene, Regensburg, Germany, [2]University of Zurich, Zurich, Switzerland, [3]Université de Strasbourg, France, [4]Institut de Biologie Structurale, France, [5]University of Oxford, United Kingdom, [6]Academic Medical Center, Amsterdam, Netherlands

Abstract not available.

OA15.03

Novel trimer-only (TO) producing HIV-1 envelope glycoprotein constructs for inducing broadly neutralizing antibody responses by genetic vaccination

I. del Moral Sánchez[1]; E.G. Wee[2]; S.M. Koekkoek[1]; E.E. Schermer[1]; W.H. Lee[3]; S. Kumar[4]; R. Zwolsman[1]; J.D. Allen[5]; A. Torrents de la Peña[3]; M.M. van Haaren[1]; E. Reiss[1]; M. van Gils[1]; M. Crispin[5]; G. Ozorowski[3] and A.B. Ward[3]

[1]Amsterdam UMC, Medical Microbiology, Amsterdam, Netherlands, [2]The Jenner Institute, University of Oxford, Nuffield Department of Medicine, United Kingdom, [3]The Scripps Research Institute, Department of Integrative Structural and Computational Biology, United States, [4]All India Institute of Medical Sciences, New Delhi, India, [5]University of Southampton, School of Biological Sciences, Highfield Campus, Southampton, United Kingdom

Background: Soluble immunogens that properly mimic the HIV-1 envelope glycoprotein (Env), such as SOSIP and native flexibly linked (NFL) trimers, are used as constituents for a number of HIV-1 vaccine approaches. However, most NFL and SOSIP constructs are not only expressed as trimers, but also produce undesired monomers and dimers that expose non-neutralizing epitopes. Usually, chromatography procedures are used to isolate the desired trimers for vaccination. However, this also implies that conventional SOSIP and NFL constructs might be less suitable for genetic vaccination. Here, we describe a novel HIV-1 Env construct that was designed to express as trimers only (TO).

Methods: First, we combined the TO design with BG505 env to generate a BG505 TO-SOSIP construct as a proof-of-concept. Next, we used TO-SOSIP as a design template to generate a number of different HIV-1 Env immunogens: 1) TO-SOSIP in which immunodominant strain-specific glycan holes were hidden by glycan masking (GM) (TO-SOSIP.GM). 2) TO-SOSIP containing germline-targeting (GT) mutations that enable binding by naïve precursors of CD4bs broadly neutralizing antibodies (bNAbs) (TO-SOSIP.GT).

Results: Transient transfection followed by lectin affinity chromatography showed that the BG505 TO-SOSIP construct expressed only as trimers, of which >85% displayed a native-like conformation, while BG505 SOSIP and NFL-SOSIP preparations contained significant amounts of dimers and monomers. Furthermore, TO-SOSIP interacted efficiently with quaternary dependent bNAbs, such as PGT145 and PGT151, while showing weak binding to most non-NAbs. Both glycan masked and germline targeting variants also expressed only trimers. TO-SOSIP.GM showed negligible reactivity to glycan hole-targeting antibodies, while several TO-SOSIP.GT variants showed efficient binding to inferred germline versions of several CD4bs bNAbs. Remarkably, the TO design approach also allowed us to generate influenza hemagglutinin (HA) immunogens that express only as trimers without heterologous trimerization domains.