23RD EUROPEAN MEETING ON
# HIV & HEPATITIS 2025

TREATMENT STRATEGIES & ANTIVIRAL DRUG RESISTANCE

# ABSTRACT BOOK

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# ORAL ABSTRACT PRESENTATIONS

## 23rd European Meeting on HIV & Hepatitis 2025

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# Comparable Dynamics of Cell-Associated HIV-RNA, Total and Intact Proviral HIV-1 DNA in Virologically Suppressed People with HIV Switching to 2DR or Continuing 3DR Over a 18-Month Follow-up

Fiaschi L[1], Bartolini N, Compagno M, Benedetti L, El Khalili O, Biba C, Paletti C, Varasi I, Iannetta M, Malagnino V, Santoro MM, Salpini R, Svicher V, Ceccherini-Silberstein F, Sarmati L, Saladini F, Zazzi M, Vicenti I

*[1]Department of Medical Biotehnologies, University Of Siena, Siena, Italy, [2]Department of infectious Diseases, University of Rome Tor Vergata, Rome, Italy, [3]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy, [4]Department of Biology, University of Rome Tor Vergata, Rome, Italy*

**Background**: Two-drug antiretroviral therapy (2DR-ART) regimens are increasingly being used with comparable efficacy and reduced drug toxicity compared to 3DR-ART. However, little is known about the different impact of 2DR-ART or 3DR-ART regimens on HIV blood reservoir. The aim of this work was to assess, in an observational, prospective pilot study, whether switching virologically suppressed people with HIV (PWH) from 3DR-ART to 2DR-ART has an impact on their HIV reservoir.

**Materials and Methods:** PWH who continued a 3DR-ART (n=18) and PWH that switched to 2DR-ART (n=16) were investigated at enrolment (T0) and 18 months later (T1) for cell-associated HIV-RNA (CAR), HIV-DNA (CAD) and HIV intact proviruses (IP) in CD4+ T-cells. CAR was quantified using a highly sensitive digital PCR (dPCR) assay targeting LTR. CAD and IP were quantified by a triplex dPCR targeting LTR for CAD and psi and ENV regions for IP. All the assays were performed on a QIAcuity platform. Multivariate linear regression analysis was performed to identify predictors of CAR, CAD and IP changes over time (T1-T0).

**Results**: At T0, the median (IQR) age was 46.5 (14), 94% of participants were male, nadir and baseline CD4+ count was 388 (231) and 792 (430) cells/µL, respectively, with a zenith HIV-1 RNA of 4.9 (1.1) log copies/mL and therapy duration of 60.5 (76.5) months. The only difference between the treatment groups was a longer ART duration in the 3DR group (median [IQR]: 56 [86] vs. 63 [58] months for 2DR and 3DR, respectively) ($p<0.001$). In the whole dataset, CAD(T0) was 2.8 (1.0) log copies/million CD4+ cells, CAR(T0) was 4.2 (1.3) log copies/million CD4+ cells, and IP(T0) was 3.2% (17.8%), with 4/34 samples excluded from IP analyses because failed to amplify psi and/or ENV. At baseline, there were no significant differences between 2DR and 3DR groups for CAD, CAR and IP. Among the three HIV-1 molecular parameters, there was a significant correlation between CAD(T0) and CAR(T0) (Rho=0.645, $p<0.001$). No significant changes over time (T1 - T0) were observed for any of the three reservoir indicators, both in the overall population (median CAD +0.19 [+0.69] log, median CAR -0.05 [+1.8] log, median IP -1.25% [+18.4%]) and within or between the 2DR and 3DR groups.

In a regression model including treatment group, age, nadir and baseline CD4+ cell counts, zenith viral load, ART duration, and baseline CAD, CAR and IP values we found that higher baseline CD4+ counts was positively associated with changes of CAD ($p=0.043$), while zenith viral load and CAD(T0) were negatively associated with changes of IP ($p=0.017$ and $p<0.001$, respectively).

**Conclusions**: CAD and CAR were correlated with each other but not with IP at T0. CAD, IP and CAR values were stable over 18 months irrespective of treatment, reassuring that switching to 2DR does not affect the size of the viral reservoir in the medium term. Higher CAD values seem to predict larger decreases in the IP proportion. Prolonged follow-up is needed to establish the clinical utility of testing for molecular markers of the HIV reservoir before treatment simplification.

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# In Addition to Their Ability to Induce Resistance to Antiretrovirals, Integrase Inhibitors and Lenacapavir Resistance Mutations Enable HIV-1 to Evade Recognition by the Innate Immune System

Teyssou E[1], Malet I[1], Sayon S[1], Ratouit P[1], Guiraud V[1], Zafilaza K[1], Soulié C[1], Marcelin A[1], Calvez V[1]

*[1]Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière - Charles Foix, laboratoire de virologie, F-75013, Paris, France, 47-83 Boulevard De L'hôpital, France*

**Background**: Inflammasome activation, an early step in innate immunity, has been observed in response to HIV-1 infection. It plays a role in countering initial viral establishment and contributes to the activation and amplification of adaptive immunity. We have previously shown that, in addition to a direct impact on reverse transcription and an increase of adaptive immunity recognition, the M184V mutation leads to a better recognition by the innate immune cells compared to the WT virus. We pursued this study by investigating the impact of mutations that confer resistance to integrase and lenacapavir capsid inhibitors.

The aim of this study was to assess the impact of integrase inhibitors and lenacapavir capsid inhibitor resistance mutations on innate immunity through IL-1β and IL-18 secretion quantification, two of the major secreted cytokines of inflammasome.

**Materials and Methods**: HIV-1 integrase inhibitor (R263K, N155H and G140S/Q148H) and lenacapavir (Q67H, M66I, K70R and K70H) mutants were obtained by site-directed mutagenesis in the pNL4.3 vector. THP-1 monocyte-like cells were cultured with RPMI-hepes 10% FBS 0.05mM 2-mercaptoethanol. A total of 5x104 of THP-1 cells was plated per well in 96-well plate and differentiated in macrophage-like cells with 10nM of PMA for 24h. In parallel, undifferentiated THP-1 were infected with WT or mutant virus at a concentration of 0.7µg (p24) for 24h. Macrophage-like THP-1 were first primed with 1µg/ml LPS for 2h and 5x104 of undifferentiated THP-1, infected or not, were added. Cytokines were measured by chemiluminescence in the supernatant after 24 h of co-culture. Sample distribution normality was assessed using Shapiro–Wilk test. Analysis of variance (ANOVA) and t-test (parametric group comparison) were performed using GraphPad Prism software.

**Results**: We compared the IL-1β and IL-18 secretion for differentiated THP-1 cells in co-culture HIV-uninfected or HIV-infected THP-1 monocyte-like cells. Differentiated THP-1 cells in co-culture with HIV WT or mutant infected cells showed a significantly higher IL-1β and IL-18 secretion than those with uninfected cells. Moreover, we observed a significantly lower IL-1β and IL-18 secretion for the differentiated cells in co-culture with INIs and lenacapavir resistant viruses compared to those infected with WT virus. However, the only exception was for the lenacapavir K70R resistance mutation for whom we observed a similar expression of IL-1β between the WT and the K70R virus (median 294.5pg/ml IQR [212.2-310.8] and median 300pg/ml IQR [227.7-336.5], respectively, p=0.55).

**Conclusions**: This study shows that, in contrast to the M184V mutation which leads to higher inflammasome activation compared to the WT virus, the integrase inhibitor and lenacapavir capsid inhibitor resistance mutations were less recognized by the innate immune cells than the WT virus suggesting that these integrase and capsid mutations confer two properties that facilitate virus escape: increase of antiretroviral resistance combined with an escape from the innate immune system.

The only exception is the capsid K70R mutation, which is recognized by the immune system at the same level as the WT virus. Interestingly, lenacapavir trials showed that K70R capsid mutation was never observed alone but always in association with the Q67H in virological failure contrary to the K70H.

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# Unveiling Novel Microbiome Signatures for Natural Virological Control in HIV-1 Elite Controllers

Brodin D, Nowak P, Fagerström-Billai F, Matussek A, Sönnerborg A, Bai X

*[1]Karolinska Institutet, Stockholm, Sweden, [2]Oslo Unviversity Hospital and University of Oslo, OSLO, Norway*

**Background**: Elite controllers (EC) represent a rare subgroup (<1%) in people with HIV-1 (PWH) who have the ability to maintain undetectable plasma HIV-1 load overtime in the absence of antiretroviral therapy. EC phenotype has been used by researchers as an ideal model to resolve HIV disease progression and to identify novel therapeutical strategies. Yet the underlying mechanisms of this natural virological control are incompletely known. A few studies based on 16S rRNA gene sequencing have shown that EC possess a different gut microbiota profile from that of treatment-naive HIV-1 viremic progressors (VP), suggesting that the gut microbiome may play a role in HIV-1 control. This study aims to redefine the gut microbiome signatures for this natural virological control in EC using deep shotgun metagenome sequencing on the well-defined EC cohort from Sweden, and using updated pipelines which include both known and uncultured microbes.

**Materials and Methods**: Fecal samples from a cohort of untreated HIV-1-infected EC (n=14), VP (n=16), and HIV-1-negative individuals (HC, n=10) were collected from the InfCareHIV cohort Sweden. Clinical data was retrieved from medical record. Whole shotgun metagenome sequencing was performed. Taxonomic profiling was done using MetaPhlAn 4.0, an approach integrating metagenome-assembled genomes of both known and uncultured microbes. Functional profiling was done by HUMAnN3 to characterize microbial genes and metabolic pathways. Statistical and correlation analyses were performed to identify microbiome biomarkers for the natural virological control in EC.

**Results**: Significant differences in microbial alpha and beta diversity were observed in EC compared to VP. A number of species were significantly enriched in EC compared to VP and HC. Remarkably, the strongest EC-specific microbial species are previously uncharacterized and recently identified species whose functions are rarely studied. Three newly identified species, i.e., Butyricimonas virosa, Parabacteroides massiliensis, Duodenibacillus massiliensis, were significantly enriched in EC compared to both VP and HC with and without adjustment for covariates, i.e., age, gender, sexual behavior and ethnicity. Functional analysis using several databases identified gene families, proteins, and pathways significantly differential between EC and VP. Network correlation analyses are in process.

**Conclusions**: Our findings and novel microbiome biomarkers may not only pave the gateway towards HIV-1 cure strategies, potentially leading to breakthroughs in HIV-1 treatment paradigms, but also reshape our understanding of gut microbiome, particularly the significant role of the previously uncharacterized microbes in human physiology and health.

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# WG-am: A Novel Antiviral Dipeptide from Elite Controllers as a Promising Vaginal Microbicide for HIV Prevention

Ceña Diez R[1], Giammarino F[1], Romero Castillo L[2], Papaikonomou K[3,4], Sonnerborg A[1,5,6]

*[1]Division of Infectious Diseases/ANA Futura, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden, [2]Division of Immunology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, , Sweden, [3]Department of Gynecology and Reproductive Medicine, Karolinska University Hospital, , Sweden, [4]Department of Women's and Children's Health, Karolinska Institutet, , Sweden, [5]Department of Infectious Diseases, Karolinska University Hospital, , Sweden, [6]Division of Clinical Microbiology, Department of Laboratory Medicine, , Sweden*

**Background**: Sexually transmitted infections (STIs) remain a significant global health challenge, with over one million new cases occurring daily worldwide. Women bear a disproportionate disease burden, particularly in sub-Saharan Africa where women aged 15-24 are twice as likely to be living with HIV as young men. Tryptophylglycine amide (WG-am), a novel antiviral dipeptide identified in Elite Controllers, exhibits dual inhibitory activity against HIV-1, blocking both viral entry and retrotranscription. This study investigates WG-am's prophylactic potential as a vaginal microbicide using human cervico-vaginal tissue explants.

**Materials and Methods**: Human cervico-vaginal tissue explants were obtained from women undergoing hysterectomy for benign conditions and cultured using an air-liquid interface model. Tissue viability was assessed over 10 days and after treatment with WG-am (5-15mM) using MTT/LDH assays. For viral challenge, explants were treated with 10mM WG-am and infected with HIV-1 (7500 TCID50). Viral inhibition was measured by p24 antigen ELISA and secondary infection of TZM.bl cells. Immunological effects were evaluated through flow cytometry analysis of key immune cell populations in both tissue explants and PBMCs, including T cells (CD3+, CD4+, CD8+), monocytes, dendritic cells, neutrophils, and expression of HIV co-receptors (CCR5, CXCR4). Cytokine production was analyzed using a multiplex Th1/Th2 panel.

**Results**: WG-am demonstrated excellent biosafety in cervico-vaginal tissue, maintaining >90% tissue viability at concentrations up to 10mM for 7 days. In viral challenge assays, 10mM WG-am significantly inhibited HIV-1 infection by 75-80% in human cervico-vaginal tissue explants. Flow cytometry analysis showed no significant alterations in immune cell populations after WG-am treatment in both tissue explants and PBMCs. Notably, WG-am did not induce CD4+ T cell activation and maintained stable expression of HIV-1 co-receptors (CCR5, CXCR4). In PHA-stimulated PBMCs, WG-am treatment resulted in decreased CD4+ T cell activation compared to untreated controls. Multiplex cytokine analysis revealed that WG-am reduced pro-inflammatory cytokines (GM-CSF, IL-1 beta, IL-18) while partially restoring Th2 cytokine production in HIV-infected tissues, with adaptive cytokines (IL-2, IL-12p70, IFN-gamma) remaining stable across all conditions. In this context, Langerhans cells (LCs) play a critical role in the initial stages of HIV-1 infection, being both protective and detrimental. Immature LCs restrict HIV-1 through capture by langerin, followed by internalization and degradation via TRIM5α/autophagy. However, mature LCs can be infected by HIV-1 through CD4 and CCR5 receptors. Our initial findings using ex-vivo LCs isolated from CVT show that WG-am does not alter LCs maturation.

**Conclusions**: WG-am demonstrates significant potential as a vaginal microbicide for HIV prevention. Its dual inhibitory mechanism provides broad protection against HIV regardless of viral strain or drug resistance status. The compound exhibits excellent safety in human cervico-vaginal tissue, without disrupting immune cell populations or inducing inflammatory responses. These findings highlight WG-am as a promising candidate for development as a woman-controlled prevention strategy against HIV and potentially other STIs.

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# A French National Real-World Survey of People with Multi-Resistant HIV-1 Viruses Treated with an Antiretroviral Regimen Including Fostemsavir or Ibalizumab

Stefic K[1], Tumiotto C[2], de Monte A[3], Wirden M[4], Rodallec A[5], Fofana D[6], Chaix M[7], Bellecave P[2], Garnier E[5], Sourice J[5], Vellas C[8], Raymond S[8], Lambert-Niclot S[6], Alidjinou E[9], Coulon P[9], Avettand-Fenoel V[10], Handala L[1], Alessandri-Gradt E[11], Moisan A[11], Delaugerre C[7], Marcelin A[4], Calvez V[4], Peytavin G[12], Descamps D[13], CHARPENTIER C[13]

*[1]CHU de Tours, Service de Virologie, Tours, France, [2]CHU de Bordeaux, Service de Virologie, Bordeaux, France, [3]CHU de Nice, Service de Virologie, Nice, France, [4]Service de Virologie, Sorbonne Université, INSERM, UMR-S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière-Charles Foix, Paris, France, [5]CHU Nantes, Service de Virologie, Nantes, France , [6]INSERM, Sorbonne Université, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpital Universitaire Saint Antoine, Service de Virologie, Paris, France , [7]Service de Virologie, INSERM U941, AP-HP, Hôpital Saint-Louis, Université Paris Cité, Paris, France, [8]INSERM UMR 1291, CHU Toulouse Purpan, Service de Virologie, Toulouse, France, [9]CHU de Lille, Service de Virologie, Lille, France, [10]CHU d'Orléans, Service de Virologie, Orléans, Paris, [11]CHU de Rouen, Service de Virologie, Université de Rouen Normandie UNIRouen, Rouen, France, [12]Service de Pharmacologie, AP-HP, Hôpital Bichat-Claude Bernard, Université Paris Cité, INSERM, IAME, Paris, France, [13]Service de Virologie, AP-HP, Hôpital Bichat-Claude Bernard, Université Paris Cité, INSERM, IAME, Paris, France*

**Background**: Entry inhibitors such as the attachment inhibitor fostemsavir (FTR) and the post-attachment inhibitor ibalizumab (IBA) are used in specific populations of people with HIV-1 (PWH) harboring multidrug-resistant viruses, and little data are available on their use in real-world settings. Here, we describe the population characteristics and the virological outcome of PWH initiating an FTR- or IBA-based treatment.

**Materials and Methods**: A national retrospective observational study of PWH receiving an FTR- or IBA-based treatment for at least 3 months was conducted within the ANRS|MIE virology and PK network. A virological failure (VF) was defined as two consecutive viral loads (VL) ≥50 c/mL, and a non-virological response was defined as a VL decrease <1 log10 c/mL or failure to achieve virological suppression (<50 c/mL). Cumulative genotypic resistance tests (GRT) were interpreted using ANRS|MIE algorithm.

**Results**: Of the 59 PWH receiving an FTR-based treatment, 29 were virologically-suppressed at initiation, median VL of PWH in failure at initiation was 3.7 log10 c/mL (IQR=2.5-4.6). Overall, 88% were infected with B subtype; 97%, 93%, 73%, and 68% harbored viruses resistant to at least one ARV of the NRTI, NNRTI, PI, and INSTI drug classes, respectively. Genotypic susceptibility score (GSS) of associated ARVs was <1 in 28 PWH, and median follow-up on FTR-based treatment was 20 months (IQR=12-38). A VF occurred in 7 PWH (median VL=2.3 log10 c/mL) and 7 had a non-virological response, all but 2 were in failure at FTR initiation. GRT was available for 8 PWH, showing emergence of gp120 FTR resistance-associated mutations in one case (S375N-M426L) and emergence of new DRMs to the associated ARVs in one case (K65R-M184V). Temsavir plasma levels were available for 8 PWH, showing suboptimal concentrations in 2 (<200 ng/mL). Envelope sequencing before FTR was available for 19 PWH showing a polymorphism associated with FTR resistance in 7 cases (6 S375I/T, 1 M426L, 2 M434K). No association was found between the presence of these polymorphisms and the virological outcome. Eleven failing PWH initiated an IBA-based treatment (median VL=4.3 log10 c/mL), 5 were infected with B subtype; 8, 9, 6, and 7 harbored viruses resistant to at least one ARV of the NRTI, NNRTI, PI, and INSTI drug classes, respectively. GSS was <1 in 4 PWH, and median follow-up on IBA-based treatment was 7 months (IQR=4-43). A VF occurred in 5 PWH (median VL=2.4 log10 c/mL) and 2 had a non-virological response. GRT was available for 4 PWH, showing emergence of new DRM to the associated ARVs in one (N74D in capsid). Suboptimal concentrations of associated ARVs were observed in 3 of the 5 assessable participants. Differential adherence between injectable and oral medications was observed, as only one PWH had adequate concentrations of oral ARVs.

**Conclusions**: In this real-world study, PWH receiving FTR or IBA-based treatment harbored multidrug-resistant viruses and had very limited treatment options. Virological success was achieved in 60% and 36% of PWH with detectable VL at initiation of FTR- or IBA-based treatment, respectively, and was maintained in 93% of virologically-suppressed PWH at initiation of FTR-based treatment.

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# Lenacapavir Resistance Profile in Heavily Treatment-Experienced People in the PRESTIGIO Registry

Santoro MM[1], Spagnuolo V[2], Marchegiani G[1], Paletti C[3], Lolatto R[2], Spalletta D[1], Bellocchi M[1], Carioti L[1], Cervo A[4], Di Biagio A[5], Gagliardini R[6], Fiscon M[7], Focà E[8], Rusconi S[9], Zazzi M[3], Castagna A[2], Ceccherini-Silberstein F[1], Saladini F[3]

*[1]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy, [2]Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy, [3]Department of Medical Biotechnologies, University of Siena, Siena, Italy, [4]Infectious Disease Clinic University of Modena and Reggio Emilia, AOU Policlinico di Modena , Modena, Italy , [5]Infectious Diseases, San Martino Hospital Genoa, University of Genoa, Genoa, Italy, [6]National Institute for Infectious Diseases "L. Spallanzani" IRCCS, Rome, Italy, [7]Infectious Diseases Unit, Azienda Ulss 9 Scaligera, Verona, Italy, [8]Department of Clinical and Experimental Sciences, University of Brescia-ASST Spedali Civili , Brescia, Italy, [9]S.C. Malattie Infettive, ASST Ovest Milanese, Legnano General Hospital and DIBIC, Università degli Studi di Milano, Milan, Italy*

**Background**: Lenacapavir is the first-in-class HIV-1 capsid inhibitor recently approved for the treatment of highly treatment-experienced (HTE) people with HIV (PWH) with multidrug resistance (MDR) in combination with an optimized background regimen (OBR). Several lenacapavir resistance associated mutations have been already reported. The aim of this study was to evaluate the genotypic and phenotypic resistance profile in HTE PWH with MDR treated with lenacapavir enrolled in the Italian PRESTIGIO Registry.

**Materials and Methods**: Plasma and PBMC samples from nine HTE PWH with MDR at baseline (when available) and under lenacapavir treatment were obtained from the PRESTIGIO biobank. Next generation sequencing (NGS) of gag and pol (PR/RT/IN) genes was performed by using home-made protocols on the MiSeq Illumina platform. Resistance to lenacapavir, PIs, RTIs, INSTIs was evaluated by the HIVDB Program v.9.8. In vitro susceptibility to lenacapavir was measured through a single-cycle assay in TZM-bl cells using NL4-3 based recombinant viruses harboring the clinically derived Gag-PR region.

**Results**: At baseline, no major lenacapavir mutations were found in any participants either in plasma or PBMCs, with only one case showing in PBMCs the accessory mutation T107A as minority variant (frequency: 5.9%). Mean phenotypic fold-change value was 1.1 (range: 0.5-2.5), suggesting full susceptibility to lenacapavir. Median genotypic susceptibility score of the OBR was 1.5 (1.0-2.5). Participants were treated with lenacapavir for a median of 20 [IQR 19-24] months. Two participants started lenacapavir with plasma HIV-RNA <50 copies/mL and maintained virological control. Among the seven participants who started lenacapavir during virological failure (median plasma HIV-RNA 2,010 [IQR 763-24,400] copies/mL), six achieved virological suppression, with one showing a viral blip (52 copies/mL) concomitant with the Q67K mutation as plasma minority variant (frequency: 5.7%). In the only one case of persistent viremia, NGS performed at 28 months under lenacapavir based treatment (viral load 39,400 copies/mL) revealed in both plasma and PBMCs the LEN primary mutations K70H (frequency 97.8% and 75.6%, respectively) and Q67K (frequency 98.5% and 75.7%, respectively). This pattern of mutations was associated with a fold-change of 2,081, indicating high-level resistance. All other participants did not show any lenacapavir resistance mutations in PBMCs either as majority or minority variants during lenacapavir treatment.

**Conclusions**: In this small population of HTE PWH with MDR, lenacapavir was associated with maintenance or achievement of virological suppression, with one single participant remaining viremic and showing majority lenacapavir resistant variants. The detection of the Q67K minority variant during a viral blip highlights the low genetic barrier to resistance of lenacapavir, however later resuppression suggests low fitness for this mutant.

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# Resistance Analysis of Long-Acting Lenacapavir in Heavily Treatment-Experienced People With HIV After 3 Years of Lenacapavir Treatment

Margot N[1], Pennetzdorfer N[1], Naik V[1], Jogiraju V[1], VanderVeen L[1], Ogbuagu O[2], Segal-Maurer S[1], Molina J[3], Dvory-Sobol H[1], Callebaut C[1]

*[1]Gilead Sciences Inc., Foster City, United States, [2]Yale University School of Medicine, New Haven, USA, [3]Hôpital Saint Louis, Paris, France*

**Background**: Lenacapavir (LEN) is the first-in-class HIV-1 capsid (CA) inhibitor approved for treating multidrug-resistant HIV-1 in combination with other antiretrovirals, for heavily treatment-experienced (HTE) people with HIV (PWH). CAPELLA is a pivotal Phase 2/3 study evaluating LEN with other antiretrovirals in viremic PWH with multidrug resistance. LEN, combined with an optimized background regimen (OBR), led to 61.4% virologic suppression at Week 156 (missing=excluded, 84.6%). We describe viral response and adherence to OBR in participants with CA resistance through Week 156.

**Materials and Methods**: Genotypic and phenotypic analyses of HIV-1 CA, reverse transcriptase, protease, and integrase were performed at virologic failure (confirmed virologic rebound ≥50 copies/mL, HIV-1 RNA ≥50 copies/mL and <1 log10 decline from baseline at Week 4, or HIV-1 RNA ≥50 copies/mL at last visit) using assays from Monogram Biosciences, as well as phenotypic assays conducted at Gilead Sciences. To measure OBR adherence, plasma concentrations of common OBR drugs (darunavir, tenofovir alafenamide, tenofovir, emtricitabine, dolutegravir) were quantified using validated liquid chromatography-mass spectrometry methods.

**Results**: Through Week 156, 28/72 participants met the resistance analysis criteria (including 27 participants who were analyzed through Week 104). Fourteen analyzed participants did not have emergence of resistance to LEN, including eight participants who resuppressed HIV-1 RNA 50 copies/mL after the resistance analysis through Week 156, and six participants who remained viremic (median HIV-1 RNA decrease from baseline of 1.74 log10). These 14 participants experienced a median increase of 70 CD4 cells/µL. The other 14 participants had emergence of LEN resistance-associated mutations (RAMs) through Week 156: 12 without change from Week 104 and two with additional LEN RAMs and further decreased LEN susceptibility compared to Week 104. Main LEN RAM patterns were M66I+others (n=6), Q67H+K70R+A105T or T107N (n=4), K70N+N74K+T107T/N (n=1), N74D alone (n=1), Q67H alone (n=1), and Q67H+K70R (n=1). The presence of LEN RAMs was associated with changes in susceptibility to LEN, ranging from 4.47- to >1428-fold compared to wild-type control. Emergence of LEN resistance was associated with inadequate OBR adherence (n=10; based on plasma drug levels) or with an OBR lacking fully active agents (n=4). Five of 14 participants with LEN resistance resuppressed upon resumed OBR adherence or change in the OBR to include active agents, and the nine viremic participants had a median HIV-1 RNA decrease from baseline of 0.40 log10. Participants with LEN resistance had a median CD4 cell recovery of 82 cells/µL.

**Conclusions**: No new participants had LEN RAM emergence during the third year of study. Emergence of LEN RAMs occurred in the setting of inadequate OBR adherence or an OBR lacking fully active antiretrovirals, akin to receiving LEN functional monotherapy. Some participants with LEN RAMs achieved resuppression upon renewed OBR adherence, reinforcing the general principle that treatment adherence is key to achieving and maintaining virologic suppression. These data confirm the role of LEN as an important option to treat PWH with multidrug-resistant HIV-1.

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# APOBEC-Associated Mutations in HIV Drug Resistance: Challenges in Interpretation Using Next-Generation Sequencing

Muñoz P[1], De Salazar A[1], Chaves L[1], Illescas M[1], Delgado R[2], Iborra A[3], Galan J[4], Sigcha M[2], Martinez Garcia L[4], Pereira S[5], Lopez-Causape C[6], Aguilera A[5], García García F[1]

*[1]University Hospital Of San Cecilio, Granada, Granada, Spain, [2]University Hospital 12 de Octubre, Madrid, Madrid, Spain, [3]University Hospital Virgen de la Arrixaca, Murcia, Murcia, Spain, [4]Hospital Ramón y Cajal, Madrid, Madrid, Spain, [5]University Hospital Complex of Santiago, Santiago de Compostela, Santiago de Compostela, Spain, [6]Hospital of Son Espases, Mallorca, Islas Baleares, Spain*

**Background**: This study focuses on identifying APOBEC-context drug resistance mutations (DRMs) in plasma using next-generation sequencing (NGS), emphasizing their correct interpretation to prevent misclassification as antiretroviral resistance.

**Materials and Methods**: Samples were collected from six centers, sequencing the integrase, protease, and reverse transcriptase regions, primarily from newly diagnosed, treatment-naïve PLWH. FASTQ files were centrally analyzed to identify APOBEC-signature mutations and context DRMs, assess their prevalence, evaluating stop codons and Q Scores.

**Results**: A total of 290 PLWH were analyzed, with 268 having sequences from all three genomic regions. Using a 1% cutoff, 159 exhibited APOBEC-signature mutations, while 64 (22%) harbored APOBEC-context DRMs. Both mutation types (signature and context) were found in 52 PLWH (18%) at the 1% cutoff, 17 (6%) at the 3% cutoff, and 8 (3%) at the 5% cutoff. Across all gene regions, APOBEC-context DRMs were more prevalent at the 1% cutoff and decreased as the stringency increased to 3% and 5%. The most frequently detected mutations included M184I (RT) and M230I (RT), both appearing in 32 cases at the 1% cutoff, though M230I disappeared at 5%. Notably, M184I was still detected at 5%. Among integrase mutations, R263K persisted across all cutoffs, highlighting its relevance for resistance to CAB (R), BIC (I), and DGT (I). In contrast, protease mutations were rarer, and with no major resistance impact. At the 5% cutoff, stop codons in APOBEC-signature positions were observed in 6 out of 13 cases. Q score between 20-29 was detected for 9 mutations in RT, 8 in integrase and 3 in protease.

**Conclusions**: Our study highlights the critical need to accurately distinguish APOBEC-induced mutations in HIV drug resistance analysis. The prevalence of these mutations and their potential to confound resistance profiling emphasize the importance of comprehensive mutation analysis, considering cutoff thresholds, quality scores, and co-occurring mutations. Refining NGS-based resistance reports through these factors can enhance the accuracy of HIV drug resistance interpretation and optimize treatment decisions.

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# Clonal Analysis of Near Full-Length Proviral HIV-1 Genome Enables Differentiation of APOBEC- and Drug-Resistant Populations in Highly Treatment Experienced People Living with HIV

Daeumer M[1,2], Engel N[1], Verheyen J[1], Vogelmann R[3], Thielen A[1,2]

*[1]Institute of Immunology and Genetics, Kaiserslautern, Germany, [2]SeqIT GmbH & Co. KG, Kaiserslautern, Germany, [3]University hospital Mannheim, Mannheim, Germany*

**Background**: HIV-1 genotypic resistance testing from proviral DNA can be beneficial in switch settings where therapy history is incomplete and/or viral RNA is undetectable. However, interpretation of a proviral resistance profile can be challenging, since APOBEC-induced mutations may also appear at drug-resistance-associated positions and the majority of proviral variants is defective (>90-95%). Here, we describe a near-full-length amplification and long-read sequencing approach to discriminate populations with APOBEC mutations from replication-competent populations by covariation analysis in highly treatment experienced PLWH.

**Material and Methods**: 17 heavily treatment experienced and virologically suppressed (median: 10 years) PLWH were included in this study. A single round, near full-length PCR was done from HIV-1 proviral DNA followed by long- and short-read sequencing approaches using Oxford nanopore technology and Illumina sequencing-by-synthesis, respectively. Covariation analysis was used to extract linkage information of mutations. Potential APOBEC mutations in Protease, Reverse Transcriptase and Integrase were determined using Stanford hivdb. Replication incompetence of individual variants was defined as presence of premature stop codons found anywhere in the genome.

**Results**: Comparing the two sequencing technologies revealed that the covariance analysis for differentiation of APOBEC- and drug-resistant populations is less effective on sequences from the short-read technology since sequences from the short-read technology cannot always span regions without potential APOBEC motifs in the HIV genome. No APOBEC-induced premature stop codons were observed in 7/17 samples, indicating a fully replication-competent population. 1 sample showed a population with stop codons in >99% of the genomes, indicating almost complete replication incompetence. Mixed populations with and without premature stop codons were detected in 9/17 samples. These showed a total of 89 mutations at resistance associated positions with frequencies between 1% and 99%. In 8/9 samples drug resistant populations were replication-competent with frequencies of replicative viruses between 0,2% and 89%. By using mutation linkage analysis within individual viruses, mutations of ambiguous origin such as M184I or M230I in the Reverse Transcriptase could clearly be classified as resistance- or APOBEC associated.

**Conclusions**: By using near-full-length amplification of the HIV-1 genome in combination with long-read sequencing, we were able to discriminate APOBEC- from potential replication competent populations in the proviral DNA. All 17 PLWH were virologically suppressed despite critical resistance profiles in some cases, indicating that APOBEC related stop codons may have an impact on viral replication competence of drug resistant variants. The characterization of a proviral population in terms of replication competence might be helpful in ART switch settings with limited options.

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# Progress and Challenges in Hepatitis B and C Elimination Efforts Across Europe: A Comprehensive Analysis

Ogbeke G[1], Bashorun A[1], Nwokenneya P[1]

*[1]National AIDS, Viral Hepatitis and STIs Control Programme, Abuja , Nigeria, [2]National AIDS, Viral Hepatitis and STIs Control Programme, Abuja, Nigeria*

**Background**: In 2016, the World Health Assembly set an ambitious goal to eliminate viral hepatitis as a public health threat by 2030. This study examines the current status of hepatitis B virus (HBV) and hepatitis C virus (HCV) across European Union (EU) and European Economic Area (EEA) countries, focusing on prevalence, care continuum metrics, and recent epidemiological trends.

**Materials and Methods**: Data were collected from multiple sources, including the European Centre for Disease Prevention and Control (ECDC) surveillance reports, the Global Burden of Disease Study 2019, and peer-reviewed research up to 2024. The analysis focused on key indicators such as HBV and HCV prevalence, diagnosis rates, treatment uptake, and recent outbreak trends.

**Results**: As of 2024, an estimated 5.4 million people in the EU/EEA live with chronic HBV or HCV infections. Recent estimates indicate that hepatitis B surface antigen (HBsAg) prevalence in the general population varies across 17 EU/EEA countries, ranging from under 0.5% in eight countries to over 2% in Bulgaria and Romania. In 2024, Spain reported a significant increase in hepatitis A cases, with nearly 900 diagnoses compared to 336 in 2023. This surge primarily affected young men, especially within the GBMSM (gay, bisexual, and other men who have sex with men) community. Additionally, in October 2024, a hepatitis A outbreak in Santa Eulària affected at least 19 individuals, including both adults and children, highlighting the ongoing transmission risks.

**Conclusions**: While progress has been made in monitoring HBV and HCV across Europe, significant data gaps persist, hindering a comprehensive assessment of elimination efforts. The recent hepatitis A outbreaks in Spain underscore the need for vigilant surveillance and targeted vaccination campaigns, especially among high-risk groups. Enhanced data collection, public awareness, and preventive measures are crucial to achieving the 2030 elimination targets.

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# Despite a Substantial Burden of HDV Infection, Gaps in HDV Screening Still Persists Across Europe: An Urgent Need to Strengthen Diagnostic Strategies at Pan-European Level

Salpini R[1], Grossi I[1], Alexiev I[2], Baiocchi L[3], Banica L[4], Begovac J[5], Benkoova B[6], Bopegamage S[6], Castelli C[1], Caudai C[7], D'Anna S[1], Demir T[8], Devaux C[9], Di Lorenzo A[10], Heger E[11], Kaiser R[11], Kokkinos N[12], Fernandez-Rivas G[13], Gazdikova K[6], Geretti A[12,10], Golkocheva-Markova E[2], Iserentant G[9], Lazarevic I[15], Lazureanu V[16], Leuzinger K[17], Lindh M[18], Llibre J[14], Lunar M[19], Mihale J[6], Mor O[20], Nierhoff D[21], Otelea D[4], Piermatteo L[1], Poljak M[19], Riccio A[1], Ruzic M[22], Sarmati L[10], Torre G[1], Tudor A[4], Vata A[23], Yildiran D[8], Zazzi M[24], Wensing A[25], Svicher V[1]

*[1]University Of Rome Tor Vergata Department of Biology, Roma, Italy, [2]National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria, [3]Hepatology Unit, University Hospital Tor Vergata, Roma, Italy, [4]National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Bucharest, Romania, [5]University Hospital for Infectious Diseases, Zagreb, Croatia, [6]Institute of Laboratory Medicine, Faculty of Medicine, Slovak Medical University, Bratislava, Slovak Republic, [7]Microbiology and Virology Unit, AOU Senese, Siena, Italy, [8]Ministry of Health, Public Health Institute, National Reference Laboratory for HIV/AIDS and Viral Hepatitis Department, Ankara, Turkey, [9]Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg, Luxembourg, [10]Dept of System Medicine, University of Rome Tor Vergata, Rome, Italy, [11]Institute of Virology, University of Cologne, Cologne, Germany, [12]Royal Free NHS Foundation Trust - North Mid Hospital, London , United Kingdom, [13]Microbiology Department, Clinical Laboratory North Metropolitan Area, Germans Trias i Pujol University Hospital. Universitat Autònoma de Barcelona-Badalona, Barcelona, Spain, [14]Hospital Universitari Germans Trias i Pujol, Barcelona, Spain, [15]Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [16]The University of Medicine and Pharmacy, Timisoara, Romania, [17]Clinical Virology, University Hospital Basel, Basel, Switzerland, [18]Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [19]Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia, [20]National Reference Laboratory for HIV and Viral Hepatitis, Sheba Medical Center of Tel A Viv, Tel A Viv, Israel, [21]Department of Gastroenterology and Hepatology, University of Cologne, Cologne, Germany, [22]Faculty of Medicine, University of Novi Sad, Clinical Centre of Vojvodina, Clinic for Infectious Diseases, Novi Sad, Serbia, [23]The University of Medicine and Pharmacy , Iasi, Romania, [24]Department of Medical Biotechnologies, University of Siena, Siena, Italy, [25]Translational Virology, Department of Global Health and Bioethics Julius Center for Health, Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands*

**Background**: Hepatitis D virus (HDV) infection is often underdiagnosed, despite the severity of the related liver disease. Here, we investigate the rates of HDV screening and the prevalence of HDV RNA among antiHDV+ individuals in routine care across Europe and parts of the Middle East, as well as the characteristics of individuals with active HDV infection.

**Materials and Method**: We collected data from 15,200 HBsAg+ people attending 16 tertiary centres during 2021-2023: Northwestern Europe (NWE: UK, Switzerland, Luxembourg, Germany, Sweden; N=3596, 23.6%), Southern Europe (SE: Spain, Italy, Slovenia, Croatia; N=3203, 21.1%), and Eastern Europe (EE: Romania, Serbia, Slovakia; N=4136, 27.2%) and the Middle-East (ME: Israel, Turkey; N=4265, 28.1%).

**Results**: Participants were predominantly males (59.5%), HBeAg-negative (90.7%) with a median (IQR) age of 49 (39-60) years and only 28.6% received nucleos(t)ide analog treatment. The rate of HDV screening varied widely across regions: 23.9%, EE; 59.3%, NWE; 71.5%, ME; 79.1%, SE. Overall, HDV seroprevalence was 5.9% (523/8801) with the highest rate of anti-HDV positivity in EE (9.9%), followed by ME (7.0%), NWE (5.8%) and SE (3.3%). By country, anti-HDV positivity peaked in Romania (10.4%), Turkey (8.7%), Germany (7.9%) and Italy (6.8%).

Compared to individuals with HBV infection, anti-HDV+ people had significantly higher HBsAg (median [IQR]: 3225 [591-9784] vs 2568 [242-11163] IU/ml, p<0.001), lower HBV-DNA (3.1 [2.2-3.9] vs 2.4 [1.6-3.7] log IU/ml, p<0.001) and a more advanced liver disease (Ishak score >4: 25.7% vs 4.6%, HCC: 5.9% vs 1%, p<0.001 for both). HIV coinfection was also more common (10.8% vs 4.1%, p< 0.001).

Overall, among 523 individuals with anti-HDV, 82.6% were tested for HDV-RNA and 46.9% showed active HDV infection with a median (IQR) HDV-RNA of 5.7 (4.2-7.1) log IU/ml and ALT of 92 (58-171) U/L. Strikingly, large regional variations were noted in both HDV-RNA detection (ME: 39.6%, NWE: 40%, SE: 56.1% and EE: 71.6%, p= 0.0002) and HDV-RNA levels (median [IQR] log IU/ml: 6.4 [4.9-7.2] in ME, 5.4 [3.9-6.7] in EE, 5.4 [4.0-7.4] in NWE and 4.0 [3.2-4.7] in SE, p=0.001).

Remarkably, among 344 participants tested for HDV-RNA despite anti-HDV negativity, a total of 7 (2%) from EE (N=5), SE (N=1) and Africa (N=1) had detectable HDV-RNA with median levels of 5.4 (5-6) log10 IU/ml.

**Conclusions**: Despite the substantial burden of HDV infection and related liver disease, there remain significant gaps in HDV screening across Europe, particularly in specific regions. Notably, we observed a small but appreciable proportion with HDV RNA despite lack of anti-HDV, underscoring the need for greater standardisation of HDV diagnostics. Overall, the data supports the urgent need to improve and harmonize HDV screening measures and promote the access to antiviral treatment at pan-European level.

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# Boosting HDV Testing in Spain through the Delta Double Reflex Strategy

Alberola Romano A[1], Illescas M[1,86], de Salazar A[1,46,86], Vázquez Blanquiño A, Carracedo R[2], Fuentes A[1,46,86], Sanchez-Yebra W[3], Freyre C[4], Becerril Carral B[5], Viciana I[6], López Rodríguez I[7], Trujillo Soto T[8], Domínguez Castaño A[9], Luzón-García M[10,46], Ramirez Arellano E[11], Liébana C[12], Franco Álvarez de Luna F[13], Lozano Domínguez M[14], Lerate Alba M[15], Sampedro A[16], Pérez A[17], Algarate Cajo S[18], Rojo Alba S[19], Fraile Ribot P[20], Iglesias Sánchez L[21], Martin Y[22], Vallejo Alonso A[23], Molina M[24], Aroca M[25], Castro Hernández B[26], Lara M[27], Pena M[28], Rodríguez-Fernández A[29,30], González Praetorius A[31], Blas Señalada J[32], Beteta López A[33], Gómez C[34], Rodríguez Escudero M[35], González M[36], Abreu Di Berardino M[37], Manrique González E[38], Beltrán Cifuentes I[39], Sandoval Torrientes M[40], Saez R[41], Martín T[42], Tajada Alegre P[43], Lorenzo B[44], García Valero M[45], Jiménez Sansegundo I[47], García-De Cruz S[48], Garcinuño Pérez S[49], Saiz M[50], Blázquez A[51], Fernández Natal I[52], Lorenzo Juanes H[53], Vidal-Acuña M[54], Moldes L[55], Calvo Sánchez N[56], Trigo M[57], Gude M[58], Ordóñez P[59], Cortizo S[60], Blasco M[61], Sigcha M[62], Falces I[63], Galán J[64], Arribi Vilela A[65], Alonso Fernández R[66], Iborra M[67], Del Amor Espin M[68], Cascales E[69], Pérez C[70], Fernández P[71], Navarro M[72], Núñez M[73], Cámara M[74], Gil-Gallardo L[75], Miqueleiz Zapatero A[76], Reina G[77], Elorduy Otazua L[78], López de Goikoetxea M[79], Gómez C[80], Liendo P[81], Aranzamendi Zaldumbide M[82], Ocete D[83], Alcaraz Soriano M[84], Rodríguez Díaz J[85], Aguilera A[2], García F[1,46,86]

*[1]Hospital Universitario Clínico San Cecilio, Granada, Spain, [2]Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [3]Hospital Universitario Torrecárdenas, Almería, Spain, [4]Hospital Universitario de Puerto Real, Puerto Real, Spain, [5]Hospital Universitario de Jerez, Jerez, Spain, [6]Hospital Universitario Virgen de la Victoria, Málaga, Spain, [7]Hospital Universitario Costa del Sol, Marbella, Spain, [8]Hospital Universitario Puerta del Mar, Cádiz, Spain, [9]Hospital Universitario Virgen del Valme, Sevilla, Spain, [10]Hospital Universitario Poniente, El Ejido, Spain, [11]Hospital Universitario Virgen Macarena, Sevilla, Spain, [12]Hospital Universitario de Jaén, Jaén, Spain, [13]Hospital Juan Ramón Jiménez, Huelva, Spain, [14]Hospital Universitario Virgen del Rocío, Sevilla, Spain, [15]Hospital Universitario Carlos Haya, Málaga, Spain, [16]Hospital Universitario Virgen de las Nieves, Granada, Spain, [17]Hospital Universitario Reina Sofía, Córdoba, Spain, [18]Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain, [19]Hospital Universitario Central de Asturias, Oviedo, Spain, [20]Hospital Universitario Son Espases, Palma de Mallorca, Spain, [21]Hospital Nuestra Señora de Guadalupe, La Gomera, Spain, [22]Hospital Universitario Doctor José Molina Orosa, Arrecife, Spain, [23]Hospital General de Fuerteventura, Puerto del Rosario, Spain, [24]Hospital Universitario de La Palma, La Palma, Spain, [25]Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain, [26]Hospital Universitario de Canarias, La Laguna, Spain, [27]Hospital Universitario Ntra. Sra. De Candelaria, Santa Cruz de Tenerife, Spain, [28]Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, [29]Hospital Universitario Marqués de Valdecilla, Santander, Spain, [30]Instituto de Investigación Valdecilla (IDIVAL), Santander, Spain, [31]Hospital Universitario de Guadalajara, Guadalajara, Spain, [32]Hospital Universitario de Albacete, Albacete, Spain, [33]Hospital Gral. Universitario Ntra. Sra. del Prado, Talavera de la Reina, Spain, [34]Complejo Hospitalario Universitario de Toledo, Toledo, Spain, [35]Hospital Virgen de la Luz, Cuenca, Spain, [36]Hospital General Universitario de Ciudad Real, Ciudad Real, Spain, [37]Hospital Público Santa Bárbara, Puertollano, Spain, [38]Hospital de Valdepeñas, , Spain, [39]Hospital Virgen de Altagracia, Manzanares, Spain, [40]Hospital Santiago Apóstol, Miranda de Ebro, Spain, [41]Complejo Asistencial Universitario de Burgos, Burgos, Spain, [42]Hospital Virgen de la Concha, Zamora, Spain, [43]Hospital General de Segovia, Segovia, Spain, [44]Hospital Universitario Río Hortega, Valladolid, Spain, [45]Complejo asistencial de Palencia, Palencia, Spain, [46]Ciber de Enfermedades Infecciosas (CIBERINFEC), Madrid, , [47]Hospital Nuestra Señora De Sonsoles Ávila, Ávila, Spain, [48]Hospital Santa Bárbara, Soria, Spain, [49]Hospital Clínico Universitario de Valladolid, Valladolid, Spain, [50]Hospital Santos Reyes, Aranda de Duero, Spain, [51]Complejo Asistencial de Salamanca, Salamanca, Spain, [52]Hospital de León, León, Spain, [53]Hospital El Bierzo, Ponferrada, Spain, [54]Complejo Hospitalario Universitario de Cáceres, Cáceres, Spain, [55]Complexo Hospitalario Universitario de A Coruña, A Coruña, Spain, [56]Complexo Hospitalario Universitario de Ourense, Ourense, Spain, [57]Complexo Hospitalario Universitario de Pontevedra, Pontevedra, Spain, [58]Hospital Universitario Lucus Augusti, Lugo, Spain, [59]Hospital Universitario Arquitecto Marcide-Profesor Novoa Santos, Ferrol, Spain, [60]Hospital Universitario Álvaro Cunqueiro, Vigo, Spain, [61]Hospital San Pedro, Logroño, Spain, [62]Hospital Universitario 12 de Octubre, Madrid, Spain, [63]Hospital Universitario La Paz, Madrid, Spain, [64]Hospital Universitario Ramón y Cajal, Madrid, Spain, [65]Hospital Clínico San Carlos, Madrid, Spain, [66]Hospital General Universitario Gregorio Marañón, Madrid, Spain, [67]Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, [68]Hospital General Universitario Santa Lucía, Cartagena, Spain, [69]Hospital General Universitario Rafael Méndez, Lorca, Spain, [70]Hospital Comarcal del Noroeste, Caravaca de la Cruz, Spain, [71]Hospital Virgen del Castillo, Yecla, Spain, [72]Hospital General Universitario Morales Meseguer, Murcia, Spain, [73]Hospital General Universitario Reina Sofía, Murcia, Spain, [74]Hospital General Universitario Los Arcos del Mar Menor, Pozo Aledo, Spain, [75]Hospital de la Vega Lorenzo Guirao, Cieza, Spain, [76]Hospital Universitario de Navarra, Pamplona, Spain, [77]Clínica Universidad de Navarra, Pamplona, Spain, [78]Hospital Universitario de Cruces, Baracaldo, Spain, [79]Hospital Universitario Galdakao-Usansolo, Galdakao, Spain, [80]Hospital Universitario Araba, Álava, Spain, [81]Hospital Universitario de Basurto, Bilbao, Spain, [82]Hospital Universitario Donostia, San Sebastián, Spain, [83]Consorcio Hospital General Universitario de Valencia, Valencia, Spain, [84]Hospital Clínico Universitario de Valencia, Valencia, Spain, [85]Hospital General Universitario Dr. Balmis, Alicante, Spain, [86]Instituto de Investigación Biosanitario de Granada. ibs.Granada, Granada, Spain*

**Background**: Despite its severity, hepatitis delta virus (HDV) remains underdiagnosed, highlighting

the need for standardized diagnostic protocols and improved detection strategies. The Delta Double Reflex (DDR) Spain study aims to implement a reflex testing strategy for HDV, estimate its seroprevalence and prevalence, and characterize HDV infection across Spain.

**Materials and Methods**: This is a national, multicenter, ambispective, and observational study. Data on the implementation of the double reflex testing strategy were collected for 2022, 2023, and 2024 (the year in which the prospective phase began at the start of the third quarter). The study assessed adherence to the DDR algorithm and described the diagnostic cascade, from the identification of HBsAg-positive individuals to HDV-RNA confirmation. In the prospective phase, virological, clinical, and demographic characteristics of diagnosed patients are also recorded. All patient data were stored in a password-protected database.

**Results**: The cohort included 65,763 HBsAg-positive patients from 80 centers across 16 of 17 Autonomous Communities (ACs) of Spain. Reflex testing rates increased significantly from 55.1% in 2022 (participation range: 4.2%-98.7%) to 64.8% in 2023 (25%-100%; p<0.0001), and further improved in 2024, with 85.9% of patients undergoing reflex anti-HDV testing (participation range: 38.6%-100% across ACs), representing a significant increase compared to previous years (p<0.05).
The anti-HDV seroprevalence declined from 5.6% (95%CI 5.2-6.1) in 2022 to 4.9% (95%CI 4.6-5.3) in 2023, and remained 4.8% (95%CI 4.4-5.2) in 2024, with significant regional variability. Testing for HDV-RNA among anti-HDV-positive individuals improved from 72.6% in 2022 to 90.8% in 2023, reaching 91% in 2024. The prevalence of chronic active HDV infection decreased from 40.2% (95%CI 35.5-45.1) in 2022 to 31.6% (95%CI 28.0-35.3) in 2023, and was 31.1% (95%CI 27.1-35.2) in 2024. Starting in the third quarter of 2024, the prospective phase had enrolled 144 anti-HDV-positive patients with a median age of 54 years (IQR 41–60), 59% being male and 56% of Spanish origin. Co-infection rates included 16% for HIV and 20% for HCV (1 with active hepatitis C). Elevated fibrosis markers were observed in 26% of patients with FIB-4 >2.67, and 17% presented APRI >1.5. Active HDV-RNA was detected in 37% (n=32) of cases, with a median viral load of 4.37 log IU/mL (IQR 3.14–5.1).

**Conclusions**: This is the largest study of its kind in Spain, revealing significant disparities in HDV testing across ACs. The variability in adherence to reflex testing highlights the need for standardized national guidelines and expanded professional education.

Preliminary findings show a high burden of disease, with frequent HIV/HCV co-infections and advanced fibrosis in many patients. Strengthening early detection and harmonizing diagnostic protocols will improve equity, patient outcomes, and disease management across Spain.

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# Viral Strain Networks Tracing Human or Environment-Linked Hepatitis E Virus Dispersion in Europe - HEV Molecular Signatures

Kasmi S[1], Hartard C[1], Sautrey G[2], Akand E[3], Murray J[3], Schvoerer E[1]

*[1]Virologie CHU et Université de Lorraine (UL), Vandoeuvre-les-Nancy, France, [2]Lorraine Université Laboratoire LCPME UMR-7564 CNRS, Villers-les-Nancy, France, [3]School of Mathematics and Statistics, UNSW , Sydney, NSW 2052 , Australia*

**Background**: In Europe, the epidemiology of HEV infections could be either water-linked or due to interhuman/interspecies contaminations. This study aimed (i) to explore HEV isolates from European databases, including French examplary strains, (ii) to shed light on the pathways of HEV transmission by combining classical phylogeny with mathematics as yet developed for HCV/SARS-CoV-2.

**Materials and Methods**: Phylogeny and mathematical tools analyzing viral transmission networks have been combined - Murray/Schvoerer (2013, HCV ; 2024, SARS-CoV-2), as complementary approaches to evaluate the fitness of mutational viral clones by their clustering according (i) to time, (ii) to viral amino-acid covariances and (iii) to alphaFold tool. Then, a pooling from European db of epidemiological, clinical and virological data on HEV dispersion was explored, both for viral genomes and related publications, in full-length or partial HEV genomes : phylogeny (MEGA), logo software for amino acid signatures and deep reading of the related publications.

**Results**: First, full-length HEV genomes (HEV n-FLG) from Europe, in humans, n=301, from animals, n= 79, and near-FLG for French sequences, n= 8, were collected. A global distribution according to amino acid-trees, showed that human or animal HEV sequences appeared to be randomly distributed. While partial European HEV genomes (MET-methyltransferase), showed one large network of human and animal sequences, either according to time or to a gap at residue 135, without any 135 gap in animals, as an adaptive molecular MET-135 signature, consistent with alphaFold hot points. Second, the scientific literature was analyzed, including a review/meta-analysis on HEV in waters – Takuissu (2022). Thus, 21 articles were selected as they provide access to HEV sequences accompanied by clinical and epidemiological information, giving a collection of 738 HEV sequences. After processing, 866 sequences were retained, i.e. 263 covering a partial ORF1 segment, 357 a partial ORF2 segment, 64 corresponding to complete ORF2 sequences, 65 to complete ORF3, plus 48 to ORF2/ORF3 overlap region and 69 to (n-)FLG HEV genomes. HEV clustering studied by phylogeny argued in favor of wastewaters sources (17 times) or surface waters (4 times), animal-related sources (7 times), alimentary transmissions (3 times), without excluding combined transmission pathways. The main observations on HEV sequences by bioinformatics including alphaFold were as following : (i) a possible adaptive molecular MET-135 signature (ORF1) between animals and humans, and (ii) for 0RF3 protein at residue 81, a non-H/R basic amino acid (C or P) in animals and wastewaters compared to patients.

**Conclusions**: This study by investigating HEV sequences and epidemiological, clinical information in Europe, from patients and environmental samples, showed potential HEV transmission networks between environment and humans. Molecular features were observed from environmental samples compared to patients: (i) no MET-135 gap (ORF1) in animals ; (ii) non-H/R basic amino acid at 0RF3-81 residue in animals and wastewaters. Thus, as key functional amino acid residues of HEV proteins are involved in viral biology and epidemiology, further explorations on HEV circulating strains deserve to be regularly pooled with the aim to accelerate the understanding of various HEV transmission routes.

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# Chronic HDV Coinfection is Characterized by a More Elevated Production of all Three HBsAg Isoforms Compared to HBV Mono-Infection, that Parallels HDV Replicative and Cytolytic Activity

D'Anna S[1], Piermatteo L[1], Magnapera A[1], Olivero A[2], Duca L[3], Torre G[1], Castelli C[1], Teti E[4], Di Lorenzo A[4], Malagnino V[4], Iannetta M[4], Baiocchi L[5], Francioso S[5], Lenci I[5], Milella M[6], Saracino A[6], Ciancio A[2], Ceccherini Silberstein F[3], Sarmati L[4], Lampertico P[7], Rizzetto M[2], Caviglia G[2], Svicher V[1], Salpini R[1]

*[1]Department of Biology, University of Rome Tor Vergata, Rome, Italy, [2]Department of Medical Sciences, University of Turin, Turin, Italy, [3]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy, [4]Department of Systems Medicine, Infectious Diseases Clinic, University of Rome Tor Vergata, Rome, Italy, [5]Unit of Hepatology, Policlinico Tor Vergata, Rome, Italy, [6]Department of Biomedical Sciences and Human Oncology, Clinic of Infectious Diseases, University of Bari "Aldo Moro", Bari, Italy, [7]Division of Gastroenterology and Hepatology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

**Background**: HBV surface proteins (HBsAg) is critical for HBV and HDV morphogenesis and entry into hepatocytes. Total HBsAg comprises 3 different isoforms: i) Large-HBs (L-HBs), present in mature virions and responsible for the binding to the hepatocytes, ii) Middle-HBs (M-HBs), with a still unknown role in viral lifecycle and iii) Small-HBs (S-HBs), present in both virions and subviral particles. Here, we investigate the levels of HBsAg isoforms as well as their correlations with virological and clinical factors in the setting of chronic HBV mono-infection (CHB) and HDV coinfection (CHD).

**Materials and Methods**: This study includes 322 plasma samples from HBeAg-negative patients: 192 CHD and 130 CHB. Total HBsAg is measured by COBAS HBsAg II assays (Roche), HBsAg isoforms by ad-hoc designed ELISAs (Beacle) and HDV-RNA was quantified by Robogene assay. AUROC analysis is used to define the levels of HBsAg isoforms correlated with altered ALT.

**Results**: CHD and CHB patients have comparable age and rate of NUC treatment. Compared to CHB, CHD is characterized by lower HBV-DNA (median [IQR]: 1.0 [0.0-1.9] vs 2.6 [1.3-3.5] logIU/ml; P<0.0001), higher ALT (median [IQR]: 80 [50-133] vs 29 [19-46] U/L; P<0.001) and total HBsAg levels (median [IQR]: 5011 [1229-10'094] vs 2110 [765-5751] IU/ml; P=0.004). Median (IQR) HDV-RNA is 5.3 (3.8-6.1) logIU/ml. CHD presents significantly higher levels of all three HBsAg isoforms than CHB (median [IQR] ng/ml: 3492 [737-6576] vs 919 [180-4624] for S-HBs, 853 [153-2212] vs 108 [20-457] for M-HBs and 2.4 [0.2-7.8] vs 0.2 [0.06-0.7] for L-HBs; P<0.001). Multivariable analysis confirms CHD as independent factors associated with higher levels of all three HBsAg isoforms (OR [95%CI]: 3.43 [2.03-5.77], 6.27 [3.52-11.16] and 6.54 [2.66-16.09]; P<0.0001). Among CHD patients, HBsAg isoforms positively correlate with HDV-RNA levels (Rho=0.48, 0.45, 0.44 for S-/M-/L-HBs; P<0.0001). In line with these results, patients with HDV-RNA>3logIU/ml show significantly higher levels of all HBsAg isoforms than those with lowly-replicating HDV (median [IQR] ng/ml: 4422 [1555-7479] vs 316 [17-2438] for S-HBs; 1147 [217-2307] vs 117 [2-1052] for M-HBs; 3.6 [0.4-9.0] vs 0.3 [0.06-1.0] for L-HBs; P<0.0001 for all).

Finally, by analysing the correlations between HBsAg isoforms and biochemical parameters in lowly-replicating CHD, S-HBs>400ng/ml results to be the best cut-off for predicting altered ALT (78.6% with S-HBs>400ng/ml vs 26.7% S-HBs<400ng/ml have altered ALT; PPV=78.6%, NPV=73.3%; P=0.009). A similar correlation is observed for M-HBs>200ng/ml (75.0% with M-HBs>200ng/ml vs 35.3% with M-HBs<200ng/ml have ALT>40U/L; PPV=75.0%, NPV=64.7%; P=0.04).

**Conclusions**: HBsAg isoforms composition differs in CHD vs CHB and correlates with HDV replicative activity in CHD. This can potentially reflect a variation in the proportion of circulating viral and subviral particles in CHD and CHB. Overall, the role of HBsAg isoforms in modulating HDV-related cytolytic activity deserves further investigation since this can help identifying patients at higher risk of disease progression in which anti-viral treatment could be prioritized.

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# A High Degree of Genetic Variability Characterises HDV Sub-Genotypes 1 and Can Drive the Selection of Divergent Genetic Pathways Modulating HDV Replicative Potential and Cytolytic Activity

Piermatteo L[1], Magnapera A[1], Torre G[1], Carioti L[2], D'Anna S[1], Khan S[2], Etogo L[1], Bertoli A[2,3], Paba P[3], Olivero A[4], Teti E[5], Di Lorenzo A[5], Malagnino V[5], Iannetta M[5], Baiocchi L[6], Francioso S[6], Lenci I[6], Cillo U[7], Vitale A[7], Brancaccio G[8], Gaeta G[9], Ciancio A[4], Ceccherini-Silberstein F[2], Grelli S[2,3], Sarmati L[5], Lampertico P[10], Rizzetto M[4], Caviglia G[4], Salpini R[1], Svicher V[1]

*[1]Dept. of Biology, University of Rome Tor Vergata, Rome, Italy, [2]Dept. of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy, [3]Unit of Virology, Tor Vergata Polyclinic Foundation, Rome, Italy, [4]Dept. of Medical Sciences, University of Turin, Turin, Italy, [5]Dept. of Systems Medicine, Infectious Disease Clinic, University of Rome Tor Vergata, Rome, Italy, [6]Hepatology Unit, Tor Vergata Polyclinic Foundation, Rome, Italy, [7]Hepatobiliary Surgery and Liver Transplantation Unit, Depat. of Surgery, Oncology and Gastroenterology, University of Padova, Padua, Italy, [8]Dept. of Molecular Medicine, Infectious Diseases, University of Padova, Padua, Italy, [9]Infectious Disease Unit, University L. Vanvitelli, Naples, Italy, [10]Division of Gastroenterology and Hepatology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

**Background**: So far, limited information is available on the extent of HDV genetic diversification in HDAg domains and CTL epitopes across the different sub-genotypes 1 and their correlation with virological and biochemical parameters.

**Materials and Methods**: 103 individuals with chronic HDV infection were included. Full-length HDV genome sequences were obtained by Illumina (median [IQR] reads/seq: 62045[30460-91899]). Sub-genotypes 1 were defined by phylogenetic analysis. Amino acid (aa) residues were defined conserved if not mutated in 95% of sequences. HDAg domains and cytotoxic T lymphocytes (CTL) epitopes (N=18) were defined according to Pascarella 2010 and Kohsar 2021.

**Results**: Individuals with HDV were mostly males with a median age of 54 (44-60) years coming mainly from Eastern Europe (51%) and Italy (44.8%). Serum HDV-RNA and ALT were 5.6 (4.9-6.2) logIU/ml and 94 (65-152) U/L. Sub-genotypes 1c and 1e were the most prevalent (47.1% and 45.2%), followed by 1a (4.8%), 1b (1.9%) and 1d (1%). Sub-genotype 1c predominated in individuals from Eastern Europe (77.6% vs 22.4%, P<0.001) while 1e in Italians (77.5% vs 22.5%, P<0.001). Overall, the number of conserved aa in HDAg was only 33.2% (71/214) ranging from 38.5% in RNA binding domain (RDB)1 to 36.4% in RBD2, 27.3% in coiled coil sequence (CCS), 25% in virus assembly signal (VAS), 19.1% in nuclear localization sequence (NLS) and 18.2% in RBD3, indicative of high genetic diversification. Similarly, the degree of genetic conservation ranges from 0% in CTL epitope 81-90 to 33.3% in CTL epitopes 105-112 and 194-202.

Notably, a lower degree of genetic conservation was noted in CTL epitope 170-179 from individuals with HDV-RNA >5 logIU/ml (70% of conserved aa with vs 20% without HDV-RNA <5 logIU/ml, P=0.025), suggesting that an enrichment of mutations in this CTL epitope can enhance viral replication.

Finally, despite a comparable degree of genetic conservation between sub-genotypes 1e and 1c, they were characterized by divergent genetic pathways. In particular, sub-genotype 1c was significantly associated with the selection of 7 specific mutations (I16T/V, N22S, D47E, R112K, T180A, A202S, prevalence ranging from 26.5% to 44.9% vs 0% in 1e, P<0.001). Conversely, sub-genotype 1e was significantly associated with the selection of 6 specific mutations (D29E, D46E, K113R, R131K, M171L, I188V prevalence ranging from 23.9% to 43.5% vs 0% in 1c, P<0.009). Notably, in sub-genotype 1c, the co-presence of I16V/T+D47E+A202S correlated with ALT>3ULN (100% vs 27.5%, P=0.001)

**Conclusions**: Sub-genotypes 1 are characterized by a conspicuous degree of genetic diversification that has contributed to the selection of divergent genetic signatures. The enrichment of mutations in specific CTL epitopes could potentially hamper HDV recognition by immune response and in turn enhancing viral replication.
Overall, the role of the high degree of genetic variability in affecting the proper HDV detection by the currently available diagnostic assays deserves further investigation.

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# HDV Molecular Epidemiology in Spain: Insights from Whole-Genome Sequencing

Alberola Romano A[1], Illescas M[1,2], de Salazar A[1,2,3], Chaves-Blanco L[1,2], Fuentes A[1,2,3], Vázquez Blanquiño A[1], Carracedo R[4], Luzón-García M[3,5], Freyre C[6], Becerril Carral B[7], Cabezas Fernández M[8], Vidal-Acuña M[9], Pena M[10], Pérez A[11], de la Iglesia Salgado A[12], Liébana C[13], Viciana I[14], López Rodríguez I[15], Iborra M[16], Ramirez Arellano E[17], García F[1,2,3], Aguilera A[4]

*[1]Hospital Universitario Clínico San Cecilio, Granada, España, [2]Instituto de Investigación Biosanitaria de Granada. ibs.Granada, Granada, Spain, [3]CIBER de enfermedades infecciosas (CIBERINFEC), Madrid, Spain, [4]Complexo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Spain, [5]Hospital Universitario Poniente, El Ejido, Spain, [6]Hospital Universitario de Puerto Real, Puerto Real, Spain, [7]Hospital Universitario de Jerez, Jerez, Spain, [8]Hospital Universitario Torrecárdenas, Almería, Spain, [9]Complejo Hospitalario Universitario de Cáceres, Cáceres, Spain, [10]Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain, [11]Hospital Universitario Reina Sofía, Córdoba, Spain, [12]Hospital Infanta Elena, Huelva, Spain, [13]Hospital Universitario de Jaén, Jaén, Spain, [14]Hospital Universitario Virgen de la Victoria, Málaga, Spain, [15]Hospital Costa del Sol, Marbella, Spain, [16]Hospital Clínico Universitario Virgen de la Arrixaca, Murcia, Spain, [17]Hospital Universitario Virgen Macarena, Sevilla, Spain*

**Background**: The diagnosis of HDV infection is improving thanks to programs such as DDR Spain. However, a comprehensive characterization of the virus also requires a deeper understanding of its genetic diversity and clinical implications. HDV exhibits high genetic variability with eight identified genotypes, each encompassing multiple subgenotypes that may influence disease progression and treatment response. Genotype 1 (G1) is the most widely distributed worldwide, followed by genotype 5 (G5) in prevalence. In Spain, molecular characterization of HDV has been historically limited, with the only available study dating back to 1998, making it difficult to determine the currently circulating genotypes. Investigating the molecular epidemiology of HDV is therefore crucial for assessing its pathogenic potential, improving diagnostic accuracy, and guiding the development of more effective treatment strategies. This study aimed to characterize the molecular epidemiology of HDV in Spain through whole-genome sequencing (WGS).

**Materials and Methods**: This study is divided into two distinct phases. In the first phase, from August 2019 to June 2024, genotypic analysis was conducted in centers located in Andalucía, Murcia, and the Canary Islands. From July 2024 onward, with the start of the prospective phase of the DDR Spain study, genotype analyses were expanded to also include samples from Galicia and Extremadura. Genotyping was performed on patients with confirmed HDV RNA positivity using WGS, employing overlapping primers and Illumina's tagmentation-indexing protocol on a NextSeq 1000 platform. Sequence assembly was performed using the CLC Genomics Workbench, referencing the HDVdb database for genotype determination. Phylogenetic relationships were inferred using the Neighbor-Joining method in the MEGA software. Sequences with >90% coverage were included in the analysis alongside reference sequences representing all eight recognized genotypes and their subgenotypes. Potential recombination events were assessed using the RDP5.63 software.

**Results**: A total of 140 patients were analyzed, of whom 103 (73.6%) were men and 55.4% were of Spanish origin. The median HDV RNA level was Log 4.92 (IQR: 3.97–6.16). Genotyping was successful in 133 cases (95%) with an average sequence coverage of 98% (range: 68%–100%). G1 was the most frequent (118 cases, 88.7%), followed by G5 (14 cases,10.5%), exclusively identified in patients of West African origin. One case (0.8%) belonged to genotype 7. Genotyping failed in 7 samples due to either non-amplification (1 case) or insufficient coverage (6 cases). Phylogenetic analysis was performed on 129 sequences with >90% coverage. Within G1, subgenotype 1d represented 85.6% of cases, while subgenotype 5b accounted for 57% of G5 cases. None of the 129 sequences displayed evidence of recombination.

**Conclusions**: HDV genotyping plays a key role in understanding its molecular epidemiology in Spain. In line with prior research, G1 remains the predominant genotype among Spanish patients, whereas G5 is found exclusively in individuals of West African origin. These results provide important insights into the geographical distribution and genetic diversity of HDV in the region.

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# Implementation of a Multicenter Reflex Testing Program for Hepatitis D Detection of and Registration of Positive Cases in Catalonia

Palom A[1,2], Rando-Segura A[2,3], Calatayud Samper L[4], Fernández Rivas G[5], Sellés Sanchez A[6], Mormeneo Bayo S[7], Pérez del Campo D[8], del Corral E[9], Ayats J[10], Tabernero D[2,3], Castellote J[11], Morillas R[12], Rodríguez A[13], Huelin P[14], Lopez C[15], Salazar D[16], Villaverde J[17], Abdo I[18], Marco A[18], Quer J[2,13,19], Buti M[1,2]

*[1]Servicio de Hepatología, Hospital Unversitari Vall D'Hebron, Barcelona, Spain, [2]CIBERehd, Madrid, Spain, [3]Servicio de Microbiología, Hospital Universitari Vall d'Hebron, Barcelona, Spain, [4]Servicio de Microbiología, Hospital Universitari de Bellvitge, Barcelona, Spain, [5]Servicio de Microbiología, Hospital Universitari Germans Trias i Pujol, Badalona, Spain, [6]Servicio de Microbiología, Hospital Universitari Joan XXIII de Tarragona, Tarragona, Spain, [7]Servicio de Microbiología, Hospital Universitari Arnau de Vilanova, Lleida, Spain, [8]Servicio de Microbiología, Hospital Universitari Doctor Josep Trueta, Girona, Spain, [9]Servicio de Microbiología, Hospital Universitari Verge de la Cinta de Tortosa, Tortosa, Spain, [10]Área de Microbiología, Dirección de Laboratorios Clínicos, Institut Català de la Salut (ICS), Barcelona, Spain, [11]Servicio de Digestivo, Hospital Universitari de Bellvitge, Barcelona, Spain, [12]Servicio de Digestivo, Hospital Universitari Germans Trias i Pujol, Badalona, Spain, [13]Servicio de Digestivo, Hospital Universitari Joan XXIII de Tarragona, Tarragona, Spain, [14]Servicio de Digestivo, Hospital Universitari Arnau de Vilanova, Lleida, Spain, [15]Servicio de Digestivo, Hospital Universitari Doctor Josep Trueta, Girona, Spain, [16]Servicio de Digestivo, Hospital Universitari Verge de la Cinta de Tortosa, Tortosa, Spain, [17]Servicio de Digestivo, Hospital de Viladecans, Barcelona, Spain, [18]Programa de Salud Penitenciaria, Institut Català de la Salut (ICS), Barcelona , Barcelona, Spain, [19]PADEICS, Institut Català de la Salut (ICS), Barcelona, Spain*

**Background**: In Spain, the prevalence of HBsAg is 0.22%, with a 7.7% rate of HDV coinfection. Previous research involving small cohorts has shown that introducing reflex testing for hepatitis D in HBsAg-positive cases leads to an increase in absolute diagnoses. This project aims to establish and implement a reflex screening program for hepatitis D in all HBsAg-positive samples across the public healthcare centers of the Catalan Health Institute (Institut Català de la Salut, ICS). Additionally, a registry of positive cases will be created to evaluate their clinical characteristics and treatment indications.

**Materials and Methods**: A multicenter prospective study was conducted, including all HBsAg+ samples analyzed at the seven ICS laboratories (covering more than 95% of the Catalan population). A double reflex testing for anti-HDV and HDV RNA was performed. Demographic, epidemiological, clinical, laboratory data, and patient linkage to care status for all anti-HDV positive patients were recorded on a digital platform.

**Results**: From 1/Jan to 31/Oct of 2024, 220,242 HBsAg determinations were performed, identifying 5,333 HBsAg+ samples. Anti-HDV antibodies were tested in 4,810 samples (90%), of which 185 (3.8%) were positive after removing duplicates. Of the 185 anti-HDV positive cases, 63 (35%) were also positive for HDV RNA. The program allowed testing for 2,223 HBsAg+ samples (46%) that had not been previously tested for HDV, detecting 56 new anti-HDV+ and 9 new HDV RNA+ subjects.
Of the 53 currently registered HDV RNA+ patients, 65% were male, with a median age of 53 years (range 42–59). Thirty-nine percent were Spanish, and 38% reported risk factors. Thirty-eight percent had elevated ALT levels, 51% had liver cirrhosis, 8% had a history of hepatic decompensation, and 6% had hepatocellular carcinoma. A total of 26 patients had previously received treatment with PegIFN without response.

Currently, 25 (47%) patients have started treatment with Bulevirtide. Those treated had more advanced fibrosis (≥ F3: 84% vs 50%, p = 0.01), a higher frequency of prior PegIFN treatment (80% vs 21%, p < 0.001), and a higher frequency of previously known HDV diagnosis (96% vs 82%, p = 0.195) compared to untreated patients. The reasons for not receiving treatment included refusal due to fibrosis stage <F2 (36%), no prior PegIFN treatment (21%), limitations in drug administration (14%), or other reasons (29%).

**Conclusions**: This regional program has enabled the testing for hepatitis D in 46% of HBsAg-positive samples for the first time. Half of the patients with hepatitis D are now receiving Bulevirtide, particularly those with advanced fibrosis and those who failed to prior PegIFN treatment. This ongoing screening and monitoring program, integrated into the public healthcare system, will help determine the prevalence, characteristics, and treatment response to Bulevirtide in patients with hepatitis D.

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# Global Burden of SARS-CoV-2 Infection, Hospitalization and Case Fatality Rate Among COVID-19 Vaccinated Individuals and Its Associated Factors: A Systematic Review and Meta-Analysis

Kengni Ngueko A[1,2], Nka A[1], Yagai B[1,3], Ka'e A[1], Tekoh T[1], Efakika Gabisa J[1], Chenwi Ambe C[1,2], Ngoufack Jagni Semengue E[1], Tommo C[1], Forgwei L[1], Fokou Bomgning N[1,2], Tsapi Lontsi W[1], Togna Pabo W[4], Sosso S[1], Halle Ekane E, Colizzi V[1,2,5], Yotebieng M[6], Ndembi N[7], Ceccherini Silberstein F[2], Ndjolo A[1], Perno C[6], Santoro MM[2], Fokam J[1,4,9]

*[1]Chantal Biya International Reference Center For Research On Hiv Prevention And Management (CIRCB)), Yaoundé, Cameroon, [2]University of Rome "Tor Vergata", ROME, ITALY, [3]Faculty of Medicine, UniCamillus-Saint Camillus International University of Health Sciences, ROME, ITALY, [4]Faculty of Sciences, University of Buea, BUEA, CAMEROON, [5]Evangelical University of Cameroon, BANDJOUN, CMAROON, [6]Department of Medicine, Albert Einstein College of Medicine, New York, USA, [7]Multimodal research area, Microbiology and Diagnostics of Immunology Unit, IRCCS Bambino Gesu Pediatric Hospital, ROME, ITALY, [8]Africa Centres for Disease Control and Prevention (Africa CDC) , Addis Ababa, ETHIOPIA, [9]Central Technical Group, National AIDS Control Committee (NACC), YAOUNDE, CAMEROON*

**Background**: COVID-19 response was supported by pharmaceutical measures such as vaccination and antiviral therapy to mitigate COVID-19 associated morbidity/mortality. Hence, a thorough assessment of vaccine's contribution is warranted when designing future strategies. This study aimed to evaluate global and regional prevalence of SARS-CoV-2 infection and disease severity among COVID-19 vaccinated individuals.

**Materials and Methods**: Relevant observational and cross-sectional studies conducted among COVID-19 vaccinated individuals were searched from PubMed/MEDLINE, EMBASE, Cochrane Central Register of Controlled Trials, Science direct and Cumulative Index to Nursing and Allied Health Literature. A random effect model was used to calculate the pooled prevalence and to evaluate the disease severity. Subgroup analyses were performed to further characterize data. I2 was used to evaluate heterogeneity among studies.

**Results**: Overall, 312 studies were included with 33.0% (103/312) conducted in Europe, 31.1% (97/312) in Asia, 29.5% (92/312) in America and 3.5% (11/312) in Africa, involving 65,207,338 participants (95.3% males; median age [IQR]: 52 [43-65] years). Most studies (90.7%; 283/312) involved participants presenting comorbidities while 9.3% (29/312) was not-reported. Among 98,214 (0.2%) participants who reported vaccination: 30.6% received Pfizer, 28.3% Moderna and 20.3% Johnson & Johnson. Globally, BTI prevalence was 25.2% (95% CI: 20.24-30.56) with 29.3% (95% CI: 17.92-41.90) hospitalization rate and 4.3% (95% CI: 2.76-6.09) case fatality rate. CoronaVac vaccinees had the highest BTI (65.9%) followed by Sputnik (38.9%), p<0.01. Those who received two doses and those fully vaccinated had higher BTI prevalence (85.6% and 98.2% respectively; p<0.01). Cerebral affections (35.4%) and Hypertension (17.6%) were comorbidities associated with higher risk of BTI (p<0.01). BTI was similar among age groups (<50(younger adults) and ≥50 (elder adults); 51.43% vs 49.56%; p=0.10). Case fatality rates were similar in partially and fully vaccinated individuals (p=0.21), but higher in elderly adults (92.5%(345/373) vs 7.5%(28/373) in young adults; p<0.01) and those presenting hypertension as comorbidity (55.3% (220/398) vs 44.7%(178/398) for other comorbidities, p=0.003).

**Conclusions**: Following COVID-19 vaccination, the overall BTI rate was moderate (25%) with lower CFRs among vaccinated patients without any reported comorbidity. This underscores the significance of vaccination for response alongside a thorough management of comorbidities to mitigate severity/death at the global-level.

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# Intra-Host Evolution of SARS-CoV-2 During Prolonged Infection

della Ventura C[1], Bergna A[1], Ciubotariu C[2], Liturri M[1], Corbellino M[2], Antinori S[1,2], Riva A[1,2], Zehender G[1], Lai A[1]

*[1]University Of Milan, Milan, Italy, [2]III Division of Infectious Diseases, ASST Fatebenefratelli Sacco, Milan, Italy*

**Background**: Intra-host evolution represents the mechanism causing the continued emergence of new, highly divergent SARS-CoV-2 variants. The aim of this work was to investigate the intra-host evolution and to elucidate conditions associated with the acquisition of new viral mutations through the sequencing of viral genomes.

**Materials and Methods**: We analysed 58 cases of COVID-19 with 2 or more longitudinally positive SARS-CoV-2 swabs collected at intervals of at least 7 days from the first positive swab in the period 2021-2023. All samples were subjected to whole genome sequencing with next-generation procedures.

**Results:** The median age was 83 years [73-88] and 55% were males.57 were hospitalized for a median of 23 days [16-32], and 12 died (21%) after a median of 25 days [19-34]. No one reported previous SARS-CoV-2 infection; 79% were vaccinated. 23% received more than one treatment for SARS-CoV-2 infection.

Negativization period showed longer intervals in BQ.1 (39 days) and shorter in BA.1 infections (17 days, p=.04). No drug resistance mutations were observed over time. A total of 906 intra-host single-nucleotide variants (iSNVs) were observed in all time points. Among these, 3.4% (n=31) were synonymous and 96.6% (n=875) were nonsynonymous. The highest mean of nonsynonymous variant density was observed in the S gene in both T1 and T2 (17.9, [0-27]; 17.4, [0-27] respectively), specifically in Receptor Binding Domain (RBD).

In T1 vs. T2, 38% decreased/increased number of mutations and 24% maintained the same number. Subjects with constant numbers of mutations maintained the same pattern while 50% of the acquisitions and losses were confirmed. Acquired mutations at T2 were not present as minority mutations at T1, 50% of the lost mutations did not persist at T2. In T2 vs. T3, 67% and 33% increased or maintained the same number of mutations, respectively. 75% of subjects with constant number of mutations maintained the same pattern, 50% of the acquisitions were confirmed. New mutations at T3 were not found as minority mutations at T2. Comparing T1 vs. T2, the acquisitions were prevalent in the S gene and the losses in ORF1a, in T2 vs. T3 the opposite was observed. Significant mutations disappearing was observed in all genes in BA.1 (p<.0001), while in XBB variant a significant mutation emerging was detected in ORF1a, ORF1b and S genes (p=.016). Considering the comorbidities, subjects with cardiovascular disease showed a significant acquisition of mutations over time (p=.048).

**Conclusions**: Our data suggested a multiple steps selection mechanism for the fitness of SARS-CoV-2 mutations independently of viral variant. During the first week of viral infection, an increase in genetic diversity was evidenced; in the second week a purifying selection process was observed, while a further increase in the number of new mutations was observed at week 3.

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# Early Detection of SARS-CoV-2 Variants in a School Cohort Using Next-Generation Sequencing: Insights from the EUCARE Project

Santos A[1], Pimentel V[1], Alves I[1], Sebastião C[1], Miranda M[1], Seabra S[1], Raimondi S[2], Gandini S[2], Quintanares G[3,4], Lopalco P[5], D'Ecclesiis O[2], Tomezzoli E[6], Mommo C[13], Fanti I[12], WP4 E, Bellerba F[2], Kaiser R[11], Bardeck N[11], Martins M[1], Toscano C[14], Incardona F[12,13], Viveiros M[1], Pingarilho M[1], Abecasis A[1]

*[1]Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards; Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, Lisboa, Portugal, [2]Department of Experimental Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy, [3]Departamento de Enfermedades Infecciosas, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Ciudad de México, México, [4]Paul Ehrlich Institut, Virus Safety, Virology Department, Langen, Germany, [5]Department of Biological and Environmental Sciences and Technology, University of Salento, Lecce, Italy, [6]Applied Research Division for Cognitive and Psychological Science, European Institute of Oncology (IEO), IRCCS, Milan, Italy, [7]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy, [8]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan, Italy, [9]Pediatric Department, "Vittore Buzzi" Children's Hospital, Milan, Italy, [10]Department of Biomedical and Clinical Science, University of Milan, Milan, Italy, [11]Institute of Virology, University Clinics of Cologne, Cologne, Germany, [12]IPRO-Informa PRO S.R.L., Rome, Italy, [13]EuResist Network, Rome, Italy, [14]Laboratório de Microbiologia, Centro Hospitalar de Lisboa Ocidental, Lisboa, Portugal*

**Background**: Monitoring SARS-CoV-2 variants in schools is crucial for controlling outbreaks and preventing transmission. Sentinel surveillance networks have effectively tracked infections and preventive measures in educational settings. The EuCARE project aims to determine the prevalence and transmission dynamics of viral variants in schools and to evaluate the efficacy of different screening and identification methods.

We aim to develop and adapt the NGS sequencing methodology for SARS-CoV-2, which will allow us to identify more precisely the circulating variants. This was an interventional study with a cross-over randomized controlled trial.

**Materials and Methods**: A total of 71 samples positive for SARS-CoV-2 as confirmed by qPCR (BD MAX ExK TNA-3 kit targeting Genes N1 and N2) were analyzed, 23 from Portugal and 48 from Italy. Total viral RNA was extracted from participants' plasma samples. Positive samples (Ct =28) underwent sequencing using Sanger sequencing or Oxford Nanopore technology (NGS). Complementary DNA was synthesized and amplified using multiplex PCR with ARTIC Network primers. Sequencing libraries were prepared and run on a MinION Mk1C device. Sequencing data was mapped and aligned using Geneious Prime v2024.0.5 and the consensus sequence was exported for SARS-CoV-2 variant identification using Nextclade: Clade assignment, mutation calling, and sequence quality checks version 3.8.2.

**Results**: In the school study population in Italy, the most common variants detected were CH.1.1 and XBB.1.5, with 37.5% and 31.3% respectively, with both being detected for first time in the country in the same month of our detection. In Portugal the most common variants were XBB.1.5 and JN.1 with 34.8% and 30.4% respectively, with the former being detected 3 months before the first detection by the National Institute of Health and the latter also being detected 3 days before its first reported detection. Several other variants in both countries were detected in schools but not by national epidemiological surveillance efforts.

**Conclusions**: The EUCARE school study detected SARS-CoV-2 variants in Portuguese schools earlier than national surveillance systems. Epidemiologic surveillance in schools is a reliable method for early detection of SARS-CoV-2 variants, due to the in-house NGS protocol allowing for collection, sequencing and variant identification to be all performed on the same day. This straightforward approach allows for up-to-date/daily surveillance and additionally suggests that schools can serve as effective sentinel centers for infection surveillance. Early identification of variants can facilitate prompt public health responses.

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# Evaluation of the SARS-CoV-2 Virus Genetic Barrier to Remdesivir and Nirmatrelvir In Vitro and In Vivo

Varasi I[1], Fiaschi L[1], Gidari A[2], Bastianelli S[2], Pierucci S[2], Bergna A[3], Della Ventura C[3], Biba C[1], Paletti C[1], Bartolini N[1], Saladini F[1], Lai A[3,4], Zazzi M[1], Francisci D[2], Vicenti I[1]

*[1]Department Of Medical Biotechnologies, University Of Siena, Siena, Italy, Siena, Italy, [2]Department of Medicine and Surgery, University of Perugia, Perugia, Italy, Perugia, Italy, [3]Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy, Milano , Italy , [4]Laboratory of Medical Microbiology and Virology, University of Insubria, Varese, Italy, Varese, Italy*

**Background**: Remdesivir (RDV) and Nirmatrelvir (NRM) are direct-acting antivirals (DAAs) approved for early SARS-CoV-2 treatment. Both have shown a high barrier to resistance, with few in vitro resistance mutations and rare in vivo cases. This study evaluates the emergence of in vivo resistance after DAA treatment and the in vitro genetic barrier of the ancestral B.1 and KP.3 Omicron variants to RDV and/or NRM.

**Materials and Methods**: Seventy-one paired swabs were collected between Mar-2022 and Sept-2024 from 66 and 5 subjects with short-term RDV and NRM treatment, respectively, at baseline (T0) and after 5 [4-6] days of treatment (T1). Cases with positive SARS-CoV-2 RNA within 29 Ct at both T0 and T1 underwent full-length NGS by Illumina COVID-Seq Assay with 5% threshold. Emerging mutations (prevalence shift from <5% to >20%) were identified using Stanford CoV-DB. In vitro antiviral activity (IC50) of RDV and NRM was determined in VERO-E6 using a live virus assay. Resistance selection (IVRS) experiments were performed by growing the KP.3 and B.1 variants in duplicate in presence of increasing concentrations (2-fold IC50) of RDV, NRM or both. IVRS was stopped when no viral breakthrough was observed for 3 weeks.

**Results**: The median age of the participants was 79 years [68-85], 48% were female and 88% had a median of 3 [2-4] comorbidities. Sixty-eight subjects were hospitalized and 79% needed oxygen support; 2 patients died. The longitudinal analysis included 20 subjects with Ct ≤ 29 (20±4 and 26±3 Ct at T0 and T1 respectively), most harboring XBB and BA.2.86 variants. No amino acid variations were observed in the spike region and in 3CL-Protease. Two distinct mutations emerged in 2 subjects treated with RDV reaching >20% of prevalence (V792VI: V/I = 50/49% and M794MI: M/I =60/40%; sample 31 and 6 respectively).

While the role of M794I was not previously characterized, V792I is reported as a resistance mutation in vitro and anecdotally in vivo. In vitro, B.1 and KP.3 showed a mean µM IC50 of 0.04±0.01 (RDV), 0.07±0.02 (NRM) and 0.07±0.01 (RDV), 0.15±0.05 (NRM), respectively. B.1 replication was observed up to 128-fold IC50 with each individual drug (20±4 days post infection [DPI]). KP.3 replication was observed up to 73-fold (21 DPI and still ongoing) in one RDV IVRS but was arrested at 9-fold IC50 in the second one (11 DPI) and at 11±5 fold IC50 in both NRM IVRS (11±1 DPI). Both KP3 and B.1 IVRS were arrested at 11±6-fold IC50 with combined RDV+NRM (9±3 days DPI). SARS-CoV-2 sequencing is ongoing to evaluate possible emergent resistance in all IVRS experiments.

**Conclusions**: In vitro, KP.3 seems less prone to viral breakthroughs under DAA pressure compared with B.1. However, the combination of RDV+NRM strongly inhibits viral replication with both viruses. In vivo study, emergent genotypic resistance to DAAs appears to be a rare event, with only one case detected in 18 subjects treated with RDV. Inclusion of further subjects in the study population, particularly immunosuppressed individuals with prolonged infection may help to better define the impact of resistance mutations against DAAs.

# LIGHTNING POSTER ABSTRACT PRESENTATIONS

23rd European Meeting on HIV & Hepatitis 2025

4 – 6 June 2025
Barcelona, Spain

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# Analysis of 16 Years of ARV Resistance Transmission in Newly Diagnosed HIV Cases in Spain (2007-2023): 2023 Update and the Impact of PrEP on M184V Detection

Muñoz P[1], Chaves-Blanco L, Illescas López M, De Salazar A, Salces Romero I, Pinto A, Portilla I, Masiá M, Peraire J, Alemánn Valls M, Sanchiz M, Iborra A, Aguilera A, Baza B, Olalla J, Espinosa N, Iribarren J, Gisbert L, Imaz A, Montero M, Navarro Marcotegui M, Suarez I, Riera M, Pérez Elias M, Galan J, García Fraile L, Sanz J, Ibarra Ugarte S, Bernal E, Blanco J, Navarro G, Delgado E, Thomson M, Moreno C, García García F

[1]*University Hospital of San Cecilio, Granada, Granada, Spain*

**Background**: The evaluation of resistance in newly diagnosed HIV cases in Spain has been carried out in CoRIS since 2007, with annual updates until 2022. In this study, we present the calendar trend of TDR from 2007 to 2023 in newly diagnosed HIV cases, as well as primary resistance to first-line drugs.

**Materials and Methods**: Newly diagnosed cases from CoRIS centers were included. After sequence quality control, mutations in RT/Pro/INI associated with TDR were investigated using CPR-Stanford. Additionally, we evaluated clinically relevant resistances to drugs recommended as first-line treatment in the GESIDA guidelines for the different analyzed periods. For subtyping, the Stanford tool and phylogenetic analysis with Mega were used.

**Results**: Between 2007 and 2023, 9,027 newly diagnosed people with HIV (PWH) were analyzed. TDR showed a stable trend over this period, with the highest levels observed for NNRTIs. Primary resistance to first-line ARVs showed a significant decline in 2014 when NNRTIs were no longer recommended as preferred initial regimens. In 2023, 548 PWH were analyzed, with available RT, Pro, and INI data for 514, 517, and 335 PWH, respectively. The overall prevalence of TDR was 12%, with class-specific rates of 4.3% for NRTIs (M184V, 1.17%), 8.2% for NNRTIs, 1.2% for PIs, and 0.3% for INIs. Notably, M184V was detected in 5 out of 6 individuals on PrEP, and RAMs to rilpivirine were found in 6.02% of patients. For first-line drugs, resistance rates were: 0.6% for tenofovir, 1.6% for abacavir, 1.4% for lamivudine/emtricitabine, 8.9% for efavirenz, 7% for rilpivirine, 1.8% for doravirine, 1.5% for raltegravir, and 0.3% for bictegravir and dolutegravir. Most PWH had subtype B (77.6%); among non-B subtypes, CRFs were the most prevalent (6.9%), with a representation of 2% for A6 and 2.6% for A1.

**Conclusions**: The higher prevalence of transmitted resistance to NNRTIs is confirmed, with doravirine being the drug in this class with the lowest levels of TDR. Transmitted resistance to PIs, INIs, and 3TC/FTC remains very low, with only one PWH presenting complete resistance to second-generation integrase inhibitors. In 2023, the M184V mutation was mostly detected in individuals who were currently or had previously taken PrEP. Our results reflect the epidemiological situation of TDR and the level of primary resistance to first-line ARVs in Spain.

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# Clearance of NNRTI Resistance-Associated Mutations in the HIV-1 DNA Reservoir with or without the Presence of M184V Mutation

Soulie C[1], Zafilaza K[1], Wirden M[1], Sayon S[1], Abdi B[1], Cocherie T[1], Teyssou E[1], Calvez V[1], Marcelin A[1]

[1]*Sorbonne Université, Inserm, Iplesp, Aphp Pitié Salpêtrière, Laboratoire De Virologie, Paris, France*

**Background**: Evolution of HIV antiretroviral resistance mutations in the human reservoir (host cell DNA) over time has made remarkable progress in recent years, particularly with regard to the M184V mutation. Data on non-nucleoside reverse transcriptase inhibitors (NNRTIs) are limited. The aim of our study was to describe the evolution of NNRTI-associated resistance mutations (NNRTI-RAMs), associated or not with M184V using next-generation sequencing.

**Materials and Methods**: A French single-center retrospective study included people living with HIV-1 (PLWHIV) with: age >18 years; a history of virological failure (VF) under an NNRTI-containing regimen; a plasma genotype with one or more major NNRTI-RAMs detected by Sanger sequencing; a blood sample collected after at least one year of virological suppression on antiretroviral (ARV) therapy (DNA1=2019 and DNA2=2024) available for reverse transcriptase NGS. The studied factors were as follows: zenith plasma HIV-1 viral load (VL), CD4 nadir, duration of replication under NNRTI, time since HIV diagnosis, time since VF, duration of viral suppression, HIV-1 DNA VL, zenith HIV-1 VL, HIV-1 VL at NNRTI failure, time on ART.

**Results**: Fifty-one PLWHIV met the inclusion criteria and NGS was successful in 49 (94.4%). Of the 49 PLWHIV, 40 had M184V in previous HIV-1 RNA genotypes. Participants were male (65.3%), infected with HIV for 28 years in median (IQR 23-31) and received ARV treatment for 22 years (IQR 19-26). The median time since NNRTI failure was 11 years (IQR 9-17) and the median time of viral suppression was 7 years (IQR 3-10).

At DNA1, NNRTI-RAMs and M184V were not detected in the HIV reservoir (blood samples) in 75.5% and 40.0% of PLWHIV, respectively. Overall, the presence of all studied NNRTI-RAM (K103N, V106A, Y181C, Y188L and M230L) decreased between the previous RNA and further DNA genotypes. NNRTI mutation clearance at DNA1 was associated with a shorter duration of replication under NNRTI regimen at VF (5 vs 37 months, p=0.013), with a longer duration between RNA and DNA1 genotypes (14 vs 9 years, p=0.010), and with an older age (60 vs 55 years, p=0.037) in univariate analysis. On multivariate analysis, only the duration of replication under NNRTI regimen at VF remained associated (p=0.041).
In case of NNRTI-RAMs persistence, there was no difference between DNA1 and DNA2 mutational VL for NNRTI-RAMs (3.69 vs 3.23 log10 copies/mL, p=0.117) or M184V (3.83 vs 3.82 log10 copies/mL, p=0.921).
The presence of M184V in past RNA genotypes was not associated with the persistence of NNRTI RAM at DNA1 (p=0.861). Indeed, in the M184V+ group, 25.0% of PLWHIV had NNRTI RAM and 75.0% did not, compared to 22.2% and 77.8% in the M184V- group, respectively. A similar result was observed five years later at DNA2 (p=0.440), with NNRTI RAM present in 47.5% of the M184V+ group and absent in 52.5%, compared to 33.3% and 66.6% in the M184V- group, respectively.

**Conclusions**: Overall, NNRTI and M184V mutations decreased over time in the HIV blood reservoir with no clear interaction between their evolution. Only the duration of replication under an NNRTI regimen was associated with the NNRTI RAM persistence.

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# Resistance Analyses of F/TAF, F/TDF, and Lenacapavir in the PURPOSE 1 and 2 Studies

Cox S[1], Andreatta K[1], Hendricks M[1], Li J[1], Kintu A[1], Brown L[1], Carter C[1], Callebaut C

[1]*Gilead Sciences, Inc., Foster City, USA*

**Background**: Lenacapavir is a first-in-class HIV-1 capsid (CA) inhibitor in development for the prevention of HIV-1 infection. The Phase 3 studies PURPOSE 1 (NCT04994509) and PURPOSE 2 (NCT04925752) evaluated twice-yearly subcutaneous lenacapavir for HIV pre-exposure prophylaxis (PrEP). PURPOSE 1 enrolled 5345 cisgender adolescent girls and young women aged 16-25 years. PURPOSE 2 enrolled 3271 cisgender gay, bisexual, and other men, transgender women, transgender men, and gender nonbinary people aged ≥16 years who have sex with partners assigned male at birth. At the primary efficacy analysis: for PURPOSE 1, there were 0 infections in the lenacapavir group (n=2138) compared with 39 in the emtricitabine/tenofovir alafenamide (F/TAF) group (n=2137) and 16 in the emtricitabine/tenofovir disoproxil fumarate (F/TDF) group (n=1070); for PURPOSE 2, there were 2 infections in the lenacapavir group (n=2183) compared with 9 infections in the F/TDF group (n=1088). Lenacapavir was superior to the primary comparator of background HIV incidence and the secondary comparator of F/TDF in both PURPOSE 1 and 2. Here, resistance analyses for both studies through the primary efficacy analyses are described.

**Materials and Methods**: Genotypic analyses of HIV-1 CA, reverse transcriptase (RT), protease, and integrase were performed on all participants in the resistance analysis population (RAP), which comprised participants who acquired HIV-1 infection through the primary analyses of the studies and had an HIV RNA viral load of >200 copies/mL. Additional analyses of participants who acquired HIV-1 prior to Day 1 were not part of the primary analysis, but were also included.

**Results**: The PURPOSE 1 RAP included 53 participants (37 F/TAF; 16 F/TDF), with 0 participants from the lenacapavir group acquiring HIV-1. The RT resistance mutation M184V/I was identified in 3 participants (2 F/TAF; 1 F/TDF), and the K65R RT mutation in 1 of the F/TAF participants with M184V/I. Seven participants outside of the RAP had an unrecognized HIV infection at baseline and received study drug (4 lenacapavir; 1 F/TAF; 2 F/TDF). No resistance substitutions were detected on Day 1. Follow-up data on the 7 participants demonstrated that the lenacapavir resistance substitution N74D had developed in 2 lenacapavir participants and that M184M/V had developed in 1 F/TAF participant. The PURPOSE 2 RAP included 11 participants (2 lenacapavir; 9 F/TDF). As previously reported, the N74D CA substitution was observed in both participants in the lenacapavir group, whereas in the F/TDF group, the M184V substitution was observed in 1 participant. Six participants outside of the RAP had unrecognized HIV infection at baseline and received study drug (4 lenacapavir; 2 F/TDF). Resistance data were available for 5/6 participants; no resistance substitutions were detected on Day 1, but follow-up data showed development of the N74D substitution in 2 lenacapavir participants and the M184M/V substitution in 1 F/TDF participant.

**Conclusions**: Acquisition of HIV-1 in participants who received lenacapavir was rare in the PURPOSE studies. Emergence of lenacapavir-associated CA mutations occurred in some participants in the setting of functional lenacapavir monotherapy, as has been observed with other PrEP agents. Overall, protection from HIV infection was high and resistance was rare in the PURPOSE studies.

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# Early Treatment Failure with Lenacapavir in HIV-2 Infection

Obermeier M[1], Yildirim Ö[1], Ehret R[1], Almahfoud M[2], Berzow D[3], Krüdewagen B[4], Degen O[2]

*[1]MVZ medizinisches Infektiologiezentrum Berlin AG Aufgang A09, Berlin, Deutschland, [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]Private Practice, Hamburg, Germany, [4]Deutsches Herzzentrum der Charité, Berlin, Germany*

**Background**: In vitro data suggest efficacy of Lenacapavir (LEN) against HIV-2, although mean IC50 is 11-fold higher as compared to HIV-1. We describe an early treatment failure after LEN initiation in an individuum infected with HIV-2 with so far unobserved mutations.

**Materials and Methods**: A 65 old male was diagnosed with HIV-2 in 1991(WHO/CDC C2). ART was started in 2005 and adapted due to development of resistance. Previous (Feb. 2018) genotypic resistance analysis showed mutations against NRTIs (K65R, M184V), and PIs (I50V, I54M, I64V) without INI mutations. Last ART was Dolutegravir (DTG, BID), 3TC, AZT and Lopinavir/r. In June 2023 HIV-2 VL was 10.280 IU/ml, CD4 cells were 32/µl (2%). Salvage-ART was initiated with LEN, DTG (BID), 3TC and AZT. HIV-2-VL was measured with the Altona RealStar® HIV-2 RT-PCR Kit 1.0, mutations were identified by amplicon based nanopore sequencing of the gag, pol and env region. Plasma drug levels were determined with LC-MS/MS. HIV-2 drug resistance was interpreted with the HIV2EU tool.

**Results**: After 21 days a rapid decrease in HIV-2-VL from 10,280 IU/ml to 1,400 IU/ml was documented, followed by an increase at day 35 to 1,200,000 IU/ ml (CD4+ 84 cells/µl). LEN plasma levels were between 20 and 62 ng/ml (reference C(tau) CI: 19.8–52.6 ng/ml) and DTG 9810-11400 ng/ml (reference C(tau) CI: 790–4266 ng/ml). Treatment was well tolerated with small lumps at the injection sites. In comparison to the baseline sequence a single amino acid change N73D (capsid position numbering HIV-2 BEN) at day 35 was observed. Re-analysis of the pol region at baseline confirmed mutations in the Protease and Reverse Transcriptase with additional mutations D67N and V111I. Integrase mutations T97A, Y143A, N155S were observed, of which only the N155S was detected before .

**Conclusions**: Despite the initial drop in HIV-2-VL and LEN plasma levels in the expected range, rapid resistance development was observed after 35 days of treatment. The found integrase mutations are most probably associated with resistance and were leading to subsequent LEN mono therapy. Further studies are needed to investigate the effectiveness and the use of LEN in salvage settings in HIV-2 patients. By now the observation could be confirmed by other groups and are now included in the capsid interpretation of the HIV2-EU Lenacapavir tool. (hosted at http://www.hiv-grade.de)

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# Increased Dynamics of Novel A6/B Recombinant Forms Emergence Following Full-Scale War in Ukraine

Serwin K[1], Mielczak K[1], Urbańska A[1], Aksak-Wąs B[1], Ząbek P[2], Lübke N[3], Obermeier M[4], Siwak E[2], Cielniak I[5], Jakubowski P[6], Bociąga-Jasik M[7], Mularska E[8], Szetela B[9], Parczewski M[1]

*[1]Department Of Infectious, Tropical Diseases And Immune Deficiency Pomeranian Medical University In Szczecin, Szczecin, Poland, [2]Department for Adult Infection Diseases, Medical University in Warsaw, Warsaw, Poland, [3]Institute of Virology, Düsseldorf University Hospital, Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany, [4]Medical Center for Infectious Diseases, Berlin, Germany, [5]Department of Infection, Tropical Diseases and Hepatology, Medical University in Warsaw, Warsaw, Poland, [6]Infectious Diseases, Pomeranian Hospitals, Gdańsk, Poland, [7]Department of Infectious and Tropical Diseases, Jagiellonian University Medical College, Kraków, Poland, [8]Department of Infectious Diseases, Regional Hospital Chorzów, Chorzów, Poland, [9]Department of Infectious Diseases, Liver Disease and Acquired Immune Deficiencies, Wroclaw Medical University, Wrocław, Poland*

**Background**: The influx of war migrants from Ukraine to Europe has resulted in a substantial rise in the proportion of individuals infected with HIV-1 A6 lineage, which is a known risk factor for virologic failure of long-acting Cabotegravir/Rilpivirine (CAB/RPV) treatment. Poland is at the crossroads of two HIV epidemics: one fueled by the historically dominant B subtype and the other by the spreading A6 sub-subtype, which is prevalent in Eastern Europe. The current situation creates optimal conditions for accumulating new A6/B recombinant forms. We aimed to investigate the emergence of new A6B recombinants using molecular epidemiology methods.

**Materials and Methods**: The sequences of 7,522 pol gene fragments sampled nationwide between 1996 and 2024 were combined with a subtype reference alignment of publicly available sequences. The sequences were subtyped using the Comet and Geno2Pheno subtyping tools. We included subtype B and A6 sequences and all unspecific variants for this analysis, excluding other pure subtypes. Unassigned sequences and potential recombinant forms were analyzed using jpHMM (jumping profile Hidden Markov Model) and RIP (Recombinant Identification Program) to detect mosaic forms. Phylogenetic Maximum likelihood (ML) trees were inferred to examine and determine the clusters of emerging A6B mosaic forms. Finally, we performed whole genome sequencing (WGS) on selected samples to verify the emergence of new variants.

**Results**: Among the 2,295 (30.5%) sequences of lineage A6 and 4,815 (64.0%) sequences of subtype B, we identified 137 (1.8%) recombinant A6B sequences containing at least one A6-attributed segments within the pol gene. Based on the Shimodaira–Hasegawa approximate likelihood ratio test (SH-aLRT), 15 clusters (between 2 to 27 sequences) with branch support ≥0.9 and 13 singletons were identified. Sequences of A6B were found in 11 out of 16 regions in Poland. The first recombinant sample emerged in 2001; however, almost a quarter of cases (22.6%, n=31) were diagnosed after 2022. WGS analysis of sequences from distinct clusters confirmed that at least three clusters contain unique recombinant forms observed among epidemiologically unliked individuals, comprising a total of 26 sequences.

**Conclusions**: Molecular analysis showed that mosaic forms of A6 and B subtypes are commonly circulating across the country. Various distinct A6B recombinants have been identified, indicating multiple viral breakpoint events. In the recombinant forms, the A6 sequence in the integrase region may alter the CAB/RPV efficacy and require careful molecular surveillance.

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# Estimating the Genetic Barrier for Resistance to Integrase Inhibitors for the Most Frequently Circulating HIV-1 Subtypes

Crouse L[1], van Kampen J[2], Paraskevis D[3], F.B. Santos A[4], Nijhuis M[5,6], Seguin-Devaux C[7], Mesplede T[2], B. Abecasis A[4], MJ Wensing A[1,8], N.S.Miranda M[1,5]

*[1]Translational Virology, Department of Global Public Health & Bioethics, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands, [2]Department of Viroscience, Erasmus Medical Center in Rotterdam, Rotterdam, Netherlands, [3]Medical School of the National and Kapodistrian University of Athens, Athens, Greece, [4]Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation towards Global Health (LA-REAL), Institute of Hygiene and Tropical Medicine, New University of Lisbon (IHMT/UNL), Lisbon, Portugal, [5]Translational Virology, Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, Netherlands, [6]HIV Pathogenesis Research Unit, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa, [7]Infection and Immunotherapy Research group, Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg, [8]Ezintsha, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa*

**Background**: The second-generation Integrase Strand Transfer Inhibitors (INSTIs) (bictegravir (BIC), cabotegravir (CAB), and dolutegravir (DTG)) are among the preferred drugs for treatment of HIV-1 infections. Following the expanded global access to dolutegravir and the introduction of long-acting cabotegravir, cases of virological failure are being reported. For subtype A6, a higher risk of failure to cabotegravir has been reported due to a lower genetic barrier to resistance. We aim to estimate the genetic barrier to INSTI resistance for a larger subset of subtypes based on the number and type of nucleotide changes.

**Materials and Methods**: The genetic barrier was calculated as the sum of transitions (scored as 1) and transversions (scored as 2.5) needed to be selected by each consensus subtype to evolve to the nearest integrase resistance codon as listed in the IAS-USA mutation charts (2022 version). Consensus subtype (A1, A6, B, C, D, F1, G) and circulating recombinant form (CRF) (CRF01_AE and CRF02_AG) sequences as established in 2021 were derived from the Los Alamos database.

**Results**: 56.9% (164/288) of integrase codons among the consensus subtypes and CRFs analyzed had conserved nucleotide triplets. These include resistance related codons 66, 97, 121, 138, 143 and 155.

At codon 74 for sub-subtype A6, a non-synonymous difference (1 transversion plus 1 transition) was found compared to all other consensus subtypes and CRFs, resulting in the presence of resistance mutation 74I. Synonymous differences were found at resistance related codons 92 (for all subtypes and CRFs except B), 118 (only for CRF01_AE), 147 (only G), 148 (only C), 153 (only C) and 263 (only C). None of these differences affect the calculated genetic barrier for selection of integrase resistance mutations.

At codon 140 there were various synonymous differences among A1, A6, B and CRF01_AE compared to all other subtypes and CRFs. Of note, only for subtype B was there an effect on the calculated genetic barrier towards several resistance mutations at this codon. The genetic barrier for subtype B as compared to the other subtypes was lower for the selection of G140C (1 transversion vs 2 transversions) and G140S (1 transition vs 1 transition plus 1 transversion), while being higher for G140R (1 transition plus 1 transversion vs only 1 transition) and not different for G140A.

**Conclusions**: Despite variation among consensus sequences, we only found relevant differences in the calculated genetic barrier for two resistance related codons. Our analysis confirms the previously reported presence of a resistance mutation in sub-subtype A6 at codon 74. In addition, we found differences in the calculated genetic barrier for the consensus subtype B for several mutations at resistance codon 140. We are planning to investigate whether the observed changes in the calculated genetic barrier are associated with specific patterns of resistance in cases with virological failure to 2nd-generation INSTIs collected in the global ROSETTA registry.

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# Three-years Outcome After Genotyping-Guided Switch in HIV Multi-Drug Resistant Patients in Cameroon: Evidence-Based Strategies for Achieving Epidemic Control in Low-and Middle-Income Countries

Ngoufack Jagni Semengue E[1], Magnilack Kuete C[1,3], Njouendou A[3], Ambe Chenwi C[1,6], Kenfack D[1], Mekel V[1,6], Njako Matute J[4], Diabe Munge S[4], Supuen L[5], Berinyuy L[5], Eduke Njuzy S[7], Perno C[8], Fokam J[1,9], Njdolo A[1]

*[1]Centre International De Référence Chantal Biya, Yaoundé, Cameroon, [2]Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Yaoundé, Cameroon, [3]Faculty of Health Sciences, University of Buea, Buea, Cameroon, [4]Limbe Regional Hospital, Limbe, Cameroon, [5]Buea Regional Hospital, Buea, Cameroon, [6]Faculty of Medicine and Surgery, University of Rome "Tor Vergata, Rome, Italy, [7]Baptist Hospital Mutengene, Mutengene, Cameroon, [8]Bambino Gesu' Children's Hospital, Rome, Italy, [9]Central Technical Group, National AIDS Control Committee (NACC), Ministry of Public Health, Yaounde, Cameroon*

**Background**: Monitoring of HIV-infected patients with long-term treatment remains challenging in low- and middle-income countries (LMICs) due to risks of multi-class HIV drug resistance (HIVDR). Following a personalized strategy, we sought to evaluate the treatment response among patients with multi-class HIVDR following genotypic resistance guided switch in Cameroon.

**Materials and Methods**: A cohort-study was conducted in the South-West region of Cameroon among patients failing first- and second-line antiretroviral treatment (ART) (i.e. non-nucleoside reverse transcriptase inhibitors (NNRTI)- and protease inhibitor (PI)-based) from 2018-2023. Following HIV-1 genotypic resistance testing (GRT), patients were switched to most-effective therapies and viral load (VL) was monitored after 3-, 6-, 12-, 24-, and 36-months. Data analysis used Epi Info v.7.2, with $p<0.05$ considered statistically significant.

**Results**: From the 336 patients failing ART in the region, 170 (50.6%) presented with GRT results; from these, 72 were further lost-to-follow-up, 12 died and 5 defaulted, giving 81 study-inclusions (57.5% females; 47/81). Before GRT, the median CD4 and VL were 232.5 [161.25–401.25] cells/µL and 54,480 [13,932.5–220,153] copies/mL respectively; with 65.4% (53/81) participants failing PI-based versus 34.6% (28/81) failing NNRTI- and dolutegravir (DTG)-based first-line ART. Overall HIVDR rate at failure was 60.5%, and prevailing mutations were M184I/V (26.1%), K103N/S (20.7%) and M46I/L (32.8%) for NRTI, NNRTI and PI respectively; with CRF02_AG as the prevailing viral clade (71.4%). Following GRT, AZT+3TC+(LPV/r or ATV/r) (26.4%) was mostly recommended, followed by TDF+3TC+DTG+DRV/r (12.4%). Monitoring data revealed that 92.6% (75/81) of the participants respected the prescribed regimen. Viral suppression (VL<1000copies/ml) post-GRT was 80% at 3-months, 84.8% at 6-months, 81.4% at 12-months, 93.7% at 24-months and 86.7% at 36-months. Prescription of DTG-based regimen was the only determinant of good virological response (p=0.04). Furthermore, DTG-based regimens yielded significantly higher responses than DTG-sparing regimens up till 24-months (p=0.013, p=0.0015, p=0.0005, p=0.02 respectively).

**Conclusions**: Our findings highlight maintained viral suppression over three years, following GRT-guided switch in individuals with multi-class HIVDR, with DTG-based regimens yielding a higher outcome. This underscores the significance of personalizing ART management for difficult-to-treat people to achieve HIV control by 2030 in LMICs.

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# Virologic Failure and Resistance Emergence During Treatment with B/F/TAF or DTG/3TC in PWH without Prior Resistance Mutations: A Real-World Study

Drumel T[1], Kimmerlin J[1], Colin D[1], Allavena C[1], Billaud E[1], André-Garnier E[1], Raffi F[1]

[1]CHU Nantes, Nantes, France

**Background**: in phase 3 studies of B/F/TAF or DTG/3TC during first-line or maintenance therapy, rate of virological failure (VF) is very low, without resistance emergence. We evaluated, in a large real-world cohort of people with HIV (PWH) treated with B/F/TAF or DTG/3TC, rates of VF and resistance emergence.

**Materials and Methods**: from electronic medical records, retrospective collection, in a single reference center, of demographics, therapeutic and immune-virological data of PWH between January 2018 and January 2025. We included all consecutive adults treated with B/F/TAF or DTG/3TC, either as first-line or suppressive maintenance, excluding those with prior resistance mutation(s) to nucleos(t)ide reverse transcriptase inhibitors (NRTI) or integrase inhibitors (INI) or follow-up <6months. VF was defined as HIV RNA >100 c/mL or confirmed >50 c/mL, after >6months of therapy in first-line, at any time-point in maintenance. Blip was defined as a single HIV RNA 50-100 c/mL, not confirmed. At VF, plasma Sanger genotype was performed if requested by clinician. Analyses used Mann-Whitney and Fisher exact tests.

**Results**: During study period, 712 patients received B/F/TAF (197 in first-line and 515 in maintenance; CD4 nadir <200/mm3: 37%) and 541 DTG/3TC (26 in first-line and 515 in maintenance; CD4 nadir <200/mm3: 28%). Pre-treatment median HIV RNA in first-line was 5.3 and 4.3 log10 c/mL for B/F/TAF and DTG/3TC (p=0.0001), respectively. Among patients with VF, the median follow-up was 41.3 months on B/F/TAF, 31.4 months on DTG/3TC and the first HIV RNA >50 c/mL was observed at a median of 11.8 months on B/F/TAF, 12.4 months on DTG/3TC. During first-line, blips and VF occurred in 13 (6.6%) and 20 (10.2%) patients on B/F/TAF, and 0 and 3 (11.5%) on DTG/3TC, respectively. During maintenance, blips and VF occurred in 19 (3.7%) and 56 (10.9%) patients on B/F/TAF, and 8 (1.6%) and 36 (7.0%) on DTG/3TC. Genotype was available in 62/76 VF on B/F/TAF and 33/39 VF on DTG/3TC, with emergence of resistance-associated mutations in 4/62 (6.5%) and 6/33 (18.2%), respectively: on B/F/TAF, in 3 cases NRTI mutations only [K65R (n=1), K65R+M184I (n=1), M184V (n=1)] and in 1 case R263K (integrase) + failure to amplify reverse transcriptase; on DTG/3TC, M184V (6/6), associated with INI mutations in 3 cases [N155H (n=1), E138K+G140A/G+Q148K (n=1), G140S+Q148H (n=1)]. At VF, median HIV RNA (zenith) was higher on B/F/TAF than on DTG/3TC (3.3 vs 2.6 log10 c/mL; p=0.055). On B/F/TAF, but not on DTG/3TC, median CD4 nadir was lower in PWH who experienced VF (202 vs 274/mm3, p = 0.0124, and 249 vs 297/mm3, p=0.33, respectively). Antiretroviral therapy was changed, following VF, in 13% of patients on B/F/TAF and 28% on DTG/3TC.

**Conclusions**: in PWH without prior resistance mutations to NRTI and/or INI receiving B/F/TAF or DTG/3TC, VF were mainly seen during maintenance therapy. Risk of emergence of resistance-associated mutations at VF seems lower on B/F/TAF than on DTG/3TC. However, emergence of resistance during B/F/TAF or DTG/3TC treatment remains very low in real-world clinical setting.

# POSTER ABSTRACT PRESENTATIONS

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# Development of an Intact Proviral DNA Assay for Subtype A6

van Osch J[1], Overmars R[1], Mesplede D[1]

[1]*Erasmus Mc, Rotterdam, Netherlands*

**Background**: The quantification of the HIV reservoir will become a relevant clinical measure in the future, particularly in the context of long term suppression under antiretroviral drugs. A universal reservoir quantification assay that works for all subtypes remains elusive. Thus, subtype-specific assays need to be engineered. Our aim was to develop and validate such assay for the sub-subtype A6.

**Materials and Methods**: Primers and probes to detect the 3' and 5' regions of HIV-1 subtype A6 were developed and tested on two virologically suppressed persons.

**Results**: Our quantitative assay was validated on two donors, yielding proviral DNA intactness levels comparable to those found in people living with subtype B. The assay was not cross reactive with subtype B.

**Conclusions**: The development of this assay will permit a precise quantification of the HIV reservoir in people living with subtype A6, including refugees from Ukraine, and may be useful when they are treated by long-acting cabotegravir plus rilpivirine injections.

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# Molecular Characterization of Human Adenovirus Genotypes in Paediatric Patients

Colagrossi L[1], Forque L[1], Fox V[1], Scutari R[1], Mastropaolo M[1], Merli P[2], Fini V[1], Di Maio C[1], Linardos G[1], Coltella L[1], Ranno S[1], Russo C[1], Villani A[3], Perno C[1]

*[1]Microbiology and Diagnostic Immunology, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [2]Department of Pediatric Hematology/Oncology and Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [3]Emergency, Acceptance and General Pediatrics Department, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy*

**Background**: Human adenoviruses (HAdV) are classified into 116 genotypes grouped into seven species with remarkable difference in term of tropism and species-specific immune-response. HAdVs cause a wide variety of clinical manifestations that can be severe in immunocompromised patients.

The aim of our study was to characterize HAdV-species involved in pediatric infection, in order to understand their potential impact on disease progression and to guide towards the selection of therapies based on AdV-specific T-cell response.

**Materials and Methods**: From January to October 2024, 595 new HAdV diagnoses were performed in pediatric patients at the Bambino Gesù Children Hospital (Rome). Among them, 60 positive specimens (53 stool; 7 nasopharyngeal-swabs) were randomly selected for HAdV Whole-genome-sequencing (WGS) using Illumina-MiSeq.

**Results**: Among the 60 selected patients, 45 (75%) were immunocompetent with a median age of 1.4 years (IQR: 0.74–2.61) and 15(25%) were immunocompromised with a median age of 12.5 years (IQR: 4.99–15.85). Most of them (55/60, 91.5%) were hospitalized.

At HAdV diagnosis, gastrointestinal and respiratory symptoms were most prevalent in immunocompetent than immunocompromised patients (91.1% vs 72.7% and 53.3% vs 27.3%, respectively). However, the immunocompromised have shown a longer length of hospitalization and viral-infection (p<0.001).

Overall, the majority of HAdV (63.3%) belonged to species-F (specifically F41), followed by species-C (25%), -A (8.3%) and –B (3.3%). Moreover, a higher prevalence of genotype-F41 was observed in immunocompetent patients (75.6% vs 26.7%; p=0.001). Differently, immunocompromised patients had higher rates of species-A (26.7% vs 2.2%; p=0.012) and species-C (40% vs 20%; p=0.169), the latter already known to cause severe infections in children and immunocompromised patients.

**Conclusions**: Our results showed a different distribution of HAdV-strain between immunocompromised and immunocompetent patients. In particular, species-F was most prevalent in immunocompetent patients with gastrointestinal and respiratory symptoms. Otherwise HAdV species-C and species-A characterized immunocompromised patients. A closer screening and the use of WGS will be essential in immunocompromised setting to anticipate the time of HAdV diagnosis and to improve HAdV surveillance. This study is a starting point to identify which HAdV-genotypes are likely to be more severe, to identify specific genes that make a strain more harmful and to guide selection of an appropriate cell therapy.

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# Hepatitis C Virus Genotype Distribution and the Rate of Reinfection in Slovenia, 2004–2024

Lunar M[1], Mlakar J[1], Skubic L[1], Poljak M[1]

[1]*Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia*

**Background**: The treatment of hepatitis C virus (HCV) has become very effective with the advent of direct-acting antivirals (DAAs). However, complete eradication of HCV remains a major challenge. Although HCV genotyping is no longer mandatory for the use of pan-genotypic DAAs, it is still routinely performed in Slovenia for all patients awaiting treatment according to national consensus. We presented national distribution of HCV genotypes and calculated reinfection rates in Slovenia over 25 years, and discussed remaining challenges.

**Materials and Methods**: Our retrospective observational study analyzed all HCV genotype and HCV RNA data routinely collected in Slovenia between 2004 and 2024. Reinfection was defined as a genotype change or, in cases where the same genotype was detected, confirmed HCV RNA-negativity of at least 12 weeks between two successful genotyping tests.

**Results**: A total of 3,644 HCV genotyping results from samples of 2,807 individuals were included in the study. The majority of patients were men (71.0%) with a median age of 36 years at first HCV RNA-positive result. The predominant genotypes were genotype 1 (1,776, 48.7%) and 3 (1,689, 46.4%), followed by genotype 2 (113; 3.10%), and 4 (55; 1.51%). We also detected genotypes 5 and 6 in one patient each, the 2k/1b recombinant in two patients and coinfection with two genotypes in six patients. Subtype information was available for 1,227/1,776 (69.1%) of the genotype 1 samples, with subtype 1a observed in 64.5%. No major changes in genotype distribution were observed over the years. In 2,160 (77.0%) patients, only a single HCV genotyping test was performed. In the remaining patients, at least two independent samples were genotyped: 498 (17.7%) patients had two, 115 (4.10%) had three, and 34 (1.2%) had ≥four genotyping results. Amongst, reinfection with a different HCV genotype was observed in 88 patients. HCV RNA-negativity of at least 24 and 12 weeks between genotyping results was observed in additional 44 and 18 patients, respectively. Reinfection was thus identified in 5.3% (150/2,807) of patients, corresponding to a reinfection rate of 0.99 per 100 person-years, with reinfection identified after median 6.42±4.0 years.

Furthermore, we observed that reinfection occurred at an additional time point in 9/150 (6.0%) patients. Reinfection was associated with male sex (p=0.0002), younger age at first HCV RNA-positive result (p<0.0001), genotype 3 at first genotyping (p=0.0009), and HIV diagnosis (p=0.0001). HCV reinfection was detected in 11 (21.2%) of the 52 people living with HIV included in this study, six of whom were reinfected with a different genotype. The HIV diagnosis was made at the same time, before or after the HCV diagnosis in 24, 21 and 7 patients, respectively. The last HCV RNA result obtained at least 12 weeks after the last genotype test was available for 2,401 (85.5%) patients and was negative in 2,057 (85.7%), indicating a sustained virological response.

**Conclusions**: Using strict criteria, we observed a reinfection rate of at least 1 per 100 person-years. Efforts should be made to reinforce safe practices in patients with the highest risk behaviors to achieve the goal of HCV elimination.

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# The Role of Type I Interferon on Hepatitis Delta Virus (HDV) Variability in a Superinfected Mouse Model

Pacin-Ruiz B[1,2], Camps Ramón G[3], Gregori J[4], Vales Aranguren Á[5], Olague Micheltorena C[5], Tabernero D[2,4], Rando-Segura A[1,2,6], Riveiro Barciela M[2,7], Buti M[2,7,8], Rodríguez-Frías F[2,9], González-Aseguinolaza G[5], Cortese M[1,2]

*[1]Departament of Microbiology, Liver Unit, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain, [2]Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red, Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain, [3]Clinic University of Navarra (CUN)- CIMA, Pamplona, Spain, [4]Viral Hepatitis laboratory, Liver disease, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain, [5]Gene Therapy and Gene Expression Regulation program, Centro de investigación médica aplicada (CIMA), Pamplona, Spain, [6]Microbiology Department, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain, [7]Department of Hepathology, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain, [8]Universitat autonoma de Barcelona (UAB), Barceloa, Spain, [9]Science Department, International University of Catalunya - UIC, Barcelona, Spain*

**Background**: The hepatitis delta virus (HDV) is the most variable among the hepatitis viruses, mainly due to the loss of fidelity of the cellular RNA polymerase. The cellular enzyme ADAR1 is essential to edit the viral genome allowing the expression of the large isoform of the delta antigen (HDAg). Its expression can also be induced by the intracellular interferon (IFN) pathway. This study aimed to inspect the role of the type I IFN on HDV variability in an in vivo model of hepatitis B (HBV)-transgenic mice (HBVtg) super-infected with HDV and knock-out for the IFNα/β receptor (IFNAR).

**Materials and Methods**: HBVtg and HBVtgxIFNAR-KO mice were injected with 5x1010 viral genomes of adeno-associated vectors carrying the HDV genome (AAV-HDV). The intrahepatic HDV RNA quasispecies (QS) were analyzed in the HDAG coding region (nucleotides 912-1298 in viral genome) through next-generation sequencing (MiSeq Illumina) at 7-, 21-, and 90-days post-injection (dpi). The QS composition was evaluated dividing it in fractions based on the variants' relative frequency, whereas QS variability was studied by analyzing the single nucleotide variations (SNVs) detected in >2 animals. The edition of the viral genome was assessed by considering the frequency of the variants A1012G in viral genome. The groups were compared using Wilcoxon or Kruskal Wallis test.

**Results**: The composition of the HDAG QS was similar between the AAV-HDV superinfected HBVtgxIFNAR-KO and WT mice at 7-dpi. Differently, the proportion of emergent variants (frequency between 1% and the master) showed an upward trend in the next timepoints, especially in WT mice (19.6% and 30% at 21- and 90-dpi, versus 12.8% and 19.8% in KO mice, p-value <0.05). In KO mice, the edition of the viral genome was delayed related to the WT. In both groups, the most dominant changes observed at 7dpi were C-to-T transitions. Differently, at 21dpi we observed mainly A-to-G (potential ADAR1-related changes) and T-to-C transitions. This trend increased at 90dpi, when superinfected WT mice presented around 2-fold more A-to-G mutated positions than KO mice (p-value < 0.05). Among the observed SNVs, 10 were detected in several animals in both groups and at almost all the timepoints, especially the T431C (positions considering the HDAG ORF), whose frequency was higher and growing especially in WT mice (5.6±4.6%, 13.4±5.2% and 15.5±4.8% at 7-, 21- and 90-dpi respectively versus 3.1±3.8%, 9.3±5% and 8.8±3.6% in KO). When considering the SNVs not-shared between the groups, at 21- and 90-dpi the WT mice presented, again, a higher proportion of A-to-G and T-to-C changes than KO mice.

**Conclusions**: The different evolution of the composition of the viral QS between WT and KO HDV-superinfected mice suggests that the IFN may contribute to the intra-hepatic HDV variability. Most of the changes identified were A-to-G transitions, which may have been produced by ADAR1 enzyme. The SNVs repeatedly observed along the time might be indicative of the presence of hotspot of mutations in the HDV genome. Grant PID2021-126447OB-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by ERDF A way of making Europe.

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# Comprehensive Molecular Analysis of the HIV Epidemic in Hungary

Adravecz L[1,2], Zsichla L[2,3], Müller D[2,3,4], Lakatos B[5], Szlávik J[5], Müller V[2,3], Áy É[1,2]

*[1]National Reference Laboratory for Retroviruses, National Center for Public Health and Pharmacy, Budapest, Hungary, [2]National Laboratory for Health Security, ELTE Eötvös Loránd University, Budapest, Hungary, [3]Institute of Biology, ELTE Eötvös Loránd University, Budapest, Hungary, [4]Department of Bioinformatics, Semmelweis University, Budapest, Hungary, [5]National Institute of Hematology and Infectious Diseaes, Central Hospital of Southern Pest, Budapest, Hungary*

**Background**: The remarkable genetic diversity of human immunodeficiency virus (HIV) presents challenges for effective diagnostic, treatment and prevention strategies. Despite the introduction of the "Treat All" WHO recommendation in Hungary in 2016, the annual number of newly diagnosed people living with HIV (PLHIV) has stabilized rather than declining in recent years. Therefore, the aim of our study was to identify the key factors driving the Hungarian HIV epidemic by monitoring HIV-1 subtypes, recombinant forms and drug-resistant variants, as well as analysing transmission clusters.

**Materials and Methods:** Between 2008 and 2024, HIV-1 protease, reverse transcriptase and integrase sequences were obtained from 1146 plasma samples of 1064 newly diagnosed, antiretroviral therapy (ART)-naïve and 61 ART-experienced individuals by Sanger sequencing. HIV subtypes and drug resistance mutations (DRMs) were identified using partial pol sequences. Samples that yielded discordant results during subtyping were subjected to next-generation sequencing and recombination analysis. Transmission clusters and epidemiological links were revealed using phylogenetic and distance-based methods.

**Results**: Although subtype B HIV-1 strains were predominant (72.5%) in the study, non-B subtypes and circulating recombinant forms including F, A, C, G, CRF01_AE and CRF02_AG were also identified. The overall prevalence of transmitted drug resistance (TDR) was 8.9% among newly diagnosed, ART-naïve patients, though not all DRMs were relevant from clinical aspect. The majority of surveillance drug resistance mutations were detected in subtype B HIV-1 strains. The most frequent indicators of TDR were nucleoside reverse transcriptase inhibitor (NRTI)-associated mutations (5.0%), followed by mutations conferring resistance to protease inhibitors (PIs; 1.9%), non-nucleoside reverse transcriptase inhibitors (NNRTIs; 1.4%) and integrase inhibitors (INIs; 0.3%). Amino acid changes conferring resistance to more than one class of antiretroviral drugs were detected in 4 samples. Recombination analysis of 43 near full-length genome sequences revealed structurally diverse patterns of parental subtypes and recombination breakpoints, indicating the presence of possible unique recombinant forms (URFs) in Hungary. B/F1 intersubtype recombinants were observed most frequently (20.9%) with different breakpoints, followed by recombinant HIV-1 strains with complex recombination patterns from at least 9 different parental subtypes. We identified 86 transmission clusters among Hungarian HIV sequences with 7 containing at least 20 sequences. Among these 7 clusters, 4 showed growth in 2023-2024. Clusters containing Hungarian and international subtype B sequences revealed epidemiological links primarily to countries within Western and Central Europe; the Hungarian subtype F clusters are closest to sequences from Romania.

**Conclusions**: Routine genotyping for HIV drug resistance complemented with near full-length next-generation sequencing has allowed a comprehensive characterization of the molecular epidemiology of the HIV epidemic in Hungary, including international links and the tracking of transmitted drug resistance.

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# Impact of the 2020 Health and Social Crisis on Primary HIV Infections: Retrospective Study of Cases Diagnosed at Bordeaux University Hospital, France, from 2018 to 2022.

Mialon F[1], Bellecave P[1], Zemali N[1], Lafon M[1,2], Bonnet F[3], Desclaux A[4], Lazaro E[5,6], Garrigue I[1,2], Burrel S[1,2], Tumiotto C[1,2]

*[1]Service de Virologie, Hôpital Pellegrin, CHU de Bordeaux, Bordeaux, France, [2]CNRS UMR 5234 - Fundamental Microbiology and Pathogenicity, Université de Bordeaux, Bordeaux, France, [3]Service de Médecine Interne - Maladies infectieuses, Hôpital Saint-André, CHU de Bordeaux, Bordeaux, France, [4]Service de Maladies Infectieuses et Tropicales, Hôpital Pellegrin, CHU de Bordeaux, Bordeaux, France, [5]Service de Médecine Interne, Hôpital Haut-Lévêque, CHU de Bordeaux, Bordeaux, France, [6]CNRS Immunoconcept 5164, Université de Bordeaux, Bordeaux, France*

**Background**: The early 2020's were deeply impacted by the covid pandemic, both medically and socially. In France and Europe, the public health emergency, associated with preventive measures such as lockdowns led to a decline in HIV and other sexually transmitted infections (STI) testing. At the same time, mental health was adversely affected, particularly among students. This study aimed to describe and compare the clinical, biological and social characteristics of patients with primary HIV-1 infections (PHIs) before and during the covid pandemic.

**Materials and Methods**: PHIs diagnosed between January 2018 to April 2022 at Bordeaux University Hospital were retrospectively analyzed with two 26-months intervals: pre-pandemic (before March 1, 2020) and during pandemic (from March 1, 2020 onward). Clinical, virological, and social demographics were collected and compared between periods. Moreover, phylogenetic analysis was conducted using HIV-1 reverse transcriptase sequences, to identify potential transmission clusters.

**Results**: A total of 58 PHIs were identified: 25 occurred before March 1, 2020, and 33 afterwards. Social demographics differed, with students being overrepresented during the pandemic (8/33 versus 0/25, p = 0,007). Clinical and biological data were comparable between the groups, with a high prevalence of symptomatic PHI (84 %, overall) and co-occurring STI (50 %, overall), as well as a high HIV-1 RNA viral load (overall median: 6.15 log cop/mL). Phylogenetic analysis revealed that strains belonging to clades B and F1 were responsible for transmission clusters, along with a non-previously described recombinant virus (called "U recombinant virus") that was consistently resistant to rilpivirine (E138A mutation) according to ANRS resistance algorithm (https://hivfrenchresistance.org/). Three viruses showed FTC/3TC resistance (M184V/I), 2 of which were found in patients with a history of pre-exposure prophylaxis (PrEP) using TDF/FTC.

**Conclusions**: Unlike some other infectious diseases, the rate of PHIs did not appear to have declined during the covid pandemic, suggesting that sexual risk behavior persisted despite lockdowns. While clinical and biological data showed no significant differences, we observed an increase of PHI incidence in students diagnosed during the covid pandemic, likely linked to the well-documented deterioration of mental health in this population. The presence of transmission clusters highlights the importance of monitoring genotypic and phylogenetic data. In the course of the study, we identified a novel circulating recombinant form harboring a constitutive rilpivirine resistance associated mutation (under investigation). Continuous monitoring of PHIs may help public health authorities to target preventive interventions on transmission clusters.

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# Profile of Circulating miRNAs in Presence of Hepatitis Delta Virus (HDV) Infection: Difference Between Controllers and Viremic Patients

Pérez Garreta J[1,2,3], Cortese M[1,2], Palom A[2,4], Pacin Ruiz B[1], Tabernero D[1,2,5], Rando Segura A[1,2,6], Vargas Accarino E[2,4], Ruiz Cobo J[2,4], Riveiro Barciela M[2,3,4], Buti M[2,3,4]

*[1]Department of Microbiology, Liver Unit, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Barcelona Hospital Campus, , Barcelona, Spain, [2]Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Madrid, Spain, [3]Universitat Autònoma de Barcelona (UAB), Barcelona, Spain, [4]Department of Hepatology, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain, [5]Liver Unit, Liver Diseases-Viral Hepatitis, Vall d'Hebron Institut de Recerca (VHIR), Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain, [6]Department of Microbiology, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain*

**Background**: The hepatitis delta virus (HDV) causes the most severe form of viral hepatitis, however, some people living with HDV (anti-HDAg-positive) can spontaneously control the viral replication (undetectable HDV-RNA, defined as controllers). The mechanism behind this control remains unknown. The microRNAs (miRNAs) are small RNAs that are involved in a variety of biological processes, including the host's response against viral infection. This study aims to analyze the miRNA profiles of people living with HDV to identify potential biomarkers related to the control of the infection.

**Materials and Methods**: The miRNA profiles were first analyzed by MicroArray (Affymetrix) in an explorative cohort with a plasma sample from 30 people living with HDV, grouped according to the HDV-RNA detectability (15 viremics, and 15 controllers with undetectable HDV-RNA in, at least, two consecutive determinations). The most differentially expressed miRNAs were then quantified through digital PCR (QIAcuity) in the validation cohort, including 54 plasma samples from 18 untreated anti-HDAg individuals (3 longitudinal samples per individual). The cohort included a group of virological controllers with undetectable HDV-RNA (A, n=3) and a group with chronic hepatitis delta (CHD) with detectable HDV-RNA (B, n=15). Group B was further divided based on their ALT levels into: B1 with normal levels (<50, n=6) and B2 with high levels (n=9). The first longitudinal sample from group A was at HDV-RNA detectability. A group of healthy donors (HD) was used as a control (n=7).

**Results**: Six differentially expressed miRNAs (log2FC >1, raw p-value <0.05, and mean expression >2) were identified during the explorative study: miR-122-5p, miR-192-5p, miR-194-5p, miR-23b-3p, miR-26a-5p and miR-4530. Two patterns of expression were identified: miR-122-5p+ miR-192-5p+miR-194-5p and miR-23b-3p+miR-26a-5p. In the validation study, the liver-enriched miRNAs (miR-122-5p, miR-192-5p and miR-194-5p) were downregulated in controllers (group A) and viremic patients with normal ALT levels (B1) in comparison to viremic with chronic hepatitis delta (B2). Differently, miR-23b-3p and miR-26a-5p were generally downregulated in all the anti-HDAg-positive groups compared to the HD, especially in the controller's group in all the longitudinal samples (adjusted p-value <0.05).

**Conclusions**: People living with HDV who achieved the control of viral replication exhibited a downregulated miRNA profile compared to those with persistently detectable HDV-RNA, being potential biomarkers of virological control. Further studies are needed to determine the mechanism behind this dysregulation and the role of the miRNAs in HDV replication. Study supported by the projects PI20/01692 and PI23/01065, funded by Instituto de Salud Carlos III and co-funded by the European Union (ERDF, "A way to make Europe").

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# ProLINK-C: A Novel Opportunistic Strategy to Re-engage HCV Patients without Treatment Linkage

Vázquez Blanquiño A[1], Illescas M[1,4], Fuentes A[1,4,5], Franco Álvarez de Luna F[2], Pérez A[3,5], Morales C[3], Alberola Romano A[1], Pérez Rodríguez L[1,4], Chaves Blanco L[1,4], García F[1,4,5]

*[1]Hospital Universitario Clínico San Cecilio, Granada, España, [2]Hospital Universitario Juan Ramón Jiménez, Huelva, Spain, [3]Hospital Universitario Reina Sofía, Córdoba, Spain, [4]Instituto de Investigación biosanitaria Ibs.Granada, Granada, Spain, [5]CIBER de Enfermedad Infecciosas (CIBERINFEC), Madrid, Spain*

**Background**: The World Health Organization (WHO) recognizes hepatitis C as a major public health concern. In Spain, identifying and re-engaging lost patients within the healthcare system is considered a priority strategy for achieving elimination. Retrospective approaches have generally shown low effectiveness in patient re-engagement. In this study, we present the results of an "opportunistic" rescue strategy targeting patients with chronic hepatitis C who were not linked to treatment.

**Materials and Methods**: We conducted a prospective, multicenter study to assess the feasibility and effectiveness of an "opportunistic" rescue strategy for patients with previously diagnosed hepatitis C virus (HCV) infection who had no documented evidence of treatment or sustained virologic response (SVR). An automated process was implemented within different laboratory information systems (LIS) used across participating centers to generate daily lists of patients with a prior positive anti-HCV antibody (anti-HCV Ab) result and an available serum sample collected for any other clinical reason. Analytical history and electronic medical records (EMRs) were reviewed daily to determine whether patients had undergone treatment and/or achieved SVR. For patients without documented evidence of SVR, HCV RNA testing (viral load) was performed to assess active infection status.

**Results**: Between May 2023 and January 2025, a total of 3,113 patients with anti-HCV Ab positivity and available serum samples were identified. After LIS/EMR review, 90% (2,982/3,313) were found to have achieved SVR or spontaneous viral clearance, with no identified risk factors for HCV reinfection. Among the remaining 331 patients, HCV RNA testing was performed in 319 (12 could not be processed due to insufficient sample volume), yielding 108 positive cases (3.5% of the total and 33% of those tested). Of these, 70% were male, and the median age was 61 years (IQR 55-67). The primary reasons for viral load testing were: (i) 47% of cases had no record of achieving SVR and were not in clinical follow-up; (ii) 34% lacked a previous viral load test to determine their viremia status; and (iii) 19% had risk factors such as severe mental disorders or current/past intravenous drug use.

All viremic patients were reported to their requesting physician and the designated clinical specialist for treatment evaluation. To date, 70 patients (65%) have been scheduled for follow-up, with 40 (57%) initiating antiviral treatment. Ten patients declined treatment due to advanced age and comorbidities, three refused without a specific reason, four are awaiting consultation, and 13 missed their appointment but were rescheduled for further evaluation.

**Conclusions**: ProLINK-C is an effective opportunistic rescue strategy for identifying and reintegrating patients with active hepatitis C who are not linked to antiviral treatment. Since these patients are already engaged with the healthcare system for other reasons, reintegration into the hepatitis C care cascade is more straightforward and efficient. We recommend the implementation of this strategy as a complementary tool in efforts to achieve hepatitis C elimination.

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# Optimizing a HIV-1 Genotypic Resistance Assay for Diverse Subtypes and Illumina Sequencing

Fonager J[1], Willadsen L[1], Rasmussen M[1]

[1]Statens Serum Institut, 2300 Copenhagen S, Denmark

**Background**: The distribution of HIV-1 subtypes in Denmark has shifted significantly, especially among individuals infected abroad. Between 2022 and 2024, the proportion of non-B subtypes in this group increased from 59% to 85%, while those infected within Denmark saw a rise from 60% to 69%. Additionally, the prevalence of undefined recombinant strains among those infected abroad grew from 0% in 2022 to 18% in 2024. These changes stem from shifting migration patterns and declining transmission within the MSM group, which has historically been infected with subtype B. This evolving subtype distribution presents challenges for genotypic resistance assays, which are primarily designed for subtype B.

**Materials and Methods**: To address this, we designed new PCR and sequencing primers (RES24) based on the 2023 Los Alamos HIV subtype reference set, targeting mutations from Protease codon 1 to Reverse transcriptase codon 348. Apart from enhancing the assay´s subtype range, our aim was also to transition from Sanger sequencing to an Illumina-based approach for enhanced efficiency. We tested the RES24 primers on 10 non-subtype B samples from newly diagnosed patients previously analyzed with in-house assays (RES14/INHOUSE).

Amplicons were sequenced using both Sanger and Illumina methods. Sanger chromatograms were inspected manually, while Illumina reads were trimmed to exclude ambiguous bases and mapped to the HXB2 reference. Variants were incorporated into consensus sequences at cut-offs of 10%, 15%, and 20% using the CLCbio analysis platform. Both sequencing methods were analyzed in HIVdb for quality control (QC) and drug resistance mutation (DRM) identification.

**Results**: With the RES24 primers, 9 of 10 samples tested positive, with the exception of one sample with a viral load of log10 2.80 copies/mL, below the assay's normal threshold of log10 3.00 copies/mL. This performance was comparable to results from the RES14/INHOUSE sets. Notably, an A6 subtype sample with a viral load of log10 4.93 copies/mL, previously difficult to amplify, was successfully amplified with RES24.

Comparison of Sanger and Illumina consensus sequences revealed identical DRM profiles across all cut-off levels. Among other protease and reverse transcriptase mutations detected with Sanger, 99.7%, 99.1%, and 98.6% were also found in Illumina sequences at 10%, 15%, and 20% cut-offs, respectively. Additional minority variants (MV) averaged 2.1, 1.6, and 1.2 per sequence at the respective cut-off levels. Sequence QC issues, including frameshifts and stop codons, ranged from an average of 0.2 per sequence in Sanger to 0.7 at the 10% cut-off. Furthermore, 98% of mutations detected in Sanger-sequenced RES24 amplicons were also found in RES14/INHOUSE amplicons, with discrepancies attributed to MV.

**Conclusions**: We successfully designed primers for efficient amplification of non-subtype B sequences and evaluated Illumina sequencing for resistance prediction. Identical DRM profiles were observed across methods. The 10% cut-off level yielded the highest similarity to Sanger sequencing but also detected the most MV and had more QC issues. These findings support the use of Illumina sequencing for enhanced detection of resistance mutations in diverse HIV-1 subtypes.

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# Automated, Large-Scale and Secure Bioinformatics for Comprehensive Analysis of Viral Genomes Using MicrobioChek

Mohamed S[1], Porzio J[2], Reffay A[2], kolsch J[2], Léonard J[2], Sayada C[2], Errafyqy M[1], Boulmé R[1], Modol A[3], Deblir L[2]

*[1]Abl Diagnostics, Woippy, France, [2]ABL SA, Luxembourg, , [3]ABL, ,*

**Background**: High-throughput sequencing technologies are revolutionising viral genome analysis, enabling the comprehensive characterisation of viral pathogens such as HIV, RSV, and SARS-CoV-2. Efficient bioinformatics pipelines are essential for accurate genome assembly, variant calling, and evolutionary analysis, especially when dealing with large datasets.

**Materials and Methods**: In this study, we used a robust bioinformatics framework (MicrobioChek, ABL SA, Luxembourg) designed to handle any type of whole genome analysis such as HIV, RSV, and SARS-CoV-2.. The software platform integrates automated data processing workflows, using state-of-the-art tools for quality control, sequence alignment, and variant detection. The platform can handle CE-IVD marked or Research Use Only (RUO) bioinformatics pipelines. Internationally recognized expertise and curated databases such as Stanford, ANRS, Rega, and Geno2pheno were incorporated for HIV-specific variant analysis, ensuring accurate, repeatable and up-to-date results. Regular updates to the databases are performed automatically without the need for laboratory intervention, providing real-time information on known mutations. Qualification and validation of the bioinformatics pipelines and updates are part of the software support. All analyses were performed using cloud-based infrastructure in compliance with data protection and following strict data security standards such as the mandatory French certification for storage and processing of health data (certification HDS, “Hébergeur de Données de Santé”).

**Results**: MicrobioChek platform successfully processed large datasets, providing high-resolution genome sequences for HIV, RSV, and SARS-CoV-2. The platform’s ease of use allowed for rapid deployment in a large-scale environment, enabling real-time data analysis. The software also generated analysis and interpretation reports that summarised key findings in a clear and actionable format. We successfully identified several HIV-1 subtypes and mutations of interest. In addition, clades were accurately detected for both SARS-CoV-2 and RSV, highlighting the robustness of the platform in identifying viral diversity. Quality control steps ensured the accuracy and reliability of the results, with variant calling achieving high sensitivity and specificity. In addition, the cloud environment allows easy scaling up of the number of analyses to be performed in parallel, always with secure data handling in compliance with the most stringent health data protection regulation.

**Conclusions**: The bioinformatics pipelines developed for viral genome analysis demonstrate high efficiency, scalability, and ease of use, making them well-suited for large-scale applications. By integrating internationally recognised and regularly updated databases, the MicrobioChek platform ensures accurate and up-to-date variant analysis without laboratory intervention in an accredited environment. With robust security features and streamlined data processing, it provides a reliable solution for genomic surveillance and infectious disease research, while meeting stringent privacy requirements.

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# Acceptability and Interpretability of Multiplex Self-Tests for HIV, Hepatitis B and C by Key Populations and Healthcare Workers

Marbán-Castro E[1], Nikitin D[2], Thomas C[3], Bessonov S[4], Thomas C[5], Bessonova A[6], Mone Iye C[5], Japaridze M[1], Watson M[1], Denisiuk O[1], Shilton S[1]

*[1]FIND, Geneva, Switzerland, [2]Global Research Institute Foundation (GLORI), Bishkek, Kyrgyzstan, [3]University of Jakarta International, Jakarta, Indonesia, [4]Attika Harm Reduction NGO, Bishkek, Kyrgyzstan, [5]Yayasan Peduli Hati Bangsa, Jakarta, Indonesia, [6]Women's Network of Key Communities, Bishkek, Kyrgyzstan*

**Background**: Human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most common chronic viral infections globally, sharing similar transmission routes including sexual, blood contact, and injecting drug use. This study investigates the feasibility, acceptability, preferences and interpretability regarding multiplex self-tests (STs) for HIV and hepatitis among target end-users (people who inject drugs and healthcare workers).

**Materials and Methods**: This was a prospective multi-country mixed-methods study, conducted in Indonesia and Kyrgyzstan. This study employed several methodologies: 1) focus group discussions (FGDs) and semi-structured interviews (SSIs) were performed to obtain general perceptions and theoretical acceptability on multiplex ST, 2) cognitive interviews were performed to optimize instructions for use, and 3) interpretability assessments were conducted to understand participants accuracy in interpreting multiplex ST results (contrived results sent by manufacturers). For the interpretability, participants were given mock ST devices, no testing was conducted. Four different manufacturers were used; thus, results could not be compared across countries.

**Results**: In Kyrgyzstan, 56 participants took part in the qualitative data collection (27 PWIDs, 24 HCWs, 5 stakeholders), and 54 in Indonesia (24 PWIDs, 24 HCWs, 6 stakeholders). Participants valued self-testing (ST) for its convenience and privacy, showing interest in multiplex ST for screening multiple infections. Cognitive interviews with 60 participants per country (30 PWIDs, 30 HCWs) led to recommendations for improving instructions for use (IFU), including larger fonts, simplified language, clearer explanations of control lines, and proper waste disposal guidance. Video instructions were suggested to enhance understanding and provide follow-up steps. For interpretability, 222 participants were involved in each country (118 PWIDs, 104 HCWs).
In Kyrgyzstan, both PWID and HCWs demonstrated high accuracy in interpreting multiplex Dual and Triple ST results. Among PWID, 90.0% of Dual and 88.6% of Triple ST results were correctly interpreted, while HCWs showed slightly higher accuracy with 89.5% for Dual and 96.7% for Triple STs. In contrast, interpretation accuracy in Indonesia was lower, particularly for Triple STs. PWID correctly interpreted 76.5% of Dual and 42.7% of Triple ST results, and HCWs achieved 93.0% accuracy for Dual and 61.9% for Triple STs. The results that were worse interpreted were the ST containing faint positive lines and invalid results.

**Conclusions**: Our study demonstrates initial acceptability of multiplex STs for HIV and viral hepatitis among PWID, HCWs, and stakeholders. However, communication strategies are needed to increase buy-in, particularly as blood-based tests were the least preferred. Integration into existing services (e.g., STI clinics, antenatal care) alongside pharmacy and community-based distribution could enhance access. Peer-based counseling and tailored IFUs, including video instructions, may improve user experience and uptake. While result interpretation was high in Kyrgyzstan, lower accuracy in Indonesia highlights the need for targeted education. Further research is needed to understand barriers in Indonesia and expand testing models across diverse populations.

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# Evaluation of the Interoperability Between Two- and Three-Genes Assays for SARS-CoV-2 Molecular Diagnosis in Cameroon: Implications for Pandemic Preparedness

Djupsa Ndjeyep S[1], Kengni Ngueko A[1,2], Ngoufack Jagni Semengue E[1], Nka A[1], Chenwi Ambe C[1,2], Ka'e A[1], Bouba Y[1,3], Molimbou E[1,2], Etame N[1,2], Tekoh T[1], Nayang Mundo R[1], Tueguem P[1], Mekel V[1,2], Mba H[1], Abega Abega C[1], Takou D[1], Angong Beloumou G[1,2], Tommo Tchouaket M[1], Moko Fotso L[1], Gouissi Anguechia D[1], Tambe Ayuk D[1], Ngongang Ouankou C[4], Alteri C[5,6], Colagrossi L[6], Boum II Y[7], Eddy Ekane H[8], Njamnshi A[9], Ceccherini-Silberstein F[2], Colizzi V[1,2,10], Ndembi [11], Ndjolo A[1], Perno C[1,6], Santoro MM[2], Fokam J[1,8,12,13]

*[1]Chantal BIYA International Reference Centre for research on HIV/AIDS prevention and management (CIRCB), Yaoundé, Cameroon., Yaoundé, Cameroon, [2]University of Rome "Tor Vergata", Rome, Italy;, Rome, Italy, [3]Faculty of Medicine, UniCamillus—Saint Camillus International University of Health Sciences, Rome, Italy, Rome, Italy, [4]Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon, Dschang, Cameroon, [5]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy;, Milan, Italy, [6]Multimodal research area, Microbiology and Diagnostics of Immunology Unit, IRCCS Bambino Gesu Pediatric Hospital, Rome, Italy;, Rome, Italy, [7]COVID-19 National Public Health Emergency Operations Coordination Centre, Yaounde, Cameroon;, Yaounde, , Cameroon, [8]Faculty of Health Sciences, University of Buea, Cameroon;, Buea , Cameroon, [9]Brain Research Africa Initiative (BRAIN), Yaoundé, / Neuroscience Lab. Faculty of Medicine and Biomedical Sciences, The University of Yaoundé I, Yaoundé, Cameroon;, Yaoundé,, Cameroon, [10]Evangelical University of Cameroon, Bandjoun, Cameroon;, Bandjoun, Cameroon, [11]Africa Centres for Disease Control and Prevention, Addis Abeba, Ethiopia;, Addis Abeba, Ethiopia, [12]National AIDS Control Committee (NACC), Yaoundé, Cameroon;, Yaoundé,, Cameroon, [13]COVID-19 National Public Health Emergency Operations Coordination Centre, Yaounde, Cameroon., Yaounde , Cameroon*

**Background**: The scale-up of molecular assays for the accurate diagnosis of pathogens have increased in resource limited settings since the advent of COVID-19. This study aimed to compare the performances for SARS-CoV-2 diagnosis on three different platforms in Cameroon.

**Materials and Methods**: For this comparative study, nasopharyngeal samples were collected (June-2021 to May-2023) from the biobank of the Chantal Biya International Reference Centre (CIRCB) in Yaounde-Cameroon and analyzed simultaneously with DaAn gene (targeting N and ORF1ab genes), Thermofisher (targeting N, ORF1ab and S genes), and GeneXpert rRT-PCR (targeting N2 and E genes). Positivity cycle thresholds (CT) followed our national guidelines i.e. CT<37 for DaAn gene and Thermofisher and CT<40 for GeneXpert. Diagnosis performances were evaluated by the Cohen's Kappa coefficient with DaAn gene as the gold standard.

**Results**: Were included, 249 samples; 55.8% (139/249) from males, median age [IQR] 36 [27–50] years and 21% (53/249) symptomatic participants. Overall positivity was 22.1% (55/249) 53.4% (133/249), and 55.0% (137/249) with median CT of 26.60 [21.27–30.99], 32.79 [26.94–36.10], and 30.65 [23.10–35.50] for Thermofisher, DaAn gene, and GeneXpert respectively. Overall agreement with DaAn gene was 68% (169/249) with k=0.38 (95%CI:0.29–0.47) and 92% (229/249), with k=0.66 (95%CI:0.57–0.75) for Thermofisher and GeneXpert respectively. Positive and negative agreements with DaAn gene were 41.3% (55/133) and 98.2% (114/116) for Thermofisher and 84.96% (113/133) and 81.3% (94/116) for GeneXpert. Interestingly, the positive agreement with DaAn gene was 93.3% (14/15) for Thermofisher and 100% (15/15) for GeneXpert at high viral load (i.e. CT<20).

**Conclusions**: We herein highlight high agreement between two- and three-genes assays for the molecular diagnosis of SARS-CoV-2 at CT<20, suggesting interoperability of these PCR platforms to detect active and transmissible cases at community-level. This is crucial to guide decision-making for effective public health response nationwide and even beyond.

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# Automated Reflex HIV Screening Based on Diagnostic Indicators: A New Perspective from Clinical Microbiology

Vázquez Blanquiño A[1], Illescas M[1,6], Chaves Blanco L[1,6], Alberola Romano A[1], Franco Álvarez de Luna F[2], Freyre C[3], García P[3], Gómez M[4], Becerril B[4], Mazuelas P[5], García M[5], García F[1], García F[1,6,7]

*[1]Hospital Universitario Clínico San Cecilio, Granada, Spain, [2]Hospital Universitario Juan Ramón Jiménez, Huelva, Spain, [3]Hospital Universitario de Puerto Real, Puerto Real, Spain, [4]Hospital Universitario de Jerez de la Frontera, Jerez de la Frontera, Spain, [5]Hospital Universitario Virgen de la Victoria, Málaga, Spain, [6]Instituto de Investigación Biosanitaria Ibs.Granada, Granada, Spain, [7]CIBER de Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain*

**Background**: Early HIV diagnosis remains a critical global challenge, as delayed detection leads to late-stage diagnoses and continued transmission. Routine laboratory workflows can integrate automated reflex testing to address missed diagnostic opportunities. This study aimed to evaluate the feasibility and effectiveness of a pilot automated HIV screening system triggered by diagnostic tests . The protocol ensures systematic patient consent.

**Materials and Methods**: This prospective pilot study is being conducted between November 2023 and February 2025 across five hospitals in southern Spain. The study implemented an automated system integrating HIV screening into routine laboratory workflows, ensuring informed consent through the following steps: a) Physicians record consent in the electronic system; b) HIV test details are included on the laboratory test requisition form; c) patients can withdraw consent before sample collection.

For patients aged 18–65 years, the system is triggered when physicians request predefined "trigger tests" (TTs), including pneumococcal/Legionella urinary antigen testing, sexually transmitted infection panels, syphilis or hepatitis C serology, or drug-of-abuse urine screening. Whenever a TT is ordered, an automated notification prompts the physician to request patient consent for an additional HIV test alongside the primary diagnostic tests.

**Results**: Through the implementation of this automated reflex HIV screening system, a total of 9,551 HIV tests were conducted in cases where the ordering physician had not initially requested an HIV test. As a result, 10 individuals living with HIV (0.104%) who would have otherwise remained undiagnosed were identified. Among them, eight were new diagnoses—people who were unaware of their HIV status until this screening—and two were cases of individuals who had already been diagnosed in the past but had been lost to follow-up. Thanks to this system, these two patients were able to re-engage with medical care and resume appropriate follow-up and treatment.

The identified cases included seven men and three women, ranging in age from 33 to 63 years. In most cases (7 out of 10), the HIV test was triggered by a single diagnostic test, while in the remaining three cases, multiple trigger tests were responsible for initiating the screening. The most frequently associated trigger test was Hepatitis C, present in four cases, followed by syphilis and urinary antigen testing (for pneumococcus or Legionella), both found in three cases.

**Conclusions:** This study presents the utility of automated reflex screening in hospital settings. By identifying 10 undiagnosed cases, including eight new diagnoses, this approach improved early detection and facilitated patient linkage to care. The strong association with hepatitis C, syphilis, and urinary antigen tests reinforces their value as screening triggers. These findings support the integration of reflex HIV testing as an efficient strategy to reduce missed diagnoses and enhance public health outcomes.

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# Impact of Misconceptions on Discriminatory Attitudes Towards People Living with HIV/AIDS in Sub-Saharan Africa: Evidence from DHS Data (2003–2023)

Effiong F[1], Mohammed A[2], Ibiriogba D[3], Adewole D[4]

*[1]University Of Calabar Teaching Hospital (UCTH), Calabar, Nigeria, [2]APIN Public Health Initiatives, Jos, Nigeria, [3]Department of Public Health, Amrita Institute of Medical Sciences, Kochi, India, [4]Department of Health Policy and Management College of Medicine, University of Ibadan, Ibadan, Nigeria*

**Background**: Stigma and discrimination undermine the health and well-being of people living with HIV/AIDS (PLWHIV), often fueled by persistent misconceptions about HIV transmission. This study explores the role of witnessing HIV-related stigmatization and the belief in foodborne transmission in shaping discriminatory attitudes among women aged 15–49 years in HIV-endemic Sub-Saharan African (SSA) countries.

**Materials and Methods**: Data were drawn from Demographic and Health Surveys (DHS) conducted between 2003 and 2023 across seven SSA countries with HIV prevalence exceeding 10% (based on 2017 data). Discriminatory attitudes were measured using three standard indicators. Independent variables included witnessing HIV-related stigma and belief in foodborne transmission. Countries without these data were excluded. Descriptive, bivariate, and multivariate analyses were performed using SPSS version 25 to assess the predictors of discriminatory attitudes.

**Results**: The final sample comprised 57,325 women across seven countries. Discriminatory attitudes towards PLWHIV varied significantly, from 33.7% in Eswatini to 72.4% in Tanzania. Factors such as being young, unmarried, uneducated, living in rural areas, or belonging to the poorest wealth quintile were associated with higher odds of discrimination. Witnessing HIV-related stigma significantly increased discriminatory attitudes in Uganda (adjusted odds ratio [aOR]: 1.49; 95% CI: 1.34–1.65). Additionally, belief in foodborne HIV transmission was a strong predictor of discrimination in several countries, including Zimbabwe (aOR: 3.42; 95% CI: 2.92–4.01), Zambia (aOR: 3.46; 95% CI: 2.93–4.09), Lesotho (aOR: 4.56; 95% CI: 3.97–5.24), and Tanzania (aOR: 2.80; 95% CI: 2.42–3.27).

**Conclusions**: This study highlights the profound influence of stigma and misconceptions on discriminatory attitudes towards PLWHIV in SSA. Witnessing stigmatization and false beliefs about HIV transmission, such as through food, exacerbate discrimination, disproportionately affecting vulnerable populations. Comprehensive education campaigns addressing these myths, coupled with community-based anti-stigma interventions, are critical to fostering a supportive environment for PLWHIV and improving public health outcomes. By challenging misinformation and promoting social inclusivity, these efforts could transform HIV-related stigma in SSA.

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# The DePASS Initiative in Healthcare Access Units: Do not Miss the Chance to Detect HIV, HBV, and HCV

Todesco E[1], Lambert-Niclot S[2], Maylin S[3], Scanvion A[4], André Garnier E[5], Leroy P[6], Benez Pariente K[7], Saune Sandres K[8], Berthier M[9], Domenech Icard V[10], Lovery H[11], Parmeland L[12], Elmouden M[13], Vermeulen H[14], Soulet De Brugiere L[15], Scholtes C[16], Larrat S[17], Toussaint C[18], Adami B[19], De Monte A[20], Fartaoui N[21], Alloui C[22], Durand F[23], Poupon-Ferron S[23], Bui E[24], Aparicio C[25]

*[1]Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et de Santé Publique (iPLESP), Assistance Publique-Hôpitaux de Paris, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière - Charles Foix, Laboratoire de Virologie, F-75013 Paris, France, Paris, France, [2]Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique (iPLESP), Assistance Publique-Hôpitaux de Paris, APHP, Hôpital Saint-Antoine, Département de Virologie, Paris, France., Paris, France, [3]Laboratoire de Virologie, AP-HP, Hôpital Saint Louis, Paris, France, Paris, France, [4]CHU de Nantes, Hôtel Dieu, Service de Médecine Interne, Permanence d'Accès aux Soins de Santé, Nantes, France., Nantes, France, [5]CHU de Nantes, Virology Department, Nantes, France., Nantes, France, [6]Groupe hospitalier Sud Île-de-France, Site Melun-Sénart, Melun, France., Melun, France, [7]CHU de Toulouse, Hôpital La Grave, Permanence d'Accès aux Soins de Santé, Toulouse, France., Toulouse, France, [8]CHU de Toulouse, Institut Fédéral de Biologie de Purpan, Département de Virologie, Toulouse, France., Toulouse, France, [9]Hospices Civils de Lyon, GH Centre, Service Permanence d'Accès aux Soins de Santé, Lyon, France. , Lyon, France, [10]Hospices Civils de Lyon, Hôpital de la Croix-Rousse, Department of Virology, Institut des Agents Infectieux, Centre National de Référence des Virus des Infections Respiratoires, Lyon, France. , Lyon, France, [11]Centre hospitalier Saint Joseph - Saint Luc, Permanence d'Accès aux Soins de Santé, Lyon, France, Lyon, France, [12]Centre hospitalier Saint-Joseph - Saint-Luc, Laboratoire de biologie, Lyon, France., Lyon, France, [13]Centre Hospitalier du Dr Jean Eric TECHER, Permanence d'Accès aux Soins de Santé, Calais, France., Calais, France, [14]Centre Hospitalier de Boulogne-sur-Mer, Boulogne-sur-Mer, France. , Boulogne-sur-Mer, France, [15]Hôpital Le Vinatier, Permanence d'Accès aux Soins et à la Santé, Bron, France. , Bron, France, [16]Virology Department, Hospices Civils de Lyon (HCL) and Université Claude-Bernard Lyon 1 (UCBL1), Lyon, France; INSERM U1052, CNRS UMR-5286, Cancer Research Center of Lyon (CRCL), Lyon, France; University of Lyon, Université Claude-Bernard (UCBL), Lyon, France; The Lyon Hepatology Institute (IHU EVEREST), Lyon, France. , Lyon, France, [17]CHU Grenoble Alpes, Institut de Biologie-Pathologie, Laboratoire de Virologie, Grenoble, France. , Grenoble, France, [18]CHU Grenoble Alpes, Permanence d'Accès aux Soins de Santé, Grenoble, France. , Grenoble, France, [19]CHU de Nice, Hôpital Pasteur, Permanence d'Accès aux Soins de Santé, Nice, France., Nice, France, [20]CHU de Nice, Laboratoire de Virologie, Nice, France., Nice, France, [21]Assistance Publique-Hôpitaux de Paris, AP-HP, Hôpital Avicenne, Permanence d'Accès aux Soins de Santé, Bobigny, France. , Bobigny, France, [22]Assistance Publique-Hôpitaux de Paris, AP-HP, Hôpital Avicenne, Laboratoire de Virologie, Bobigny, France., Bobigny, France, [23]Gilead Sciences S.A.S, 65 Quai Georges Gorse, 92100 Boulogne-Billancourt, France, Boulogne-Billancourt, France, [24]Assistance Publique-Hôpitaux de Paris, AP-HP, Hôpital Saint Antoine, Policlinique Médicale - Permanence d'Accès aux Soins de Santé, F-75012 Paris, France, Paris, France, [25]Assistance Publique-Hôpitaux de Paris, AP-HP, Hôpital Lariboisière, Policlinique Médicale - Permanence d'Accès aux Soins de Santé, Paris F-75010, France., Paris, France*

**Background**: HIV, hepatitis B (HBV), and hepatitis C (HCV) infections represent major public health concerns, especially among vulnerable populations such as individuals without healthcare insurance, who often go undiagnosed. However, systematic testing for HIV and viral hepatitis in medico-social structures for precarious patients remains inconsistent.

In this context, the DePASS initiative aimed to raise awareness among healthcare professionals working in healthcare access units about the importance of HIV/HBV/HCV systematic screening, assess the prevalence of these infections in these settings, and identify barriers and facilitators in implementing routine HIV/HBV/HCV testing in these centers.

**Materials and Methods**: Between December 2022 and May 2024, a nine-month screening campaign was conducted in 12 hospital-based healthcare access units across metropolitan France. Staff meetings were organized in each unit before and during the campaign to promote HIV/HBV/HCV testing, plus a final one after the center concluded the campaign.

All individuals attending these healthcare access units were offered testing using a 4th generation HIV ELISA test, HBsAg (HBV surface antigen), and total anti-HCV antibodies. For those testing positive for HBsAg, an additional hepatitis Delta virus (HDV) serological screening was performed. A healthcare workers (HCWs) survey was also conducted to gather their opinions and practices regarding the screening process and the campaign.

**Results**: Among 6,602 individuals enrolled, seroprevalence rates were 1.8% for HIV, 2.3% for HCV, and 5.8% for HBV, with 6.5% of HBsAg-positive individuals co-infected with HDV. Post-diagnosis follow-up was initiated for 90.3% of HIV-positive patients.

Of the HCWs surveyed (6 responding centers, N=45), 33% were medical doctors, 42% nurses and 11% social workers. Prior to the campaign, 18% of

professionals surveyed estimated that 80-100% of patients were tested for HIV/HBV/HCV. This figure almost tripled after 9 months of the DePASS initiative, to 51%, and 91% of the HCWs considered the campaign useful.

Nevertheless, 62% of them reported challenges finding sufficient time to conduct the screenings. When prescribed, a significant portion of individuals were referred to external facilities for blood sampling; only 33% of the HCWs performed 80-100% of the sampling on site. Additionally, 24% of HCWs felt uncomfortable discussing testing with patients.

**Conclusions**: Healthcare centers for vulnerable people play a crucial role in detecting undiagnosed infections and in initiating patient care. With seroprevalence rates from 7 to 9 times higher compared to the French general population, these results highlight the importance of systematically offering testing in this population. It is also an opportunity not to be missed to talk about prevention of these infections.

Despite its feasibility and perceived usefulness, barriers such as time constraints and provider discomfort need to be addressed. Strengthening healthcare provider training, improving on-site testing capacity, and integrating systematic screening into routine are essential to enhance early diagnosis and linkage to care for vulnerable populations.

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# An Audit of Cases of Vertical HIV Transmission in Programmatic Settings in Malawi

Chikuse E[1], Balakasi K[1], Smith D[1,2], Phiri S[1,3], Njala J[1], Phiri K[1], van Oosterhout J[1,2]

*[1]Partners in Hope Malawi, Lilongwe, Malawi, [2]University of California Los Angeles, David Geffen School of Medicine,, Los Angels, USA, [3]School of Global and Public Health, Kamuzu University of Health Sciences, Lilongwe, Malawi*

**Background**: Major improvements in the prevention of vertical HIV transmission (PVT) have taken place in sub-Saharan Africa. To identify remaining gaps in PVT program implementation, we reviewed PVT services among Malawian pregnant and breastfeeding women (PBFW), whose infants tested HIV-positive between 0-24 months post-partum.

**Materials and Methods**: At 123 Partners in Hope (PEPFAR/USAID clinical implementing partner)-supported health facilities in 9 Malawian districts across, we conducted a clinical records audit of mother-infant pairs of which the infant tested HIV-positive between October 2022-September 2023. Routinely collected data were extracted from registers, treatment charts and electronic monitoring systems. We used summary statistics to describe antenatal care (ANC) attendance, HIV testing, pre-exposure prophylaxis (PrEP), ART initiation, interruptions in treatment (IIT), viral load (VL) monitoring, and antiretroviral prophylaxis for HIV-exposed infants.

**Results**: During the audit period, 138 mothers with 138 new HIV-positive infants were identified. 51% were diagnosed at the 2-months testing milestone, 30% at 12 months and 19% at 24 months. The following gaps were observed in PVT services: 52% of mothers did not attend ANC; 42% were not HIV tested in the third trimester while eligible; among women who tested HIV-negative and categorized as high risk, 89% were not offered PrEP; 36% of women had started ART before pregnancy, 24% during pregnancy, 8% during labour/delivery and 32% during breastfeeding; 36% of women on ART experienced IIT (>28 days late for appointment) during pregnancy and breastfeeding; 40% of mothers newly initiated on ART did not undergo VL testing when indicated; among infants eligible for antiretroviral prophylaxis, 18% received no prophylaxis and 42% did not receive the recommended type of prophylaxis.

**Conclusions**: A broad spectrum of gaps in PVT services contributed to cases of vertical HIV transmission in programmatic settings. Ensuring comprehensive PVT services for high-risk mother-infant pairs requires innovative interventions that need to be tested in implementation research trials.

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# Assessment of Machine Learning in HIV Prediction and Prevention Services in Key and Vulnerable Population Nairobi, Kenya

Mirera I[1]

[1]Partners For Health & Development In Africa, Nairobi , Kenya

**Background**: Machine learning (ML) has proven effective in predicting HIV risk and identifying individuals most suitable for HIV testing and prevention. In Kenya, UNAIDS (2022) reports that key populations, including men who have sex with men, sex workers, and people who inject drugs, are 55% more likely to be infected with HIV. NSDCC(2023) report indicates a 5.6% HIV prevalence among Key population groups in Nairobi, highlighting the need for targeted interventions. ML can help detect high-risk individuals, enabling personalized healthcare strategies and improving service delivery to enhance health outcomes for vulnerable populations.

The main Aim of this study is to determine utilization of Kenya EMR machine learning model For Provision of Prevention Services Among Key and Vulnerable populations in sex workers outreach program clinics in Nairobi Kenya.

**Materials and Methods**: A retrospective case study design was implemented from October 2023 to September 2024, focusing on sex workers and men who have sex with men. A total of 9 out of 10 clinics were selected for sampling. Risk scores were categorized, and key indicators important for HIV prevention were assessed, including the number of sexual partners, risky behaviors, substance abuse, and instances of sexual gender-based violence.

**Results**: The HTS screening tool assessed 28,068 sex workers. The Kenya EMR machine learning model predicted the following risk categories: 7% (2,018) were very high risk, 15% (4,297) were high risk, 39% (10,808) were moderate risk, and 39% (10,853) were low risk. Among those identified as very high risk, 87% (1,695) were linked to prevention services, 86% (3,646) from the high-risk group, 69% (7,360) from the moderate-risk group, and 64% (6,854) from the low-risk group received services such as PrEP, PEP, risk reduction counselling and condom promotion.

**Conclusions**: Machine learning effectively predicted HIV risk, enabling healthcare providers to deliver targeted, patient-centered prevention services, reducing new HIV infections. There is a need for further capacity building among healthcare providers to enhance the use of machine learning and scale up HIV prevention services.

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# The Uptake of Post Exposure Prophylaxis Amongst Sexual and Gender Based Violence Survivors in Kenya

Muhia J[1], Mathenge J[1], Onyango A[1], Maina J[1], Mwangi K[1]

[1]Hoymas Kenya, Nairobi, Kenya

**Background**: Sexual and Gender-Based Violence (SGBV) in Kenya is a critical issue that affects many individuals, particularly women and marginalized groups. The Kenyan government has enacted several laws and policies to address SGBV, including the Sexual Offences Act 2006, the Protection Against Domestic Violence Act 2015, and the Prohibition of Female Genital Mutilation Act 2011. Despite these efforts, SGBV remains prevalent due to various social, cultural, and institutional barriers. Global data shows that as many as one in every three women has been violated and at least 2 in every 5 men have been violated.

Post Exposure Prophylaxis remains an integral part of management for SGBV survivors, this ensures that the survivors do not get infected with HIV/AIDS.

**Materials and Methods**: Programmatic national aggregate data collected and entered into national data warehouse (KHIS) through the facilities between January and December 2024 was analysed and used in this project. The data instrumental in informing key stakeholders decisions and inform active decision making and monitoring progress over time through key performance indicators.

**Results**: Data available in the KHIS shows that a total of 47,620 SGBV cases were reported between January and December 2024 with only 20,021 (42.0%) reported within the critical 72 hours after violence. Among the 20,021 eligible for post exposure prophylaxis (PEP), only 12,141 (60.6%) were initiated on post exposure prophylaxis. Subsequently, 6626 (54.57%) completed the recommended 28 days PEP regimen after exposure and only 215 cases were reported to have seroconverted by the third month of follow-up. However, data disaggregated by gender shows that females have a higher incidence rate of SGBV at 34,051 (71.5%) and males at 13,569 (28.5%)

**Conclusions**: Gender based violence is still a challenge in our society and survivors of SGBV may not be able to actively access service in the facilities due to socio-economic and cultural challenges. While men are still affected by GBV, they do not seek support compared to their female counterparts. GBV still contributes to new HIV infections in the country

**Recommendations**: Kenya should champion safe and stigma free GBV support services to survivors to allow timely and prompt care.

Capacity building for healthcare providers to provide GBV support services.
Engaging the communities in awareness and sensitization

Sensitizing and creating awareness among stakeholders and community gatekeepers.
Normalizing conversations that men are equally affected by GBV

Reinforcing legal framework and prompt action of law process.

Provision of PEP through tele-consultation especially in remote areas and during the weekends.

Integration of SGBV support services in all service delivery points.

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# Syphilis Micro-Elimination in HIV-Positive Men Who Have Sex with Men: A Critical Need in Georgia

Karchava M[1,2,3], Chokoshvili O[2,8], Dvali N[2], Gatserelia L[2,3], Dzigua L[2], Rukhadze N[2], Mechurchlishvili R[2], Todua M[4], Jamutashvili M[2,5], Kipiani N[6], Abutidze A[2,3,4], Sharvadze L[2,3,4,7]

*[1]Tbilisi Medical Academy, Tbilisi, Georgia, [2]Infectious Diseases, AIDS and Clinical Immunology Research Center, Tbilisi, Georgia, [3]Hepatology Clinical-Hepa, Tbilisi, Georgia, [4]Ivane Javakhishvili Tbilisi State University, , Georgia, [5]European University, , Georgia, [6]Tbilisi State Medical University, , Georgia, [7]The University of Georgia, , Georgia, [8]Georgian National University SEU, , Georgia*

**Background**: A systemic review and meta-analysis of syphilis among Men who have sex with men (MSM) report, that global prevalence of syphilis among MSM is 15 times higher than that of men in the general population (7.5% compared to 0.5%). These finding highlights the urgent need to assess and address syphilis burden in Georgian MSM. In this study we are reporting the trend of syphilis prevalence and incidence in Georgian HIV infected MSM over the period of 20 years in order to address syphilis epidemic for the purpose of micro elimination action plan proposal.

**Materials and Methods**: We have retrospectively analyzed syphilis laboratory testing data among HIV infected MSM and non-MSM population. RPR and/or TPHA laboratory results were extracted from national HIV health information system since 2004. The prevalence of syphilis was calculated at using test result conducted at patient registration visits at AIDS Centers throughout the country. HIV cumulative incidence was calculated by person month using R packages “tidycmprsk”. Maximum follow-up period was 15 months.

**Results**: Laboratory testing data for syphilis were available for 87% MSM (1283 from 1476) compared to 68% of the non-MSM group (6399 from 9324). Among tested MSM cohort, 37.7% (484/1283) had positive syphilis results, whereas only 11.4% (731/6399) of the non-MSM group. This difference was statistically significant (X-squared = 553.24, 95% CI 0.23–0.29, $p < 0.001$). Interestingly, since 2016 there has been a notable and sustained increase in the prevalence with rates rising to 51% among MSM and 25% among non-MSM population. We suspect that the observed doubling of syphilis prevalence among the non-MSM group may be attributable to the presence of a hidden MSM population within non-MSM cohort. A total of 484 new syphilis cases were diagnosed during the study period, yielding an observed incidence rate of 11.3 per 100 person-years (95% CI: 10.3 – 12.3).

**Conclusions**: Our study identifies a significant increase in syphilis prevalence and incidence over time, particularly among men who have sex with men (MSM), underscoring the critical need for continued surveillance and targeted interventions, such as the implementation of DozyPEP, to mitigate transmission and facilitate micro-elimination within this population.

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# Adherence and Retention on ART in Cross Boarder Counties. A case of Kuria East Sub County Migori County, Kenya

Ochieng J[1], Okeyo I[2], Okeyo P[2]

*[1]Ministry of health Migori County, Migori, Kenya, [2]Tukicheki Program, Migori, Kenya*

**Background**: Adherence to and retention on ART are the key factors in improving treatment outcome ,lowering the risk of transmission and disease progression. Kenya is near to epidemic control hence it is important to identify and address any risk factor that will reverse this gains. Sub-optimal treatment retention is one of such risks that has led to design of a campaign dubbed project "HIFADHI" i.e. HIV treatment continuity using full accountability of data, health information systems and program interventions. A strategy to help the PEPFAR program implement interventions towards preventing, detecting and addressing IIT (treatment interruption) with a goal of reducing overall IIT to less than 1% by end of FY24. Nevertheless, HIFADHI strategy has not worked optimally for cross-border areas.
A case of Migori bordering Tanzania republic where migration, intermarriage, trade, transnational sexual activities, unstable communication networks ,difference regimen guidelines and different education calendar to implement OTZ are factors that can be attributed to sub optimal adherence to ART/retention .This is also replicated in Psycho-social groups with biased attendance from Kenya and inconsistent participants from Tanzania due to challenges of networks during invitations. The programmatic intervention of community ART distribution and physical defaulter tracing have not been successfully implemented for cross border clients due to diplomatic reasons.

**Materials and Methods**: Desk review was conducted to analyze clients that have interrupted treatment during July 2023 to June 2024. Trends of EMR machine learning to predetermine probability of clients missing appointments. For returning clients back to treatment, we sought to use peers, expert clients and adherence counselors to trace clients. This was done by use of phone calls and physical tracing.

**Results**: The result revealed that as at June 2024, we had 100 patients who had interrupted treatment between July 2023 and June 2024, of which 30 were lost to follow up, 9 stopped treatment and 51 transfer out. Of the 100 IIT patients 12% had viral-load result of more than 1000 copies 16% of the clients are Tanzanian and 75% of the clients were female. Physical tracing and phone calls managed to return 32 clients to treatment. 22% of the clients returned to treatment cited that they missed treatment as a result of forgetting their appointment dates of which 18% of them were from Tanzania. 4% of the missed appointment were due to lack of transport from Tanzania to Kenya. The result also highlighted that some clients 47% had drugs,19% had travelled and 8% were busy at work.21 clients phone calls attempt failed because of network.

**Conclusions**: Communication and lack of defined strategies in managing clients across boarders, Hinders optimal HIV client management
Cross-border management for HIV clients should be given special consideration in programming of HIV epidemic control.

Strengthen inter-country collaborations for efficient follow-up of HIV clients e.g. through cross-border multi-sectorial approach and the use of Machine learning in prediction of treatment defaulters

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# Missed Opportunities: Addressing Low HIV Testing Uptake Among High-Risk Adults in a Community Outreach

Effiong F[1], Ikechukwu E[1], Anaele B[1], Anyabolo E[1], Mohammed A[2], Ogar F[1]

*[1]University Of Calabar Teaching Hospital (UCTH), Calabar, Nigeria, [2]APIN Public Health Initiatives, Jos, Nigeria*

**Background**: HIV testing is a critical step in prevention and treatment, yet barriers persist in reaching high-risk populations. This study investigates HIV testing uptake, prevalence, and associated factors during a community health outreach conducted on December 13, 2024.

**Materials and Methods**: Data were collected from 128 adults (mean age = 41 years, SD = 14). The primary outcome variable was self-reported HIV testing history. Logistic regression analyzed the association between testing and demographic, behavioral, and health-related factors. HIV prevalence was calculated from the reactive cases identified during the outreach.

**Results**: Among the participants, 78.13% reported having ever been tested for HIV, while 21.87% had not. The outreach identified three reactive cases, yielding an HIV prevalence of 2.34%. Education level was significantly associated with HIV testing ($p = .039$, Exp(B) = 0.180, 95% CI [0.035, 0.920]). Contrary to expectations, behavioral risk factors, such as unprotected sex, history of STIs, and intravenous drug use, did not significantly predict testing uptake. This indicates that individuals with higher risk exposures are not being adequately reached by current HIV testing efforts.

**Conclusions**: The low testing uptake among high-risk individuals and the observed HIV prevalence underscore critical gaps in public health outreach. These findings highlight the need for targeted interventions to improve accessibility and utilization of testing services, particularly among vulnerable groups. Addressing barriers such as stigma, awareness, and service availability is essential to enhancing HIV prevention and reducing undiagnosed cases.

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# Community-Led Approaches to HIV Prevention: Strategies and Implementation at Wasaib Sanwaro

Ilyas M[1]

[1]Wasaib Sanwaro, Multan, Pakistan

**Background**: Wasaib Sanwaro, a grassroots organization based in Multan, Pakistan, has been at the forefront of HIV prevention, addressing the needs of key populations, including transgender individuals, men who have sex with men (MSM), and people living with HIV (PLHIV). With a focus on evidence-based strategies and person-centered care, we have pioneered community-led initiatives to mitigate the spread of HIV in vulnerable communities.

**Objectives**: Our work aims to:
1. Enhance awareness of HIV prevention through targeted behavioural change communication.
2. Expand access to HIV testing, treatment, and linkage to care.
3. Reduce stigma and discrimination against marginalized populations.
4. Advocate for policy reforms to ensure equitable health services for all.

**Materials and Methods**: Using a multifaceted approach, Wasaib Sanwaro implements:
Peer-led education and outreach programs to disseminate accurate information on HIV prevention.

Mobile HIV testing units to increase accessibility in underserved areas.

Drop-In Centers (DICs) offering safe spaces, counselling, and harm reduction services.

Partnerships with local and international organizations to strengthen community systems and sustain interventions.

**Results**: Between 2020 and 2024, our initiatives have achieved measurable outcomes, including:
Reaching over 15,000 individuals with HIV prevention messages.

Conducting 5,000 HIV tests, with 90% of PLHIV successfully linked to treatment.
Establishing a support network for 1,200 transgender individuals, promoting health and well-being.

Advocacy efforts leading to increased funding for community-led HIV responses.

**Conclusions**: Our experience demonstrates that community-led strategies are vital to combating HIV. By empowering key populations, fostering collaboration, and leveraging cultural competency, Wasaib Sanwaro has created an effective model for HIV prevention. Scaling up such interventions can significantly contribute to achieving UNAIDS targets and ending the HIV epidemic in Pakistan and beyond.

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# A Holistic Approach to HIV Prevention, Care, and Treatment: The Impact of Support Groups on Health Outcomes and Well-being for People Living with HIV.

Wambui M

[1]Eneza Imani Community Shelter and Rescue Centre, Nairobi, Kenya

**Background**: Despite advancements in HIV treatment, gaps remain in ensuring comprehensive care for people living with HIV (PLHIV). While biomedical interventions have significantly improved health outcomes, psychosocial challenges, stigma, and poor adherence continue to hinder treatment success. Many individuals struggle with mental health, social isolation, and limited community support, making it difficult to access and remain on treatment.

A holistic approach integrating prevention, treatment, and peer support is essential for improving retention in care and achieving viral suppression. Support groups have emerged as critical tools for enhancing adherence, mental well-being, and empowerment. However, healthcare systems often overlook the role of peer support, focusing primarily on clinical interventions. This study explores the effectiveness of peer-led support groups in improving health outcomes for PLHIV.

**Materials and Methods**: This study assesses the impact of community-based support groups on HIV prevention, care, and treatment outcomes using a mixed-methods approach.

Data was collected from structured support groups in health facilities, community centers, and virtual platforms. These groups focused on treatment literacy, stigma reduction, mental health support, and male engagement programs.

The study included qualitative interviews with PLHIV, healthcare providers, and facilitators to assess the benefits and challenges of support groups in HIV care.

**Results**: Findings show that peer-support models significantly improve treatment adherence and retention in care. PLHIV in structured support groups demonstrated higher viral suppression rates, better treatment knowledge, and improved mental health outcomes.

Male engagement programs effectively reduced stigma and increased male participation in HIV care, leading to higher uptake of HIV services. Support groups also played a key role in HIV prevention education, increasing PrEP awareness and risk reduction strategies.

Participants reported feeling more empowered, less isolated, and more confident in managing their health. Many described the groups as a safe space to openly discuss their experiences.

However, challenges remain, including funding constraints, limited healthcare integration, and persistent stigma. These barriers limit the sustainability and expansion of peer-led support initiatives.

**Conclusions**: Holistic, community-driven approaches that integrate peer support, mental health services, and prevention education significantly enhance HIV care outcomes. Support groups provide psychosocial support, treatment literacy, and empowerment, leading to better adherence and overall well-being.

To maximize impact, healthcare systems must integrate support groups into routine HIV care. Policymakers and organizations should invest in community-led initiatives to create sustainable, patient-centered HIV programs.

By prioritizing holistic care, strengthening peer-led networks, and addressing social determinants of health, we can bridge gaps in HIV prevention and treatment. A future where PLHIV receive comprehensive, stigma-free care is achievable through collaborative, multi-sectoral approaches.

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# Low Prevalence of Archived Drug Resistance Among Ukrainian Migrants Entering Care with Suppressed Viraemia

Mielczak K[1], Urbańska A[1], Serwin K[1], Aksak-Wąs B[1], Karasińska-Cieślak M[1], Leszczyszyn-Pynka M[2], Witak-Jędra M[2], Parczewski M[1]

*[1]Department of Infectious, Tropical Diseases and Immune Deficiency, Pomeranian Medical University, Szczecin, Poland, [2]Department of Infectious, Tropical Diseases and Immune Deficiency, Regional Hospital, Szczecin, Poland*

**Background**: Proviral DNA resistance testing and subtyping may be useful for patients with undetectable or low-level viremia (LLV), in whom historic genotype is not available. Subtype variability and drug resistance analyses, including integrase mutations also are of primary importance among populations where genotyping were not performed, such as displaced people from countries where dolutegravir based regimens are commonly used. To address this knowledge gap we aimed to analyse the subtype variability, prevalence of drug resistance mutations (DRMs) and APOBEC introduced variability editing mutations among treatment-experienced war-displaced Ukrainian patients entering care on effective antiretroviral treatment.

**Materials and Methods**: The study included 106 antiretroviral treated Ukrainian individuals (with VL <200 copies/mL), from whom samples were collected at care entry between 2022-2024. HIV-1 DNA was extracted from PBMCs with protease (PR), reverse transcriptase (RT), and integrase (INT) sequences obtained through Sanger sequencing. Subtypes were initially assessed using the online algorithms Comet and HIV Blast and subsequently confirmed through phylogenetic analyses. Major drug resistance mutations, APOBEC-context drug resistance mutations and APOBEC editing mutations (APOBEC-related mutations, stop codons) were identified using Stanford HIVdb algorithm, version 9.8. All sequences containing at least one APOBEC editing mutation were considered defective.

**Results**: Most of the individuals were female (55.66%), with a median age of 42 years (IQR: 18–60) with heterosexual transmission route being predominant (63.21%). Viral load was <50 copies/mL in 86.79% of individuals, while 13.21% had LLV (50–200 copies/mL). A total of 66.98% were treated with INSTIs, 59.43% receiving dolutegravir. Based on both PR/RT/INT sequences subtype A6 was the most prevalent (82.08%), followed by subtype B (10.38%), while in 6.60% sequences discordant variants in PR/RT and INT were observed. At least one major DRM was detected in 13.21% of individuals (1.89% NRTI, 6.60% NNRTI, 5.66% INSTI). Resistance to two drug classes (NRTI+NNRTI) was found in 1.89% of patients. The majority of DRMs for NRTIs and NNRTIs had a prevalence below 1%, except for NNRTI mutations K103 (1.89%) and E138A (4.72%). For integrase the following resistance patterns were observed: T66A, G118R, E138K (1.89%, each) and G140R (3.77%).

APOBEC editing substitutions were found in 26.42% of individuals (9.43% PR/RT and 18.87% INT). Additionally, the association between the presence of defective PR/RT/INT sequences and sex, age, transmission route, subtype, CD4 cell count, viral load, and previous drug exposure was analyzed but not significant.

A total of 27 APOBEC-context DRMs were identified in 19.81% of individuals: 5.66% in PR/RT (half with APOBEC-related mutations/stop codon) and 15.09% in INT (62.5% of these had APOBEC editing substitutions, while 37.5% did not).

For NRTI- 2/4 (50.00%) major DRMs were APOBEC-context mutations, only one occurred in defective sequence. For NNRTI- 4/12 (33.33%) were APOBEC-context mutations, 3 in defective sequence. For INSTI- 8/10 major mutations (80.00%) were APOBEC-context mutations, of which 4 (all G140R) occurred in defective sequence.

**Conclusions**: As expected, subtype A6 was the most common among war-displaced Ukrainian migrants. PR/RT mutations were infrequent. Integrase resistance was found more commonly than expected but was associated with APOBEC-context mutations with low likelihood of clinical significance.

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# Command-Line Tool to Classify Patients as Exposed or Naïve to Antiretrovirals Based on the HIV-1 Pol Gene Using Hidden Markov Models

Almeida M[1], Frazão M[1], S. Sebastião C[1,2,3], Santos A[1], N Miranda M[1], Alves I[1], Pingarilho M, Giovanetti M[4], B. Abecasis A[1], Pimentel V[1]

*[1] 1 Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health (LA-REAL), Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa (UNL), Lisbon, Portugal, [2]Centro Nacional de Investigação Científica (CNIC), Luanda, Angola, [3]Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola, [4]Department of Science and Technology for Humans and the Environment, University of Campus Bio-Medico di Roma, Rome, Italy*

**Background**: High-potency antiretroviral therapy has increased life expectancy and reduced HIV transmission, but its expansion has revealed resistance mutations. In Portugal, over 40% of new HIV diagnoses involve migrants, many of whom already know their HIV status and have had prior therapy but report as new cases due to stigma. This results in "false" transmitted resistance, complicating first-line treatment guidelines. This study aimed to develop a machine learning algorithm to classify patients as treated or naïve based on pol gene sequence analysis.

**Materials and Methods**: 25,275 sequences (subtypes B, CRF02_AG) from the Los Alamos database, covering Protease and Reverse Transcriptase regions, were aligned using MAFFT and manually edited to obtain an 1100 nt fragment (positions 2253-3363 relative to HXB2). Sequences were translated, and major resistance codons (WHO-2009 list) were removed. Hidden Markov Models (HMMs) using Match (M), Insertion (I), and Deletion (D) states were trained, with transition and emission probabilities estimated from labelled sequences. The Forward algorithm computed a log odds ratio, comparing the sequence's probability under the motif model to a null model, providing a score for motif likelihood. Model performance was assessed using accuracy, precision, recall, and F1 score.

**Results**: The HMM classifier was efficient in identifying naïve vs. treated individuals, with average (from 35 iterations) performance metrics for subtype B: accuracy (67.77%), precision (71.47%), recall (67.77%), and F1 score (66.32%). For subtype CRF02_AG: accuracy (65.97%), precision (74.39%), recall (65.97%), and F1 score (62.75%).

**Conclusions**: Bayesian classification of therapeutic status offers a simple, resource-efficient approach to improve HIV management in populations with uncertain treatment histories, addressing challenges posed by stigma and resistance mutations.

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# Emerging NNRTI Mutations and Their Potential Impact on Novel Therapies: A Focus on Doravirine and Islatravir in Treated-Experienced PLHIV.

Ribeiro M[1], Veloso M[1], Rocha S[3], Fernandes S[1], Diogo I[1], Costa I[1], Cruz Alves I[2], Cabanas J[1], Gonçalves F[1], Pimentel V[2], Pingarilho M[2], Abecasis A[2], Gomes P[1,4]

*[1]Molecular Biology Laboratory, LMCBM, SPC. ULSLO - Hospital Egas Moniz, Lisbon, Portugal, [2]Universidade NOVA de Lisboa, UNL, Lisbon, Portugal, [3]Serviço de Doenças Infeciosas e Medicina Tropical, ULSLO - Hospital Egas Moniz, Lisbon, Portugal, [4]Egas Moniz Center for Interdisciplinary Research, Egas Moniz School of Health & Science , Caparica, Almada, Portugal*

**Background**: The development of once-a-week therapies represents a promising advancement in ART, with the co-formulation of Doravirine and Islatravir emerging as a key candidate. However, pre-existing resistance mutations could affect the efficacy of these novel regimens. This study aims to analyze the prevalence of NNRTI and NRTI resistance mutations in a population of people living with HIV in Portugal, over the past three years, assessing their potential impact on the future implementation of once-weekly Doravirine-Islatravir therapy. Identifying relevant resistance patterns is crucial to optimize treatment strategies and ensure long-term regimen durability.

**Objective**: Analyse resistance patterns to NNRTIs and NRTIs among treatment-experienced PLHIV in Portugal over the past three years and assess their potential impact on the effectiveness of once-weekly Doravirine-Islatravir therapy.

**Materials and Methods**: 2642 HIV-1 PLHIV were tested between February 2022 and December 2024 at the Molecular Biology Laboratory of the Unidade Local Saúde Lisboa Ocidental, Portugal. This laboratory performs resistance testing on patients from the centre, south, and island regions, thus providing a representative sample of Portugal. Among patients in the study, 798 were treated and underwent resistance testing due to suspected virological failure. HIV-1 genomic RNA was sequenced using the NGS Vela Diagnostics System. Sequences were analysed with vela software and results were analysed using Excel. Only quasispecies greater than 5% were considered.

**Results**: During this period, 798/2642 were treated PLHIV (30,2%). Of those 278 (34,8%) harbored a drug-resistance mutation. These included 166 PLHIV with NNRTI major mutations (20,8%). Overall, subtype B was most frequent (n=228; 29%), followed by CRF02_AG (n=118, 15%) and CRF14_BG (n=115, 14%). 252 (42%) were Portuguese, 96 (16%) Brazilan, 83 Angolan (14%) and 79 (13%) from Guinea-Bissau. K103N was present in 93 (11,7%) of PLHIV. Mutations that may impact Doravirine were observed at low frequencies: V106A/M (n=7; 0,88%), Y188L (n=17; 2,13%), G190E (n=3; 0,38%), M230L (n=3; 0,38%), and F227C/L (n=4; 0,5%). Regarding NRTI resistance, 148 (18,55%) PLHIV had major mutations. M184V and K65R mutations, which affect Islatravir, were found in 122 (15,3%) and 13 (1,6%) respectively. Only 15 (1,9%) PLHIV simultaneously harbored either M184V or K65R along with any of the identified NNRTI mutations (V106A/M, Y188L, M230L, G190E, F227C/L), indicating that dual-class resistance remains rare.

**Conclusions**: Our study indicates that, apart from K103N, resistance-associated mutations are present at a low prevalence in ARV-treated PLHIV, suggesting that the once-weekly Doravirine/Islatravir regimen could remain a viable option for many pretreated individuals. Key NNRTI mutations that may impact Doravirine were detected at low frequencies, such as V106A/M (0,88%), Y188L (2,13%), and M230L (0,38%). Similarly, while M184V (15,3%) and K65R (1,6%)—which affect Islatravir—were more common, only 1,9% of patients harbored both NNRTI and NRTI resistance mutations simultaneously, indicating that dual-class resistance remains rare. These findings suggest that most ARV-treated PLHIV do not have resistance profiles that would significantly compromise the effectiveness of the Doravirine/Islatravir regimen. However, resistance testing remains essential to guide treatment decisions. Continued surveillance will be crucial to track resistance trends and ensure the long-term viability of this novel therapeutic approach.

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# Prevalence and Impact of NNRTI Resistance Mutations in ART-Naïve PLHIV: Implications for Doravirine and Islatravir Use

Veloso M[1], Ribeiro M[1], Rocha S[3], Costa I[1], Fernandes S[1], Diogo I[1], Cruz Alves I[2], Cabanas J[1], Gonçalves F[1], Pimentel V[2], Pingarilho M[2], Abecasis A[2], Gomes P[1,4]

*[1]Molecular Biology Laboratory, LMCBM, SPC. ULSLO - Hospital Egas Moniz, Lisbon, Portugal, [2]Universidade NOVA de Lisboa, UNL, Lisbon, Portugal, [3]Serviço de Doenças Infeciosas e Medicina Tropical, ULSLO - Hospital Egas Moniz, Lisbon, Portugal, [4]Egas Moniz Center for Interdisciplinary Research, Egas Moniz School of Health & Science , Caparica, Almada, Portugal*

**Background**: Transmitted drug resistance (TDR) remains a critical concern in antiretroviral therapy, particularly as new treatment strategies, such as once-a-week regimens, emerge. The co-formulation of Doravirine and Islatravir is a promising new treatment option. However, pre-existing resistance mutations may impact its long-term effectiveness in treatment-naïve individuals. This study evaluates the prevalence of TDR mutations in a population in Portugal over the past three years, assessing their potential impact on the efficacy of once-weekly Doravirine-Islatravir therapy. Understanding the extent of TDR in this setting is essential to guiding future implementation and optimizing treatment efficacy.

**Objective**: To assess the prevalence of transmitted drug resistance (TDR) mutations in treatment-naïve individuals in Portugal, over the past three years, and evaluate their potential impact on the efficacy of once-weekly Doravirine-Islatravir therapy.

**Materials and Methods**: 2642 HIV-1 PLHIV were tested between February 2022 and December 2024 at the Molecular Biology Laboratory of the Unidade Local Saúde Lisboa Ocidental, Portugal. This laboratory serves the central, southern, and island regions, providing a representative sample of Portugal. Among patients in the study, 1841 were naïve to treatment and did the resistance test after being diagnosed with HIV infection. HIV-1 genomic RNA was sequenced using the NGS Vela Diagnostics System. Sequences were analysed with vela software and results were analysed using Excel. Only quasispecies greater than 5% were considered.

**Results**: During these three years, 1841/2642 were ART-naïve PLHIV (70%). Of those 225 (12,2%) harbored drug-resistance mutations (TDR/PDR). 137 (7,4%) naïve PLHIV had NNRTI major mutations. Overall, the most prevalent subtype was B (n=616, 34%) followed by CRF02_AG (n= 303, 17%) and C (n=186, 10%). From these naïve PLHIV, 484 (40%) were Portuguese, 268 (22%) Brazilian, 178 (15%) from Guinea-Bissau and 104 (9%) from Angola. K103N was the most frequently found mutation present in 95 (5,2%) ART-naïve PLHIV. From the mutations that can impact Doravirine, we found V106A/M in 9 (0,5%), Y188L in 6 (0,3%), G190E in 3 (0,3%), M230L in 1 (0,1%) and F227C/L in 4 (0,2%) naïve PLHIV. 49 (2,7%) naïve PLHIV had major mutations to the NRTI class. We found M184V in 16 (0,9%) and K65R in 2 (0,1%) naïve PLHIV that may impact Islatravir. 3 (0,2%) naïve PLHIV had simultaneously either M184V or K65R with any of V106A/M, Y188L, M230L, G190E and F227C/L.

**Conclusions**: Our study demonstrates that the prevalence of resistance-associated mutations impacting the once-a-week Doravirine/Islatravir regimen remains low among ART-naïve PLHIV. While some key mutations, such as V106A/M, Y188L, and M230L (affecting Doravirine) and M184V and K65R (affecting Islatravir), were identified, their overall frequency was low, and only 0,2% of individuals harboured both NNRTI and NRTI resistance mutations simultaneously. These findings support the potential use of Doravirine/Islatravir once-a-week in this population as first-line therapy, with minimal concerns regarding pre-existing resistance. Continued surveillance remains essential to monitor resistance trends and ensure the long-term success of this novel regimen.

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# Hepatitis B Immune Escape and Drug Resistance Mutations Among Blood Donors in Gabon: Public Health Implications for Viral Hepatitis Elimination in Sub-Saharan Africa

Maulot-Bangolat D[2], Fokam J[1], Ngoufack Jagni Semengue E[1], Svicher V[4], Salpini R[4], Takou D[1], Angong Beloumou G[1], Mangala C[2], Ambe Chenwi C[1], Nka A[1], Fainguem N[1], Gar Wamba A[3], Penlap V[6], Ajeh Awoh R[5], Tayou C[6], Colizzi V[9], Ndembi N[7], Perno C[8], Ndjolo A[1]

*[1]Centre International De Référence Chantal Biya, Yaoundé, Cameroon, [2]Virology Department, National Public Health Laboratory , Libreville, Gabon, [3]Virology Department, National Blood Transfusion Centre , Libreville , Gabon, [4]Faculty of Medecine and Surgery, University of Rome Tor Vergata, Rome, Italy, [5]Coordination and Subvention Unit for Global Funds and Partners for HIV/AIDS, Tuberculosis and Malaria, Ministry of Public Health, Yaounde, Cameroon, [6]Clinical Haematology Laboratory, University Teaching Hospital, Yaoundé, Cameroon, [7]Africa Centres for Disease Control and Prevention, Addis Ababa, Ethiopia, [8]Department of Microbiology, Bambino Gesu Pediatric Hospital, Rome, Italy, [9]UNESCO Chair of Biotechnology, University of Rome Tor Vergata, Rome, Italy*

**Background**: Hepatitis B virus (HBV) and specifically occult HBV infection (OBI) remains concerning in transfusion medicine in many countries. This study aimed to assess the burden of overt and occult HBV infection among blood donors and circulating viral genotypes in Gabon.

**Materials and Methods**: A facility-based study was conducted among blood donors at the Gabon National Blood Transfusion Centre in 2022. Screening for HBsAg and anti-HBc was done using ELISA; characterization of OBI (defined as HBsAg- but anti-HBc+) was done using HBV DNA viral load (VL); and HBV Pol/S sanger-sequencing was performed for the analysis of immune-escape and drug resistance mutations. Data were analyzed with $p<0.05$ considered significant.

**Results**: Overall, 283 participants were enrolled: 218 (77.0%) males, median age 31 [26-35] years and 264 (93.3%) frequent donors. HBV seropositivity revealed 25.1% (71/283) overt infection (i.e. HBsAg+ and anti-HBc+) and 5.7% (16/283) OBI (HBsAg- but anti-HBc+). All OBI cases (16/16) had HBV DNA VL<10UI/ml versus 37.5% (6/16) VL<10UI/ml, 25% (4/16) VL between ]10-500] UI/ml, and 37.5% (6/16) VL>500UI/ml among controls. Genotyping was successful for 15.6% (5/32) participants with VL>500UI/ml (p=0.02). Following genotyping, 80% (4/5) participants (VL>2000UI/ml) had at least one immune escape mutation (sT131N, sR122K, sG145A) and rtI169L mutation associated with drug resistance was detected in one donor. Molecular phylogeny revealed genotypes A (80%) and E (20%).

**Conclusions**: Our study revealed low prevalence of OBI (<6%), suggesting a low transfusion-risk among blood donors in Gabon. However, overt infection (~25%) is characterized by high replication and mutations associated with both immune escape and treatment failure, translating potential circulation of viruses with important clinical implications at community-level.

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# A Machine Learning Approach for Classifying Integrase Inhibitor Resistance in HIV-1B Based on Viral Genetic Sequences Using a Random Forest Algorithm

Leonor Frazão M[1], Almeida M[1], S. Sebastião C[1,2,3], Santos A[1], N Miranda M[1], Alves I[1], Pingarilho M[1], Giovanetti M[4], B. Abecasis A[1], Pimentel V[1]

*[1]Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health (LA, Lisbon, Portugal, [2]Centro Nacional de Investigação Científica (CNIC), Luanda, Angola , [3] Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola, [4]Department of Science and Technology for Humans and the Environment, University of Campus Bio-Medico di Roma, Roma, Italia*

**Background**: Effective treatment with antiretroviral therapy (ART) reduces viral load as well as the morbidity and mortality associated with HIV in infected patients. Despite the widespread global availability of ART, virological failure remains a significant public health challenge. In this context, the application of predictive algorithms based on machine learning holds promise for optimizing and anticipating the needs of individuals living with HIV. The present study utilized the Random Forest algorithm to classify the resistance profile to integrase inhibitors, based on the analysis of regions with a low association with INSTI resistance.

**Materials and Methods**: A total of 3,833 integrase sequences from the Los Alamos database were selected and aligned using the MAFFT algorithm, resulting in 866-nucleotide fragments (corresponding to positions 4230-5096 relative to HXB2). Sequences were labeled using the Stanford HIV Drug Resistance Database, and the major resistance-associated codons (66, 92, 118, 138, 140, 143, 147, 148, 155, 263) were excluded. Subsequently, a Random Forest model was trained to develop a predictive model, leveraging its effectiveness in assessing feature importance. To balance the dataset, 419 sequences from each class (resistant and non-resistant) were used for training via under sampling. For testing, 767 sequences (105 with resistance, 662 without) were used. The model's performance was evaluated using Accuracy, Precision, Recall, and F1-Score.

**Results**: The Random Forest model was used to predict virological failure, achieving an accuracy of 0.96. For non-resistant sequences, the F1-Score, Recall and Precision were 0.97, 0.97 and 0.98, respectively, while for resistant sequences, they were 0.85, 0.87 and 0.83. Furthermore, an analysis of feature importance revealed the top 10 most significant predictors, including codons 157, 160, 234, 97, 112, 101, 230, 74, 232, and 265, ranked by their contribution to the model.

**Conclusions**: The Random Forest classifier effectively predicted and identified relevant predictors of virological failure. The results of this study may be beneficial for other possible neglected resistance-related codons.

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# HIV GRADE Drug Resistance Interpretation Web-Tool Update for Capsid- and Post-Attachment-Inhibitors

Obermeier M[1], Ehret R[1], Thielen A[2], Däumer M[3], Jensen B[4], Elsner C[5], Dächert C[6], Knops E[7], Geringer E[8], Heger E[7], Wolf E[9], Wiesmann F[10], Walter H[11], Hauser A[12], Hoffmann C[13], Görzer I[8], Verheyen J[3], Eberle J[14], Metzner K[15], Korn K[16], Lengauer T[7], Meixenberger K[17], Stürmer M[18], Münchhoff M[6], Braun P[10], Esser S[19], Klimkait T[20], Kaiser R[7], Lübke N[21]

*[1]MVZ medizinisches Infektiologiezentrum Berlin AG, Berlin, Germany, [2]Seq-IT, Kaiserslautern, Germany, [3]Institut für Immunologie und Genetik, Kaiserslautern, Germany, [4]Klinik für Gastroenterologie, Hepatologie und Infektiologie, Düsseldorf, Germany, [5] Institut für Virologie Universitätsklinikum Essen, Essen, Germany, [6]Max von Pettenkofer-Institut, Ludwig-Maximilians-Universität München, Munich, Germany, [7]Virologie, Uniklinikum Köln, , Germany, [8]Zentrum f. Virologie, Medizinische Universität Wien, Wien, Austria, [9]Clinical Research, MUC research, Munich, Germany, [10]PZB Aachen, , Germany, [11]Medizinisches Labor Stendal, , Germany, [12]Virology expert, Berlin, Germany, [13]ICH Hamburg, Hamburg, Germany, [14]Virology expert, Freising, Germany, [15]Paul Ehrlich Institute, Langen, Germany, [16] Universität Erlangen, Erlangen, Germany, [17] Robert-Koch Institut, , Germany, [18]IMD Labor Frankfurt, Frankfurt, Germany, [19]Zentrum für HIV, AIDS, Proktologie und Geschlechtskrankheiten, Universitätsmedizin Essen, Essen, Germany, [20]Biomedicine, University of Basel, Basel, Switzerland, [21]Institut für Virologie, Universitätsklinikum Düsseldorf, Düsseldorf, Germany*

**Background**: With the approval of new drug classes with different modes of action and new drug resistance mutations in targets that are currently not covered by the existing analysis tools an update for these regions was necessary and implemented. Mutations for capsid inhibitors can be found in the gene region of gag coding for the capsid protein, while mutations against post-attachment inhibitors can be found in the env gene gp120 region.

**Materials and Methods**: The existing framework of the HIV-GRADE (Genotypic Resistance-Algorithm Deutschland) tool, a web-web based tool to identify and interpret viral drug resistance (https://www.hiv-grade.de/cms/grade/) was extended to recognize and correctly highlight the amino acid positions of the capsid region and the gp120 region. Subtype specific consensus sequences were gathered from Los Alamos National Laboratories (LANL) HIV sequence database. These sequences were then realigned and patterns for recognition of the gene regions extracted (92 each for capsid and gp120). Test sequences were also retrieved from LANL HIV sequence database (459 each). Interpretation rules were generated by the HIV-GRADE expert team based on published data and own clinical experience. The generated rules were than translated into ASI-XML files. The test sequences were then entered into the HIV-GRADE tool and the output was manually reviewed for plausibility and correctness

**Results**: All of the 459 test sequences for each gene region could be correctly identified and interpreted. Known drug resistance mutations were identified and drug resistance was predicted based on the ruleset defined by the HIV-GRADE team. This includes subtype specific mutations that can lead to pre-existing drug resistance.

**Conclusions**: With the upgrade of the HIV-GRADE tool v.02/25 drug resistance interpretation of new drug classes can now be substantially simplified. This allows for more accessible drug resistance interpretation in individuals with limited treatment options.

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# HIV-1 Drug Resistant Minority Mutations Detection by Using MGI DNBSEQ-E25 Next-Generation Sequencing Platform

Mohamed S[1], Drali R[1], Doudou A[1], Arliaud J[1], Mercadal M[1], Deblir L[2], Sayada C[2], Modol A[3], Oleskovs F[4], Wurdeman B[4], Sheng Jiang A[4], Eurlings L[4], Pellegrino L[4]

*[1]Abl Diagnostics, Woippy, , [2]ABL SA, Luxembourg, , [3]ABL, , , [4]MGI Tech GmbH, ,*

**Background**: Subtype and drug-resistance mutations were mostly assessed routinely using Capillary Electrophoresis (CE) sequencing which does not detect co-infection or minor variants (frequency below 15-20%). Next Generation Sequencing (NGS) has become the new standard for genotypic drug resistance testing. The objective of this study was to evaluate the performances of DNBSEQ-E25NGS platform using HIV-1 Quality Control for Molecular Diagnostics (QCMD) samples.

**Materials and Methods**: A total of 5 HIV RNA positive plasmas (QCMD) at a range of 4.20 to 5.31 Log10 copies/mL were amplified and sequenced using both Illumina and MGI sequencing. The protease, reverse transcriptase and integrase genes was amplified, and library were prepared using the DeepChek® HIV Assays (121A and 122A, ABL) and the whole genome was amplified using the DeepChek® HIV assay (170B, ABL). The HIV libraries were sequenced using the NGS iSeq100 (Illumina). For MGI sequencing, libraries were prepared and sequenced using DeepChek® native kit for MGI (203A and 204A, ABL). MGI's libraries were sequenced using the NGS DNBSEQ-G99 and DNBSEQ-E25 (MGI). DeepChek® HIV software (ABL) was used for the interpretation of subtype and drug resistance according to the French ANRS v35 (National Agency for AIDS Research), and HIVdb 9.6 3-2024.

**Results**: The median coverage per sample was 50.000, 15.000.000 and 5.000.000 of reads for iseq100 (Illumina), G99 (MGI), and E25, respectively. The Q30 was 85%, 91%and 89%for iseq100 (Illumina), G99 (MGI), and E25 (MGI) respectively. A better Q30 was achieved with MGI than with Illumina. All samples were accurately genotyped. Two subtypes B, one subtypes C and two recombinant forms 02_AG and 57_BC were identified using three NGS platforms. 100 % of concordance was found for the detection of drug-resistant mutations (>5%) for the protease, reverse transcriptase, and integrase regions between the three platforms. All minority mutations were found with the DNBSEQ-E25 (MGI).

**Conclusions**: This study is the first evaluation of HIV-1 QCMD samples using the DeepChek® assays and NGS MGI's E25 platform. These results suggest that the performances of the DNBSEQ-E25 is comparable to the Illumina iseq100 and DNBSEQ-G99. The DNBSEQ-E25 can identify HIV-1 minor variants (3-20%) conferring drug resistance. The NGS should occupy a major place in HIV, HCV and HBV applications testing for subtyping, mutation determination and analysis, and drug resistance surveillance.

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# Susceptibility to Lenacapavir Among Newly Diagnosed HIV-Positive Patients Followed up in Mozambique that Presented with Primary Antiretroviral Resistance to Other Classes

S. Sebastiã C[1,2,3,4], Bathy J[5], Nhampossa T[5,6], Santos A[1], N Miranda M[1], Magode Manhiça N[7], Bila R[7], Vubil D[5], Seabra S[1], O Martins M[1], Giovanetti M[8], Gomes P[9,10], Pingarilho M[1], B. Abecasis A[1], Pimentel V[1]

*[1]Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health (LA-REAL), Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa (UNL), Lisbon, Portugal, [2]Centro Nacional de Investigação Científica (CNIC), Luanda, Angola, [3]Centro de Investigação em Saúde de Angola (CISA), Caxito, Angola, [4]Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola, [5]Centro de Investigação em Saúde de Manhiça (CISM), Manhiça, Mozambique, [6]Instituto Nacional de Saúde, Ministério da Saúde (INS), Maputo, Mozambique, [7]Serviço Distrital de Saúde, Mulher e Ação Social (SDSMAS) de Manhiça, Manhiça, Mozambique, [8]Department of Science and Technology for Humans and the Environment, University of Campus Bio-Medico di Roma, Rome, Italy, [9]Laboratório de Biologia Molecular (LMCBM, SPC, CHLO-HEM), Lisbon, Portugal, [10]Egas Moniz Center for Interdisciplinary Research (CiiEM), Egas Moniz School of Health & Sicence, Almada, Portugal*

**Background**: Multidrug-resistant HIV patients have limited ART options. Lenacapavir (LEN) is a capsid inhibitor that exhibits substantial antiviral activity in patients with therapeutic failure but is also proposed for PrEP. Herein, we assessed LEN susceptibility among ART-naïve HIV patients with drug resistance in Mozambique.

**Materials and Methods**: In this study, 63 patients with DRM against PIs, NRTIs, NNRTIs, and INSTIs were included. The gag (p24) and env fragments were amplified with a low-cost in-house protocol and sequenced with nanopore. HIVDR database from Stanford University was used to assess LEN resistance and geno2pheno to assess viral tropism and protease/maturation inhibitor-associated mutations.

**Results**: A total of 59 patients were successfully sequenced. About 29% had DRMs to PIs, 5% to NRTI, 83% to NNRTI, and 2% to INSTI. No DRMs to LEN were detected. Additionally, 42% of the sequences presented protease/maturation inhibitor-associated mutations. A relationship was observed between the E138A/G mutation and protease/maturation inhibitors (p=0.004). We identified changes at the first codon position of position 56 of the p24 gag gene, which represents a key site for resistance to LEN. Also, codon 66 was highly conserved.

**Conclusions**: Our results support the potential effectiveness of lenacapavir as a PrEP regimen or rescue therapy for patients with any drug resistance mutations.

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# The Prevalence of HIV-1 Multi-Class Drug Resistance in Russia

Kirichenko A[1], Lapovok I[1], Shlykova A[1], Kireev D[1]

[1]Central Research Institute of Epidemiology, Moscow, Russian Federation

**Background**: Successful antiretroviral therapy (ART) has made significant progress in reducing morbidity and mortality among people living with HIV (PLWH), as well as in reducing the risk of HIV transmission. However, increased coverage of ART has led to increased drug resistance (DR). Multi-class DR (MDR), i.e., DR to three or more classes of drugs, can jeopardize the effectiveness of ART, increase morbidity and mortality, deteriorate of health among PLWH, and limit treatment options. This study aimed to assess the prevalence of HIV-1 MDR in Russia.

**Materials and Methods**: We analyzed sequences uploaded to the Russian HIV database (https://ruhiv.ru/) by Central Research Institute of Epidemiology and derived from 403 ART-experienced and 467 ART-naïve patients during 2020-2024. The sequences covered the HIV pol gene regions encoding the protease and fragment of the reverse transcriptase (2253–3369 bp according to HXB-2), and the integrase (4230–5093 bp according to HXB-2).

HIV-1 DR was determined using the Stanford HIV DR Database (v 9.8) as sequences with a Stanford Penalty Score of 15 or higher, indicating a low to high DR level.

**Results**: Among 403 ART-experienced patients, HIV-1 DR was detected in 70.5% (95% CI, 65.8-74.7%) of cases. HIV-1 MDR to three drug classes was found in 10.6% (95% CI, 8.0-14.1%) of cases:

- 8.2% (95% CI, 5.9-11.3%) to NRTI+NNRTI+INSTI;
- 1.5% (95% CI, 0.06-3.3%) to PI+NRTI+ NNRTI;
- 0.7% (95% CI, 0.15-2.3%) to PI+NRTI+INSTI;
- 0.2% (95% CI, 0.01-1.5%) to PI+NNRTI+INSTI.

HIV-1 MDR to four drug classes (PI+NRTI+NNRTI+INSTI) was detected in 1.2% (95% CI, 0.4-3.0%) of cases.
Among 48 patients with HIV-1 MDR, drug resistance was most frequently detected the following drugs:

- NRTI: ABC (97.9%), FTC/3TC (95.8%), DDI (87.5%);
- NNRTI: NVP (89.6%), EFV (85.4%);
- INSTI: CAB/ EVG/RAL (87.5%).

In contrast, among 467 ART-naïve patients, HIV-1 DR was detected in 21.2% (95% CI, 17.7-25.1%) of cases, without any cases of MDR.

**Conclusions**: Our findings highlight the significant burden of HIV-1 MDR among ART-experienced patients and emphasize the urgent need for effective DR monitoring, improved patient adherence to ART, and alternative treatment strategies.

Although HIV-1 MDR was not detected among ART-naïve patients, the high prevalence of DR in this group indicates that pretreatment resistance testing remains essential to optimizing ART outcomes.

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# Pretreatment Drug Resistance Analysis of HIV-1 Variants Circulating in Azerbaijan in 2022-2024

Lapovok I[1], Kirichenko A[1], Shlykova A[1], Syrkina A[1], Lopatukhin A[1], Amirov R[1], Babazade S[2], Ibrahimova S[2], Cafarova G[2], Aliyev S[2], Safarova A[2], Kireev D[1]

*[1]Central Research Institute of Epidemiology, Moscow, Russian Federation, [2]Republic Center of the struggle against AIDS, Baku, Azerbaijan*

**Background**: In the past, the HIV-1 epidemic in Azerbaijan had the same features as the epidemic in all former Soviet Union (FSU) countries: HIV-1 of sub-subtype A6 was the dominant genetic variant. Rare cases of infection by subtype B, CRF03_A6B, CRF63_02A6 and other genetic forms have been revealed in different studies.
Analysis of HIV-1 pretreatment drug resistance (PDR) performed with samples collected in 2017-2019 showed the PDR prevalence to any drug class in Azerbaijan was 4.2% and only to EFV and NVP. The aim of our study was the analysis of HIV-1 genetic variants circulating in Azerbaijan in 2022-2024, including PDR analysis.

**Materials and Methods**: HIV-1 in plasma blood samples from 182 ART-naïve patients were sequenced using the AmpliSens HIV-Resist-Seq kit, and nucleotide sequences encoding protease and fragment of reverse transcriptase (2253-3368 bp according to HXB-2) were analyzed. HIV-1 subtype determination was carried out using HIVBlast and phylogenetic analysis in Mega 6.0., and HIV-1 drug resistance (DR) analysis was performed using the Stanford HIV DR Database (v 9.8). Resistance level was determined based on a Stanford Penalty Score calculation. The drugs to which resistance was studied were drugs including to first-line antiretroviral therapy (ART) regimens.

**Results**: At total, 151/182 (82.97%) of HIV-1 samples belonged to sub-subtype A6. Meanwhile, 14 (7.69%) of samples were HIV-1 of different AG-recombinant forms, including CRF63_02A6, and unique recombinant forms (URFs). Finally, we revealed 6 (3.30%) subtype B samples, 1 (0.55%) sample of CRF03_A6B and 10 (5.49%) samples of rare for Azerbaijan HIV-1 gene forms: 4 – to BF1-recombinants genetically close to CRF14_BF1, 3 – to subtype G, and 3 – to subtype C, subtype D and CRF55_01B, respectively.

At total, 20 (10.99%) samples harbored viruses with DR to at least one class of drugs . There no viruses with DR to major protease inhibitors (PIs). One A6-sample had high-level resistance to NRTIs, and two – low-level resistance to drugs of this class. The most frequent DR was the resistance to NNRTIs. DR to EFV and NVP was found only in sub-subtype A6 samples: 13 (7.14%) viruses harbored high-level DR to EFV and NVP, 3 (1.65%) – high-level DR to NVP and middle-level DR to EFV, 3 – middle and low-level DR to both drugs.

**Conclusions**: Although HIV-1 sub-subtype A6 dominated in Azerbaijan in 2022-2024 HIV-1 genetic diversity was gradually increasing. The circulation of rare and unique recombinant gene forms may be explained by relationship of Azerbaijan with foreign countries. Fortunately, we didn't find HIV-1 DR in non-A6 viruses studied as well.

Revealed PDR level to major ART-drugs (10.99%) was higher than in 2017-2019 (4.2%) which must be investigated further studies.

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# Selection Rate of Resistance to Long-Acting CAB/RPV in a Real Life French Cohort with Strict Inclusion and Monitoring Criteria

LAMBERT S[1], ABDI B[2], MARGUERON C[3], SOURICE J[4], DE MONTE A[5], SOULIE C[2], ALESSANDRI GRADT E[6], CHARPENTIER C[7], RAYMOND S[8], WARNAKULASURIYA F[9], MARQUE-JUILLET S[10], TUMOTTIO C[11], PEYTAVIN G[12], MARCELIN A[2], DESCAMPS D[7], CALVEZ V[2], MORAND-JOUBERT L[1], LAMBERT-NICLOT S[1]

*[1]Santé Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique APHP, Hôpital Saint-Antoine, Département de Virologie, Paris, France, [2]Santé Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique APHP, Hôpital de la Pitié Salpetrière, Département de Virologie, Paris, France, [3]Hospices Civils Lyon, laboratoire de virologie, CHU Lyon, Lyon, France, [4]CHU Hôtel Dieu, laboratoire de virologie , Nantes, France, [5]CHU NICE, Laboratoire de Virologie, Nice, France, [6]Laboratoire de Virologie, CHU Rouen, Rouen, France, [7]Evolution (IAME) INSERM UMR 1137, Université Paris Cité, Laboratoire de virologie Hopital Bichat, Paris, France, [8]Plateau Technique d'Infectiologie, Pôle biologie Institut Fédératif de Biologie -Laboratoire de virologie, hopital Purpan, Toulouse, France, [9]Laboratoire de virologie, Groupe Hospitalo-Universitaire Paris-Sud, Hopital Paul Brousse, Villejuif, France, [10]Laboratoire de virologie, Hopital de Versailles, Versailles, France, [11]Laboratoire de virologie, CHU de Bordeaux, Bordeaux, France, [12]Laboratoire de Pharmacologie, Hopital Bichat, Paris, France*

**Background**: People with HIV infection (PWH) are confronted with considerable challenges in maintaining the efficacy of ART under the pressure to take daily drugs. The Long-Acting antiretroviral (LA-ARVs) may overcome several problems associated with the daily use of oral pills and privacy of patients taking drugs. Cabotegravir (CAB) + rilpivirine (RPV) dosed intramuscularly monthly or every 2 months is a complete LA regimen for the maintenance of HIV-1 virological suppression. Clinical trials have reported few virological failures (VF), but in these cases, the selection of resistance mutations is common. We set out to characterize real-life CAB/RPV LA failures through a French national cohort.

**Materials and Methods**: We collected data on 956 PWH receiving CAB/RPV LA strategy between 2020 and 2024 with at least 6 months of follow-up in a national retrospective observational cohort. The definition of VF was to have 2 consecutive VL>50 copies/mL or one VL>200 copies/mL, a non-virological response was defined as failure to achieve virological suppression (VL <50 c/mL). To be included in the cohort, PWH had to have pre-CAB/RPV LA strategy genotypes. Sequences of reverse transcriptase (RT) and integrase (INSTI) gene were interpreted according to the ANRS algorithm (V35).

**Results**: We reported 19 (2 %) VF out of 956 PWH receiving CAB/RPV LA strategy. Among these 19 PWH with VF, 4 of them harboured a VL>50 copies/mL at baseline (6168, 250, 608 and 104 copies/mL). At failure, 15 patients harboured VL>200 copies/mL (median of 512 copies/mL ;min 241, max 16 000) at the first VL measurement and 4 patients had 2 consecutive VL>50 copies/mL (median on 2nd sample=81 copies/mL), for 2 patients the failure are a non-virological response. Two PWH had BMI>30 kg/m2 and none had A1 or A6 subtype virus (7 subtype B, 9 subtype CRF02_AG and 3 others non-B subtypes). All but one pre-CAB/RPV LA strategy genotypes, did not show any resistance mutations to the strategy, except for APOBEC-linked mutations accompanied by stop codons in the DNA in two cases. Only one PWH harboured a pre-CAB/RPV LA strategy (Y181C on RT gene). At failure for 2 patients genotype was missing and for one only RT gene. At VF, resistance mutations emerged in viruses from 6 PWH (E138K on RT; G140S, N155H on INSTI; E138A on RT; E138K on RT and E138K, Q148R on INSTI; K101P on RT and E138K, Q148R on INSTI; R263K on INSTI). None of the pre-treatment APOBEC-linked mutation was present on failure genotype. At the time of failure, 5 PWH were maintained on the ARV strategy and 14 patients switched ARV treatment. Two thirds of PWH subsequently achieved a viral load <50 copies/mL.

**Conclusions**: The proportion of real-life VF in our cohort is low, similar to clinical trials. The selection of resistance mutations was evidenced even in absence of VF risk factors but at relatively low proportion (31%) (in clinical trial this proportion was >50%); probably in relation with more stringent definition of failure, inclusion criteria and better monitoring. We did not observe any pre-existing APOBEC-linked mutations emerging at failure.

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# Integrase Resistance Profiles in HIV-1 Subtype F1 Infected Patients in Romania

Paraschiv S[1,2], Nicolae I[1], Banica L[1,2], Patrascu R[1,2], Otelea D[1]

*[1]National Institute for Infectious Diseases "Prof. Dr. Matei Bals", Bucharest, Romania, [2]Carol Davila University of Medicine and Pharmacy , Bucharest , Romania*

**Background**: Second generation Integrase Inhibitors (INSTI) are currently recommended both as first line of therapy and in treatment-experienced patients. Romania has a unique cohort of long surviving patients infected with subtype F1 in their early childhood with heavy treatment experience.

**Materials and Methods**: A number of 1004 integrase gene sequences collected between 2019-2024 in the reference center for HIV therapy monitoring laboratory in Romania were analysed. The sequencing was performed with Viroseq kit/in house protocol and Sanger technology. Detection and prediction of drug resistance mutations to INSTI were done with publicly available algorithm from the Stanford HIV drug resistance database.

**Results**: We have identified 29 patients that present INSTI mutation profiles predicted to be fully resistant to all drugs (n=18) or intermediate resistant to second generation drugs (n=11). The main pattern observed among the patients fully resistant to INSTI consists in G140A/S and Q148R/K/H, frequently associated with E138A/K and L74I/M/F. N155H mutation was present in two cases together with substitutions in positions 138, 147 and 148, but not in 140. This profile was present in patients failing second generation INSTI that had received raltegravir. In strains with intermediate resistance we observed a different profile with selection of S147G and N155H mutations, solely or in combination with E92Q or E138K mutations. The dolutegravir associated mutation R263K was found in only one patient.

**Conclusions**: During the last six years, the rate of complete resistance to second class integrase inhibitors was rather low in Romanian F1 subtype infected patients and mainly associated with raltegravir history. Different mutation profiles were observed in strains fully or intermediatly resistant to second generation INSTI. Before initiation of injectable cabotegravir and rilpivirine therapy, possibly preexisting resistance should be carefully assesed.

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# HBV Reactivation Post-Switch to HBV-Inactive ART: A Scoping Review

Geretti A[1,2,3], Ring K[4,5], Parczewski M[6], Boffito M[7], Garcia F[8], Orkin C[4,5]

*[1]University Of Rome Tor Vergata, Rome, Italy, [2]Royal Free London NHS Foundation Trust, London, UK, [3]King's College London, London, UK, [4]SHARE Collaborative, Blizard Institute, Queen Mary University of London, London, UK, [5]Barts Health NHS Trust, London, UK, [6]Pomeranian Medical University, , Poland, [7]Chelsea and Westminster Hospital NHS Trust, , UK, [8]Hospital Clinico Universitario San Cecilio, , Spain*

**Background**: Hepatitis B virus (HBV) reactivation in people living with HIV and evidence of past HBV infection is an important consideration when evaluating the risks and benefits of discontinuing HBV-active antiretroviral therapy (ART) in favour of other treatment regimens. We aimed to summarise available evidence on HBV reactivation risk after switching to HBV-inactive regimens, and to identify predictors that may guide clinical practice.

**Materials and Methods**: We searched electronic databases (PubMed, Embase, Cochrane, 1996–2024) and conference abstracts (2021–2024) for studies and case reports describing HBV reactivation in treated people switching to tenofovir-free ART. Reactivation was defined as hepatitis B surface antigen (HBsAg) and/or HBV DNA detection by routine diagnostic assays in individuals who tested HBsAg negative and anti-hepatitis B core (anti-HBc) positive pre-switch. Reactivation in the context of ART interruption was excluded.

**Results**: We initially identified six studies and seven publications describing eight case reports. The studies reported 133 reactivations after 11,731 switches (1.1%) among 11,507 individuals, with two US cohorts contributing most data. However, 2,854 of 11,731 switches allowed post-switch use of lamivudine or emtricitabine and only one reported accordingly, noting no reactivations over 144 weeks among 71 people on dolutegravir/lamivudine. In four studies reporting on switches to fully HBV-inactive ART, there were 99 reactivations after 6,093 switches (1.6%; 95% CI 1.3–2.0). with proportions ranging widely across studies. Reactivations were typically less common with pre-switch anti-HBs ≥10 IU/L (range 0–11%) compared with lack or loss of anti-HBs (3.2–14%). Although reactivations occurred across a wide range of CD4 counts and HIV RNA loads, the risk was higher with HIV viraemia, low CD4 counts, and nadir CD4 counts <100 cells/mm3, as well as with HBV DNA detection pre-switch. In studies and case reports, the earliest detection of HBsAg and/or HBV DNA ranged from 3 weeks to 14 months post-switch and was variably associated with alanine aminotransferase (ALT) increases, including severe hepatic flares. These typically resolved after re-introduction of HBV-active agents, with HBsAg loss documented within 2–5 months. Pre- and post-switch HBV investigations were variable in routine care.

**Conclusions**: Moderate-to-low quality evidence indicates that HBV-inactive ART carries a low but notable risk of HBV reactivation in people with HIV and a past HBV infection. A thorough evaluation of HIV and non-HIV related reactivation risk factors is essential before switching and should continue post-switch, accompanied by a pre-defined monitoring plan. Detection of HBsAg and/or HBV DNA post-switch signals a heightened risk of hepatic flares requiring prompt confirmation and management. Standardisation of pre- and post-switch HBV monitoring practices remains a critical need.

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# Cost-Effectiveness of Cabotegravir Long-Acting for HIV Pre-Exposure Prophylaxis (PrEP): A Systematic Review of Modelling Studies

Effiong F[1], Ekpor E[2], Dine R[3], Olawuyi D[4], Adewole D[5]

*[1]University Of Calabar Teaching Hospital (UCTH), Calabar, Nigeria, [2]School of Nursing and Midwifery, University of Ghana, Accra , Ghana, [3]Rinda-Ubuzinma, Kigali, Rwanda, [4]Faculty of Clinical Sciences, College of Medicine, University of Ibadan, Ibadan, Nigeria, [5]Department of Health Policy and Management, College of Medicine, University of Ibadan, Ibadan, Nigeria*

**Background**: Cabotegravir long-acting (CAB-LA) is a promising HIV prevention strategy; however, its cost-effectiveness compared to oral pre-exposure prophylaxis (PrEP) varies across settings. This systematic review examines the economic viability of CAB-LA interventions using modelling studies in diverse populations.

**Materials and Methods**: We searched literature databases for modelling studies on the cost-effectiveness of CAB-LA in various settings. The search was executed in PubMed, Web of Science, Scopus, and the Cochrane Library. The search was conducted in January, 2025, and was limited to English studies; but there was no limitation on year of publication. Quality assessment was based on the 2022 CHEERS checklist for economic evaluation studies. A narrative synthesis was conducted to summarize the findings. Key outcomes to be extracted included study characteristics and design, incremental cost-effectiveness ratios (ICERs), adherence rates, and willingness-to-pay (WTP) thresholds.

**Results**: The search retrieved 19 results, but only six modeling studies meeting predefined inclusion criteria were included. These studies evaluated CAB-LA among various populations, including heterosexual men, women at high risk of HIV, men who have sex with men (MSM), transgender women (TGW), and large simulated cohorts. The studies employed static epidemiological models, deterministic compartmental models, and Markov cohort models to evaluate CAB-LA alongside oral PrEP. Quality assessment results show that studies were of moderate and high quality. CAB-LA demonstrated potential cost-effectiveness under specific conditions. In Sub-Saharan Africa, CAB-LA achieved ICERs below $1,000 per disability-adjusted life year (DALY) averted at adherence rates exceeding 75%. In high-income settings, ICERs for CAB-LA remained below $98,000 per quality-adjusted life year (QALY) when drug costs were reduced to $4,100/year. Low-income settings required annual costs below $16 for cost-effectiveness. Epidemiological benefits included a 30%-40% reduction in HIV incidence with optimal adherence. Drug pricing, adherence, and quarterly monitoring were identified as key determinants of cost-effectiveness. Comparisons with oral PrEP indicated that CAB-LA could be more cost-effective in populations with low adherence to oral regimens.

**Conclusions**: CAB-LA is a cost-effective HIV prevention intervention under specific economic and adherence scenarios. Reducing drug costs and enhancing adherence strategies are critical to optimizing its economic and epidemiological impact.

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# Add-on Efficacy of Doravirine to Antiretroviral Treatment in People Living with HIV-1 Harboring a Classical Virological Failure and Those with Persistent Low-Level Viremia

Calvez V[1], Wirden M[1], Charpentier C[2], Alidjinou E[3], Bouvier-Alias M[4], De Monte A[5], Maillard A[6], Montes B[7], Trabaud M[8], Raymond S[9], Teyssou E[1], Soulié C[1], Peytavin G[10], Descamps D[2], Marcelin A[1]

*[1]Sorbonne Université, INSERM, Institut Pierre Louis d'Epidémiologie et de Santé Publique, AP-HP, Hôpitaux Universitaires Pitié Salpêtrière - Charles Foix, laboratoire de virologie, F-75013., Paris, France, [2]Service de Virologie, AP-HP, Hôpital Bichat-Claude Bernard, Université Paris Cité, INSERM, IAME, Paris, France, [3]Service de Virologie, CHU de Lille, Lille, France, [4]Service de Virologie, CHU Henri Mondor, Créteil, France, [5]Service de Virologie, CHU de Nice, Nice, France, [6]Service de Virologie, CHU de Rennes, Rennes, France, [7]Service de Virologie, CHU de Montpellier, Montpellier, France, [8]Service de Virologie, CHU de Lyon, Lyon, France, [9]Service de Virologie, CHU de Toulouse, Toulouse, France, [10]Service de Pharmacologie, AP-HP, Hôpital Bichat-Claude Bernard, Université Paris Cité, INSERM, IAME,, Paris, France*

**Background**: Strategies involving the addition of a single molecule (add-on) to antiretroviral treatment in patients with virological failure can be used when a conventional treatment has failed and there is no desire to change the entire treatment but simply to add a molecule. The aim of this study was to measure the efficacy of adding Doravirine (DOR) alone to people living with HIV-1 (PLWHIV) with virological failure (at least two successive VL > 50 cp/ml).

**Materials and Methods**: This was a retrospective and observational study where PLWHIV with viruses susceptible to DOR received an addition of DOR to their treatment. Two groups of patients were studied: #A 74 PLWHIV for whom that the plasma viremia was successfully suppressed and then harbored a recent VL rebound (recent viral rebounders with two VL >50cp/ml in the last month) and #B 32 patients with prolonged virological failure despite attempts to change treatment for at least one year (all these patients harbored a low-level viremia between 50 and 200 cp/ml). All these PLWHIV received DOR alone without any other change in treatment, and virological efficacy was measured at M6 (VL< 50 cp/ml). The effect of the GSS (number of still active drug in the regimen before adding DOR), current CD4 number and nadir, VL Zenith were assessed. Genotypic resistance testing was performed at the end of the study in plasma of PLWHIV that did not harbor a complete virologic success.

**Results**: Of the 74 PLWHIV with recent failure, 88% had virological success at M6 (100% if the GSS of the molecules associated with DOR was 2 or 3, 50% if the GSS was 1 (in all cases of success, the only molecule still sensitive in combination with DOR was DTG) and 0% if the GSS was zero). In these 74 PLMHIV with recent failures, only 2 (2.7%) selected mutations to INNRTs after addition of DOR leading to DOR resistance in two cases. Of the 32 PLWHIV with prolonged failure, 9 out of 32 showed a complete virological response at M6 (all these patients had a GSS associated with DOR ≥ 2 and a CD4 Nadir > 250 cell count In these 32 PLMHVI with prolonged failures, only 5 (15%) selected mutations to INNRTs after addition of DOR leading to DOR resistance in 3 (9%) cases.

**Conclusions**: In cases of recent virological failure, the addition of DOR alone is sufficient to control viremia if the GSS of the associated molecules has a favorable profile, which may make it possible to avoid a complete change of treatment. In PLWHIV with prolonged virologic failures for whom treatment is known to be difficult (frequent adherence problems, residual replication in the viral reservoir…) the addition of DOR can be a solution in PLWHIV with a nadir > 250 CD4 cell count. In these two groups, resistance selected for DOR is possible but remains at a low frequency.

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# Real-world Effectiveness and Safety Outcomes in People With HIV-1 Switching to Dolutegravir + Lamivudine (DTG + 3TC) with Unknown Prior Genotype: A Systematic Literature Review

Boulos J[1], Fraysse J[1], Fernvik E[2], Jones B[3], Verdier G[4], Priest J[1], Letang E[5]

*[1]ViiV Healthcare, Durham, USA, [2]ViiV Healthcare, Stockholm, Sweden, [3]ViiV Healthcare, , UK, [4]ViiV Healthcare, Montréal, Canada, [5]ViiV Healthcare, Tres Cantos, Madrid, Spain*

**Background**: In phase 3 interventional studies, DTG/3TC demonstrated durable efficacy as an antiretroviral therapy (ART) switch option for people with HIV-1 with virologic suppression and no pre-existing resistance-associated mutations (RAMs) or prior virologic failures (VFs). However, historical or baseline genotypes are not always available in clinical practice. In a pooled TANGO/SALSA post hoc analysis, high proportions of participants who switched to DTG/3TC maintained virologic suppression (VS) regardless of availability of prior genotype, and results from several clinical trials (SOLAR-3D, ART-PRO, VOLVER) and a meta-analysis of interventional and observational studies suggested that switching to DTG + 3TC with known pre-existing M184V/I may not impact virologic effectiveness. We describe real-world outcomes from observational studies to further evaluate frequency and impact of RAMs in people with HIV-1 switching to DTG + 3TC with unknown prior genotype.

**Materials and Methods**: A systematic literature review (SLR) was conducted, searching Ovid MEDLINE®, Embase, and Cochrane databases and relevant congresses for observational studies reporting DTG + 3TC use (published January 2013-November 2024) per Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. Publications reporting effectiveness and/or safety outcomes for people switching to DTG + 3TC with unknown prior genotype were included.

**Results**: The SLR identified 310 publications representing 61,334 people with HIV-1 using DTG + 3TC after accounting for population overlap. Of these, 13 publications reported outcomes for 3402 unique individuals switching to DTG + 3TC with unknown prior genotype in Europe (n=7), Asia (n=4), and South America (n=2). Eleven studies (n=3380) reported effectiveness outcomes at time points ranging from 6 months to 96 weeks (or ~24 months). High VS rates (96%-100% per study) were maintained after switch. Overall, 0.30% (10/3380) of individuals experienced VF or discontinued for virologic reasons. M184V was detected at VF in 0.06% (2/3380) of individuals, and 1 person had T97A, E138K, and N155H, which confers low-level resistance to DTG, detected at treatment discontinuation for virologic reasons (0.03%; 1/3380; HIV-1 RNA 540 copies/mL). Safety and/or tolerability outcomes were reported in 5 of the 13 studies at time points ranging from 30 weeks (median follow-up) to 36 months (n=356). Among these studies, 0.84% (3/356) of individuals discontinued DTG + 3TC due to adverse events, all of which were considered unrelated to study drug (n=2 deaths, n=1 diarrhea). Additionally, 2 studies reported no serious adverse events, and 2 studies reported no discontinuations for any reason.

**Conclusions**: Real-world effectiveness and/or safety outcomes were reported in 3402 people with HIV-1 switching to DTG + 3TC with unknown prior genotype. All studies reporting effectiveness outcomes showed high VS rates and low VF rates. On-treatment RAMs detected at VF were rare, and 0.03% (1/3380) of individuals had integrase resistance detected at VF. Treatment with DTG + 3TC was well tolerated, with no drug-related discontinuations. These results are consistent with interventional studies and may provide reassurance for considering DTG + 3TC as a potential switch option if genotype is unknown.

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# Very Slow Decay of Total HIV-1 DNA During Suppressive ART: A Routine Cohort Report

Alidjinou E[1], Coulon P[1], Diarra A[2], Tetart M[2], Guigon A[1], Cappeliez B[2], Derdour V[2], Aissi E[2], Viget N[2], Hober D[1], Meybeck A[2], Robineau O[2]

*[1]Laboratoire de Virologie ULR3610, Univ. Lille, CHU de Lille, Lille, France, [2]CH de Tourcoing, Service Universitaire des Maladies Infectieuses, Tourcoing, France*

**Background**: The HIV-1 reservoir is the major hurdle to a cure. The quantification of total HIV-1 DNA was shown to be a clinically relevant marker for the HIV-1 reservoir and can be implemented in routine. Levels of total HIV-1 DNA in patients with virologic suppression (VS) largely represent integrated HIV-1 genomes and were shown to correlate with the levels of inducible virions and viral rebound after treatment interruption. Determinants of HIV-1 reservoir long-term dynamics during suppressive ART are not fully understood. In this routine cohort study, we aimed to investigate HIV-1 DNA decay and the associated factors in patients with VS.

**Materials and Methods**: People living with HIV-1 (PLWH) on suppressive ART who underwent two HIV-1 DNA measures were included in this study. The first quantification was performed after at least 1 year of VS (T1), and the second at least 1 year after the first one (T2). Plasma viral load (PVL) was undetectable between both timepoints, and only a blip (PVL <200 copies/mL) was allowed. Total DNA was extracted from whole blood, and HIV-1 DNA quantification was performed using the real-time qPCR targeting the 5'LTR region. Linear and logistic regressions were used to investigate associations.

**Results**: A total of 631 PLWH met the inclusion criteria. The median age was 57 years, and they were mainly male (73%). Most of them were infected by a subtype B virus (69%). At T1, PLWH have been treated for 10.5 years and suppressed for 4.3 years. The median HIV-1 DNA viral load (VL) was 2.40 log copies/106 cells. In multivariate analysis, HIV-1 DNA levels were negatively associated with CD4 nadir, and time since VS ($p<0.0001$). T2 was performed 3.7 years after T1, and the median VL was 2.38 log copies/106 cells. In multivariate analysis, the change in HIV-1 DNA level was negatively associated with HIV-1 DNA VL at T1 and the delay between T1 and T2 ($p<0.0001$). Assuming that a 0.5 log change is significant at individual level, no significant change was observed in 82.7% of patients. A significant decrease was recorded in 11.4% and an increase in 5.9%. A comparison of these two later subgroups confirmed that the decrease was positively associated with HIV-1 DNA VL at T1 ($p<0.0001$).

**Conclusions**: This study supports a very slow but steady decline of total HIV-1 DNA during suppressive ART. The size of reservoir and the time on suppressive ART were found to be major determinants of total HIV-1 DNA decay.

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# Safety and Efficacy of Tenofovir DF-Containing, Ainuovirine-Based Regimen (ANV/3TC/TDF) vs Tenofovir Alafenamide-Containing, Cobicistat-Boosted Elvitegravir-Based Regimen (E/C/F/TAF) for Maintaining Virologic Suppression in Adults Living with HIV-1: Week 96 Results from the Phase 3, Noninferiority SPRINT Randomized Trial

Zhang F[1], Wu H[2], Cai W[3], Ma P[4], Zhao Q[5], Wei H[6], Lu H[7], Wang H[8], He S[9], Chen Z[10], Chen Y[11], Wang M[12], Fu H[13], Yun X[13], WANG Y[13]

*[1]Clinical and Research Center for Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing, China, [2]Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University,, Beijing, China, [3]Infectious Disease Center, Guangzhou Eighth People's Hospital, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [4]Department of Infectious Diseases, Tianjin Second People's Hospital, Tianjin, Tianjin, China, [5]Department of Infectious Diseases, The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [6]Department of Infectious Diseases, The Second Hospital of Nanjing, Najing, China, [7]National Clinical Research Centre for Infectious Diseases, The Third People' s Hospital of Shenzhen, The Second Affiliated Hospital of Southern University of Science and Technology, Shenzhen, China, [8]Department of Infectious Diseases, Shenzhen Third People's Hospital, Shenzhen, China, [9]Department of Infectious Diseases, Public Health Clinical Medical Center of Chengdu, Chengdu, China, [10]Drug Clinical Trial Institution, Public Health Clinical Medical Center of Chengdu, Chengdu, China, [11]Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing, China, [12]Institute of HIV/AIDS, The First Hospital of Changsha, Changsha, China;, [13]Clinical Research & Development, Jiangsu Aidea Pharmaceutical Co., Ltd, Yangzhou, China*

**Background**: The SPRINT study demonstrated that immediate switch to tenofovir DF- (TDF-) containing, ainuovirine- (ANV) based regimen (ANV/3TC/TDF) resulted in non-inferior virologic efficacy, and improved cardiometabolic conditions compared to that to tenofovir alafenamide-containing, cobicistat-boosted elvitegravir-based regimen (E/C/F/TAF) at 48 weeks.

**Materials and Methods**: In the extensional study between weeks 48, and 96, people with HIV-1 (PWH) on ANV/3TC/TDF continued the assigned regimen (immediate switch arm), while those on E/C/F/TAF re-switched to ANV/3TC/TDF FDC (delayed switch arm). The E/C/F/TAF arm (weeks 0 to 48) was used as comparator for the extensional study. The primary efficacy end point was proportion of PWH with HIV-1 RNA titer at 50 copies per millimeter or above at a non-inferiority margin of 0.05. The safety outcomes of primary interest included absolute changes in body weight, and fasting serum lipids between weeks 48, and 96.

**Results**: The primary efficacy end points were both 3.4% (13/381, and 13/377) for the two intervention arms at week 96, noninferior to that of the comparator arm at week 48 (1.6%; estimated treatment difference [ETD], 0.018, 95% confidence interval [95% CI], -0.005 to 0.043, P <0.001; 0.019, -0.004 to 0.044, P<0.001). The treatment-emergent adverse events were generally similar between the two intervention arms during the extensional period. The delayed switch arm showed modest reduction in body weight (mean± standard error of mean [SEM], -0.66±0.80 kg), in contrast to the immediate switch arm with slight weight gain from week 48 (0.05±0.82 kg; -0.71 kg, -1.18 to -0.24, P=0.003 for difference). Fasting serum low-density lipoprotein cholesterol remained generally unchanged in the immediate switch arm (0.01±0.04 mmol/L) but improved greatly in the delayed switch arm at week 96 from week 48 (-0.37±0.08 mmol/L; -0.38, -0.46 to -0.31; P<0.001).

**Conclusions**: Both immediate and delayed switches to ANV/3TC/TDF maintained high viral suppression through 96 weeks. Delayed switch could offset weight gain, and lipid dysmetabolism associated with previous exposure to E/C/F/TAF.

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# Hepatitis Delta Virus Infection: Prevalence, Diagnostic Delay, and Treatment Needs in an Infectious Diseases Center

Gomes Rocha S[1], Inácio Santos C[1], Antunes C[1], Leal P[1], Miranda A[1], Baptista T[1], Mansinho K[1]

[1]*Egas Moniz Hospital, Lisbon, Portugal*

**Background**: Hepatitis delta virus (HDV) infection occurs exclusively in individuals with hepatitis B virus (HBV), as it depends on the latter for replication. The global prevalence of HDV among HBsAg-positive individuals is estimated at 4.5–13%, affecting 48–60 million people. HBV/HDV co-infection leads to more severe liver disease, doubling hepatocellular carcinoma (HCC) risk compared to HBV monoinfection. Individuals with triple infection (HIV/HBV/HDV) have a sixfold higher HCC risk than those with HIV/HBV. HBV and HDV share transmission routes with HIV, with an estimated 5–6% of people living with HIV co-infected with HBV, of whom 7–15% also have HDV. Despite this, HDV remains underdiagnosed and is often detected late.

**Objective**: To characterize HBV/HDV and HIV/HBV/HDV co-infected patients followed at an Infectious Diseases and Tropical Medicine Center in Lisbon, focusing on diagnostic delay, treatment needs, and therapeutic choices.

**Materials and Methods**: An ongoing retrospective analysis of clinical records from patients evaluated at our center from 1 January 2023 and 31 December 2024 is being done and, so far, 113 HBsAg-positive patients were identified. Patients who were deceased were excluded, and in cases of repeated records, only one entry was retained. Final results will be updated at the meeting. Data analysis was performed using Microsoft Excel.

**Results**: Among 15 patients with chronic HDV, 5 had HBV co-infection alone, and 10 had HIV/HBV/HDV. Most were male (n=10, 66.7%), of African origin, 9 (60%) from Guinea-Bissau and 3 (20%) from Angola. The mean age at HDV diagnosis was 42.2 years, and the most frequent transmission route was sexual (n=7, 46.7%).

Regarding HBV infection, only one patient was HBeAg-positive (HIV/HBV/HDV). Two patients had active hepatitis (both HBeAg-negative), one of whom was HIV-positive with cirrhosis. All HIV-positive patients and the HIV-negative cirrhotic patient (n=11) started tenofovir-based therapy, with 71.4% achieving undetectable HBV DNA levels at the last follow-up.

A diagnostic delay of 6 or more years after HBV infection was noted in most cases (n=9, 60%). Among these, five were diagnosed more than 15 years later, all with HIV/HBV/HDV co-infection. Of the 15 patients, only four met criteria for HDV-specific treatment. One received pegylated interferon (Peg-IFN), while three were on bulevirtide, not due to treatment failure but previous interferon therapy for hepatitis C or chronic kidney disease.

The HIV-positive subgroup (n=10) had an average follow-up of 17.9 years. At HDV diagnosis, only one patient had a CD4+ T-cell count below 200 cells/µL, with a mean CD4+ count of 317.5 cells/µL. At the latest follow-up, the average CD4+ count was 621.1 cells/µL, and all had undetectable HIV RNA levels.

**Conclusions**: This study highlights that HDV infection remains underdiagnosed, with significant diagnostic delays often exceeding six years. In HIV-positive patients, this delay may result from the prioritization of HIV care, leading to the oversight of HBV/HDV screening and postponing diagnosis and treatment. Given HDV's rapid progression to cirrhosis, early detection through systematic reflex testing in all HBsAg-positive patients is crucial. Strengthening hepatitis B vaccination programs remains the most effective measure to prevent HDV infection, particularly for high-risk populations.

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# Barriers and Challenges in Assessing Health-Related Quality of Life in People with HIV.

Menozzi M[1], Milic J[2], Fontana B[2], Benassi L[2], Ricciardetto M[2], Ricciardetto M[2], Cuomo G[1], Mancini G[1], Guaraldi G[2], Mussini C[2], Cervo A[1]

*[1]Infectionus Disease Clinic and Modena HIV Metabolic Clinic - University Hospital of Modena, Modena, Italy, [2]Modena HIV Metabolic Clinic - University of Modena and Reggio Emilia, Modena, Italy*

**Background**: The fourth 90, i.e. assessment of health related-quality of life (HR-QoL), has been introduced as the ultimate goal of the HIV care. The objective of the study was to identify people with HIV (PWH) with unknown HR-QoL status and analyse their socio-demographic and clinical characteristics that may pose barriers to HR-QoL evaluation in the clinical setting.

**Materials and Methods**: This was a cross-sectional study of antiretroviral therapy (ART)-experienced PWH of Modena HIV Metabolic Clinic (MHMC) from 2015 to 2024. People with HIV (PWH) were invited via email to complete online questionnaires on HR-QoL. Demographic, anthropometric, HIV-related, and other clinical characteristics were collected according to the standard of care at MHMC. Logistic regression was used to identify risk factors associated with a higher likelihood of response to online questionnaires.

**Results**: Of 2804 PWH, 2066 (73.7%) were males, with a median age of 59 years (Q1, Q3: 54, 64) and a median HIV duration of 21.3 years (Q1, Q3: 12.6, 27.8). Median nadir and current CD4 counts were 209 and 716 cells/µL, respectively. Overall, 2526 (90.1%) completed the questionnaire.

Table 1 summarizes characteristics of PWH according to presence or absence of response to questionnaires.

Older PWH (59 vs 58 years, p<0.01) and those with longer HIV duration [22 (Q1, Q3: 13, 28) vs 18 (Q1, Q3: 11, 25) years, p<0.01] were more likely to respond. However, lower education levels (12 (Q1, Q3: 8, 13) vs 8 (Q1, Q3: 8, 13) years, p0.027], unhealthy lifestyles [current smoking [117(49%) vs 843(34%), p<0.01], moderate/severe alcohol intake [105(43%) vs 871(35%), p<0.01]], higher ART pill burden (1.39±0.98 vs 1.05±1.23, p<0.01), frailty index [0.20 (Q1, Q3:0.16, 0.27) vs 0.16 (Q1, Q3:0.11, 0.24), p<0.01] and impaired neurocognitive function [57 (47.9%) vs 154 (12.5%), p<0.01] were linked to non-response. At logistic regression impaired neurocognitive function (lower total global deficit score) was associated with non-response (OR=0.43, 95%CI:0.26-0.72, p=0.001), while higher ART pill burden favoured it (OR=2.10, 95%CI:1.10-4.12, p=0.033). The model was adjusted for age, sex, HIV duration, waist circumference, BMI, alcohol intake, visceral adipose tissue, physical performance, and frailty index.

**Conclusions**: A higher ART pill burden was associated with higher access to HR-QoL questionnaires, while impaired neurocognitive function, measured by the total global deficit score, were linked to a greater likelihood of non-response to HR-QoL questionnaires. However, these individuals may benefit the most from assessing patient-reported outcomes, especially HR-QoL. Efforts should be made to reduce barriers to HR-QoL assessment and ensure access for all PWH who require interventions to achieve the fourth 90 goal.

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# Causes of Death of Patients Living with HIV in the Infectious Diseases Department of Casablanca

Badi H[1], Bencherifi W[1,2], Sodqi M[1,2], Marih L[1,2], Oulad Lahsen A[1,2], Ihbibane F[1,2], Marhoum El Filali K[1,2]

*[1]Infectious Diseases unit, University Hospital of Ibn Rochd, Casablanca, Morocco, [2]Faculty of medecine and pharmacy, Hassan II university , Casablanca, Morocco*

**Background**: AIDS is a disease known to be fatal due to opportunistic diseases secondary to immunosuppression induced by the invasion of HIV into immune cells.
This study aims to describe and analyze the causes of death in patients living with HIV (PLHIV) followed at the Infectious Diseases Department of Casablanca.

**Materials and Methods**: This is a retrospective analytical study on the causes and characteristics of adult PLHIV patients who died between January 2015 and November 2021 at the Infectious Diseases Department (SMI) of Ibn Rochd University Hospital. The data were collected and analyzed using an Excel file containing medical records and Nadis computerized records.

**Results**: Of the 4,500 patients followed at the SMI, 352 died (7.8%). The average age at the time of death was 41.7 years. Two hundred and eight patients were male (59%), and 338 patients were classified as stage C. At the time of death, the mean CD4 count was 51.8 cells/mm3 (0–1031), and the mean viral load was 1,519,392 copies/ml. Two hundred and thirty-seven patients were receiving antiviral therapy (67.3%). In 72% of cases, death occurred during the first year after diagnosis of HIV infection. The main causes of death were tuberculosis in 59%, pneumocystosis in 33.3%, cerebral toxoplasmosis in eight cases (24.5%), cryptococcosis neuro meningitis in 17.5%, cytomegalovirus infection in 15%, Kaposi's sarcoma in 8.9% and lymphoma in 5.2%. Non-AIDS-classifying diseases were responsible for death in 22% and immune reconstitution inflammatory syndrome in 5.2% of cases.

**Conclusions**: In Casablanca, almost all deaths among PLHIV occur at stage C of the infection, with tuberculosis as the main cause. A collaboration between the national tuberculosis control program and the AIDS control program has been established to improve the prevention, screening, diagnosis, and management of HIV/tuberculosis coinfection.

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# Patient-Reported Outcomes (PROs) in Treatment-Experienced (TE) People with HIV After Switching to Bictegravir/Emtricitabine/Tenofovir Alafenamide (B/F/TAF): A Pooled Analysis from Observational Cohort Studies

Antinori A[1], Yokomaku Y[2], Elinav H[3], Pullukçu H[4], de Wet J[5], Antela A[6], Lu P[7], Sabranski M[8], Kim Y[9], Bonnet F[10], den Hollander J[11], Jackson A[12], Choy C[13,14], Cai W[15], Zhang [16], Thorpe D[17], Marongiu A[17], Harrison R[17], Jarrett J[17], Boffito M[18]

*[1]National Institute for Infectious Diseases, Lazzaro Spallanzani IRCCS, Rome, Italy, [2]Clinical Research Center, NHO Nagoya Medical Center, Nagoya, Japan, [3]Hadassah University Medical Center, Jerusalem, Israel, [4]Ege University Medical Faculty Hospital, İzmir, Türkiye, [5]Spectrum Health, Vancouver, BC, Canada, [6]Hospital Clínico Universitario de Santiago de Compostela, Universidad de Santiago de Compostela, Madrid, Spain, [7]Kaohsiung Medical University, Kaohsiung, Taiwan, [8]ICH Study Center, Hamburg, Germany, [9]Chungnam National University School of Medicine, Daejeon, Republic of Korea, [10]CHU de Bordeaux and University of Bordeaux, INSERM U1219, Bordeaux, France, [11]Maasstad Hospital, Rotterdam, the Netherlands, [12]Cork University Hospital, Cork, Ireland, [13]National Centre for Infectious Diseases (NCID), Singapore, Singapore, [14]Tan Tock Seng Hospital, Singapore, Singapore, [15]Guangzhou Eighth People's Hospital, Guangzhou Medical Hospital, Guangzhou, China, [16]Beijing Ditan Hospital Capital Medical University, Beijing, China, [17]Gilead Sciences Europe Ltd, Uxbridge, UK, [18]Chelsea and Westminster Hospital NHS Foundation Trust, London, UK*

**Background**: PROs provide insights into individuals' health and well-being and are often included in clinical trials. However, PRO data from observational settings are limited. Here, we report PROs from two large observational cohort studies in treatment-experienced people with HIV switching to B/F/TAF in routine clinical practice.

**Materials and Methods**: Mental/physical health and HIV treatment satisfaction were self-reported by participants using the 36-Item Short Form health survey and the HIV Treatment Satisfaction Questionnaire (HIVTSQ; status [s] and change [c]), respectively. Data were pooled from the BICSTaR cohort study (Asia/Canada/EU/Israel/Japan) and a similarly designed Chinese cohort study (GS-CN-380-5759), representing participants from 15 countries. Descriptive statistics and linear mixed models adjusted for potential confounders and interactions, with bootstrapped confidence intervals (CIs), were used to analyze PROs through 24 months (M). Baseline demographics, clinical characteristics, mental/physical component summary (MCS/PCS), and HIVTSQ scores are presented overall and in underreported populations.

**Results**: This analysis included 3724 treatment-experienced participants (2133 [57.3%] from China), 3004 of whom had MCS/PCS data; 3029 had baseline HIVTSQs data; 2109 had 12M HIVTSQc data. Most participants were Asian (64.2%) and male (89.0%); median age was 41 years. At baseline, 54.6% of participants had comorbidities, including 10.3% with a neuropsychiatric disorder (NPD). The most common prior HIV treatments were integrase strand transfer inhibitors (58.0%) and tenofovir disoproxil fumarate–based regimens (38.6%).

Model-predicted unadjusted MCS and PCS scores increased over time in the overall population; MCS score by +1.8 (95% CI: 1.3–2.3) from mean 48.2 at baseline to 50.0 at 24M, and PCS score by +0.6 (0.2–1.0) from 54.5 to 55.2. Baseline MCS scores were below the population average of 50 despite antiretroviral therapy (ART); PCS scores were above the population average.

In participants with and without baseline NPDs, the predicted-adjusted MCS scores increased from baseline to 24M by +2.5 (1.0–3.6; from 39.9–42.4) and +2.0 (1.5–2.5; from 46.2–48.2), respectively. Predicted-adjusted PCS scores changed by +0.7 (0.4–1.1; from 54.9–55.6) for those with NPDs and by +0.7 (0.4–1.1; from 55.6–56.4) for those without NPDs.

In people with and without baseline comorbidities, the predicted-adjusted MCS scores increased from baseline to 24M by +1.1 (0.5–1.7; from 46.2–47.3) and by +3.8 (3.0–4.6; from 45.7–49.5), respectively. The predicted-adjusted PCS score increased by +0.2 (-0.2–0.6; from 55.1–55.3) and by +1.7 (1.2–2.3; from 55.4–57.1) for those with and without comorbidities, respectively.

Baseline HIVTSQs scores (possible range: 0–60) were high (median: 55 [IQR: 49–60]). Participants reported increased treatment satisfaction at 12M following switch to B/F/TAF, versus their previous regimen (median: HIVTSQc +27 [IQR: 19–30]; possible range -30 to +30).

**Conclusions**: In this large cohort analysis of PROs in people with HIV who switched to B/F/TAF in routine clinical practice, self-reported mental and physical health scores showed small increases over time, along with increases in HIV treatment satisfaction. Further studies are needed to better understand the clinical relevance of changes in PROs in people with HIV receiving ART in routine clinical practice.

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# Post-Hospital Discharge Outcomes of People Living with HIV in Rural Tanzania - Prospective Observational Study

Enzigard E[1]

[1]Ifakara Health Institute (IHI), Morogoro, Tanzania, United Republic of

**Background**: Despite widespread antiretroviral therapy availability and progress toward the 95-95-95 targets, the burden of HIV inpatients in sub-Saharan Africa remains significant. Although East and South Africa have seen a 57% reduction in new HIV infections and a 58% decrease in AIDS-related deaths since 2010, in-hospital mortality for people with HIV (PWH) remains high (10–45%). Late diagnosis, poor adherence, and opportunistic infections such as tuberculosis are major contributors to both in-hospital and post-discharge mortality. Furthermore, patients discharged from hospitals often face high risks of poor outcomes, with up to 50% mortality reported in a,Tanzanian study conducted before widespread ART implementation. However, little is known about post-discharge outcomes in rural settings, despite these areas accounting for a significant proportion of Tanzania's population.

**Materials and Methods**: This study will be nested in the KIULARCO cohort at SFRH and will follow adult PWH admitted between February 2024 and 2026. Data will be captured using OpenMRS, an electronic medical record system. The primary outcome will be the mortality rate within 90 days of discharge. Secondary outcomes include symptom resolution, readmission rates, and lost followup. Data will be analyzed using descriptive statistics, with Mann-Whitney U tests for continuous variables and chi-square tests for categorical data.

**Results**: Out of 140 patients, 53% were female, with a median age of 48 years. The median hospital stay was 5 days, and 24% died during hospitalization Of the 103 patients discharged, 64% completed 90-day follow-up. Post-discharge mortality was 17%, with deaths occurring after a median of 22 days. In-hospital deaths were mainly due to sepsis, tuberculosis, and cryptococcal meningitis. After discharge, most deaths were caused by cardiovascular events, cancer, and kidney failure. These results show a high risk of death during and after hospitalization,highlighting the need for better follow-up care and support for PWH.

**Conclusions**: Our preliminary findings reveal high in-hospital and post-discharge mortality among PWH at SFRH, with sepsis, tuberculosis, and NCDs as major causes. The high 90-day post-discharge mortality highlights the need for better follow-up and continuity of care, especially in rural areas.Ongoing data collection aims to identify risk factors and guide strategies to improve outcomes..

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# Profiles of Body Weight, Glucose, and Uric Acid in PWH on Ainuovirine-Based Antiretroviral Regimen: Week 96 Results from SPRINT, A Randomized Phase 3 trial

Cai W[1], Wu H[2], Zhang F[3], Ma P[4], Zhao Q[5], Wei H[6], Lu H[7], Wang H[7], He S[8], Chen Z[8], Chen Y[9], Wang M[10], Yun X[11], Fu H[11], WANG Y[11]

*[1]Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [2]Beijing Youan Hospital, Capital Medical University, Beijing, China, [3]Beijing Ditan Hospital Capital Medical University, Beijing, China, [4]Tianjin Second People's Hospital, Tianjin , China, [5]The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [6]The Second Hospital of Nanjing, Najing, China, [7]Shenzhen Third People's Hospital, Shenzhen, China, [8]Public Health Clinical Medical Center of Chengdu, Chengdu, China, [9]Chongqing Public Health Medical Center, Chongqing, China, [10]The First Hospital of Changsha, Changsha, China, [11]Jiangsu Aidea Pharmaceutical Co., Ltd., Yangzhou, China*

**Background**: In the SPRINT study, switch to ainuovirine, a novel non-nucleoside reverse transcriptase inhibitor, combined with lamivudine and tenofovir DF (ANV/3TC/TDF), resulted in favorable changes in body weight (BW) and serum uric acid (SUA), but constant fasting serum glucose (FSG), compared to that to cobicistat-boosted elvitegravir with emtricitabine and tenofovir alafenamide (E/C/F/TAF), in virologically suppressed (VS) people with HIV-1 (PWH). In the extensional study, PWH continued or switched to ANV/3TC/TDF until 96 weeks. This secondary analysis aimed to evaluate the changes in BW, FSG, and SUA of PWH switching to ANV/3TC/TDF through 48 to 96 weeks.

**Materials and Methods**: Out of 762 randomized participants, 376 participants originally assigned to ANV/3TC/TDF (376/381, 98.7%) and 371 participants originally to E/C/F/TAF (371/381, 97.4%) completed the extensional study, respectively. The safety outcomes of special interest were absolute changes from 48 in BW, FSG, and SUA at 72 and 96 wk.

**Results**: Modest decline in BW was seen in both treatment arms at 72 wk (mean, ANV/3TC/TDF vs. E/C/F/TAF→ANV/3TC/TDF, -0.47 vs. -1.14 kg), to a lesser extent at 96 wk (-0.03 vs. -0.59 kg) at 96 wk. FSG remained constant in both arms from 48 wk through 96 wk (72 wk, 0.7 vs. -0.10 mmol/L; 96 wk, 0.04 vs. 0.00 mmol/L). Compared to that in ANV/3TC/TDF arm, SUA showed a marked and constant reduction in E/C/F/TAF→ANV/3TC/TDF arm at both 72 wk (24.4 vs. -17.8 µmol/L), and 96 wk (-2.9 vs. -37.6 µmol/L).

**Conclusions**: Continuation of ANV/3TC/TDF treatment resulted in constant profiles of BW, FSG and SUA for VS PWH. However, switch from E/C/F/TAF favored profiles of BW and SUA in the SPRINT extensional period in contrast to that to E/C/F/TAF in the randomized study.

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# Bone Mineral Density in People Living with HIV

Matsiyeuskaya N[1], Gvazdeluk V[1], Alizarovich J[2]

[1]Grodno State Medical University, Grodno, Belarus, [2]Grodno City Clinical Hospital of Emergency Medical Care, Grodno, Беларусь

**Background**: Osteoporosis is a metabolic skeletal disease characterized by decreased bone mass, impaired bone microarchitecture and, as a consequence, fractures with minimal trauma. HIV infection is a proven risk factor for the development of secondary osteoporosis. It is known that the incidence of decreased BMD- by 6.4 times, and osteoporosis - by 3.7 times higher in people living with HIV (PLHIV) compared to uninfected individuals of the same age (I. V. Polozhaeva, 2018).

**Objective**: To present the characteristics of PLHIV with decreased BMD.

**Materials and Methods**: The BMD parameters of 64 adult PLHIV (aged 22 to 68 years; 33 men, 31 women) were assessed using a DMS/Stratos DR X-ray osteodensitometer and statistically analyzed in Excel 2010. The patients were divided into 2 groups: Group 1 included 26 patients with impaired BMD (9 men, 17 women, average age 49.38 years); Group 2 included 38 patients without impaired BMD (24 men, 14 women, average age 40.57 years).

**Results**: Decreased BMD according to densitometry results was found in 26 of 64 (40.63%) patients, among them 22 patients (34.38%) had osteopenia, 4 (6.25%) had osteoporosis.. In general, among PLHIV included in the study, the frequency of impaired BMD among women was 54.8%, among men - 27.3%, p<0.05. In this case, In the 1st group: the average age of women was 52.11 years. Of the 17 women, 10 (58.82%) were in menopause, the average age of which was 48.3 years, the menopause experience was 11.6 years; the average age of men was 44.22 years.

In the 2nd group, women were younger, since the average age was 39.6 years, p < 0.05. Among the 14 women, 2 (14.29%) were in menopause (p<0,05 in compare with the 1st group), the average age of which was 46 years, the menopause experience was 7.5 years; the average age of men was 41.13 years.

The duration of ART in the 1st and 2nd groups was 6 years. The average "delay" in prescribing ART in group 1 was 4 years, in group 2 - 2 years (p<0,05). Tenofovir-containing regimen was received by 73.1% of patients in group 1 and 73.7% of patients in group 2, p> 0.05.

**Conclusions**: The proportion of PLHIV with decreased BMD in the study group was 40.6%, including 54.8% among women and 27.3% among men. The age of osteopenia development in PLHIV was lower than expected (44.22 instead of 60 years for men and 52.1 instead of 55.0 years for women). Impaired BMD among PLHIV was associated with classic risk factors for osteopenia - older age, the presence and duration of menopause in women. Later prescription of ART is also associated with decreased BMD. All these factors need to be taken into account when monitoring PLHIV receiving ART.

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# Distribution of CCR5-Delta32, CCR2-64I, and SDF1-3'A Host Genetic Factors in HIV-Infected and Uninfected Individuals in Luanda, Angola

Sebastião C[1], Pimentel V[2], Jandondo D[3], Kinanga J[3], Sacomboio E[4], Pingarilho M[2], Brito M[5], Cassinela E[1], Vasconcelos J[3], Abecasis A[2], Morais J[3]

*[1]Centro Nacional de Investigação Científica (CNIC), Luanda, Angola, Luanda, Angola, [2]Global Health and Tropical Medicine, GHTM, Associate Laboratory in Translation and Innovation Towards Global Health, LA-REAL, Instituto de Higiene e Medicina Tropical, IHMT, Universidade NOVA de Lisboa, UNL, Rua da Junqueira 100, 1349-008 Lisboa, Portugal, Lisboa, Portugal, [3]Centro de Investigação em Saúde de Angola (CISA)|Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola, Luanda, Angola, [4]Instituto de Ciências da Saúde (ICISA), Universidade Agostinho Neto (UAN), Luanda, Angola, Luanda, Angola, [5]H&TRC - Health & Technology Research Center, ESTeSL - Escola Superior de Tecnologia da Saúde, Instituto Politécnico de Lisboa, Lisbon, Portugal, Lisboa, Portugal*

**Background**: The HIV/AIDS pandemic remains a public health concern. Studies on host genetic polymorphisms that confer resistance to HIV-1 infection or delay HIV disease progression are scarce in African countries. Herein, we investigate the allelic frequencies of the AIDS-related polymorphisms CCR5-Delta32, CCR2-64I, and SDF1-3'A in HIV-infected and uninfected individuals in Luanda, the capital of Angola, a sub-Saharan African country.

**Materials and Methods**: This was a cross-sectional study conducted with 284 individuals, of which 159 were HIV-negative and 125 HIV-positive. The CCR5-Delta32, CCR2-64I, and SDF1-3'A genotypes were detected by conventional PCR and visualized on 2% agarose gel. A Chi-square test determined the frequency of each genetic variant and was deemed significant when $p<0.05$.

**Results**: The frequency of CCR5-Delta32, CCR2-64I, and SDF1-3A were 0% (0/272), 60.2% (154/256), and 42.5% (114/268), respectively. CCR2-64I and SDF1-3A polymorphisms were statistically related to HIV infection ($p<0.001$). Statistically significant was observed between ABO blood groups ($p=0.006$) and HIV-1 subtype ($p=0.015$) with CCR2-64I. Also, the age group ($p=0.024$) and RH blood group ($p=0.018$) were statistically related to the distribution of SDF1-3A polymorphism.

**Conclusions**: We found no CCR5-Delta32 allele, while CCR2-64I and SDF1-3'A were found and presented a relationship with HIV infection, age, ABO/RH blood group, and HIV-1 subtypes. Further studies investigating biological and non-biological factors related to susceptibility to HIV infection and AIDS progression or death should be conducted in Angola.

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# Trends in Active Hepatitis B Virus Infection and Associated Risk Factors Among Blood Donor Candidates from Luanda, Angola

Sebastião C[1], Machado M[2], Vigário J[2], Comandante F[2], Armando A[3], Silva M[4], Sebastião J[5], Manico E[2], Machado D[2], Pimentel V[6]

*[1]Centro Nacional de Investigação Científica (CNIC), Luanda, Angola, Luanda, Angola, [2]Instituto Nacional de Sangue (INS), Ministério da Saúde, Luanda, Angola, Luanda, Angola, [3]Centro de Estudos, Investigação Científica e Pós-Graduação, Universidade Privada de Angola, Luanda, Angola, Luanda, Angola, [4]Faculdade de Medicina (FM), Universidade Katyavala Bwila (UKB), Benguela, Angola, Benguela, Angola, [5]Centro de Investigação em Saúde de Angola (CISA)|Instituto Nacional de Investigação em Saúde (INIS), Luanda, Angola, Luanda, Angola, [6]Global Health and Tropical Medicine (GHTM), Associate Laboratory in Translation and Innovation Towards Global Health (LA-REAL), Instituto de Higiene e Medicina Tropical (IHMT), Universidade NOVA de Lisboa (UNL), Rua da Junqueira 100, 1349-008 Lisboa, Portugal, Lisboa, Portugal*

**Background**: Hepatitis B virus (HBV) infection is a global public health concern with a high burden in the African region. Assessing trends in HBV infection over time can provide insights into the effectiveness of prevention/treatment strategies in different settings. Herein, we investigate trends in active HBV infection and associated risk factors among blood donor candidates from Luanda, the capital city of Angola, a sub-Saharan Africa country.

**Materials and Methods**: This was a retrospective study conducted with 96654 medical records of blood donors consulted between 2018 and 2022 at the Angolan National Blood Institute. Participants were screened for hepatitis B surface antigen (HBsAg). Chi-square and logistic regression were used to analyse interactions between demographic variables with significance at $p<0.05$.

**Results**: Active HBV infection was 10% (95% CI: 9.4–10.6). Men (AOR: 1.38, $p<0.001$), employed (AOR: 3.25, $p<0.001$) and non-urbanised regions (AOR: 1.16, $p=0.019$), were more likely to contract the HBV, while aged 30 years or older (AOR: 0.78, $p<0.001$) and married (AOR: 0.66, $p<0.001$), were less likely to contract the infection. From 2018 to 2022, HBV infections increased from 18.2 to 21.9%. Infections increased in under 30 years (31.3% to 52.5%, $p<0.001$), males (91.1 to 91.8%, $p=0.149$), urbanised regions (2.7 to 3.3%, $p=0.538$), and unmarried (93.9 to 95.8%, $p=0.019$).

**Conclusions**: We revealed a highly active HBV infection over the past 5 years in the adult population of Luanda, Angola. Age, sex, occupation, place of residence and marital status have influenced the dissemination of HBV in Angola. Our findings may facilitate the planning and evaluation of the HBV control program in Angola.

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# Cardiometabolic Comorbidity and Pharmacotherapy Burden on Virologically Suppressed People with HIV-1: Week 96 Results from SPRINT, A Randomized Phase 3 Trial

Wu H[1], Zhang F[2], Cai W[3], Ma P[4], Zhao Q[5], Wei H[6], Lu H[7], Wang H[7], He S[8], Chen Z[8], Chen Y[9], Wang M[10], Yun X[11], Fu H[11], WANG Y[11]

*[1]Beijing Youan Hospital, Capital Medical University, Beijing, China, [2]Beijing Ditan Hospital, Capital Medical University, Beijing, China, [3]Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [4]Tianjin Second People's Hospital, Tianjin, China, [5]The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [6]The Second Hospital of Nanjing, Nanjing, China, [7]Shenzhen Third People's Hospital, Shenzhen, China, [8]Public Health Clinical Medical Center of Chengdu, Chengdu, China, [9]Chongqing Public Health Medical Center, Chongqing, China, [10]The First Hospital of Changsha, Changsha, China, [11]Jiangsu Aidea Pharmaceutical Co., Ltd., Yangzhou, China*

**Background**: Cardiometabolic safety has become the primary therapeutic concern among people with HIV-1 (PWH), even those virologically suppressed. This secondary longitudinal analysis aimed to evaluate cardiometabolic comorbidity, and pharmacotherapy burden on PWH virologically suppressed on antiretroviral (ARV) treatment, including non-nucleoside reverse transcriptase inhibitor- (NNRTI), and boosted integrase strand transferase inhibitor- (INSTI) based, in the SPRINT study, a multi-center, randomized, active-controlled phase 3 trial.

**Materials and Methods**: A total of 923 PWH were screened for eligibility of participation in the SPRINT study, comparing efficacy and safety of switch to ainuovirine, a novel NNRTI, plus 3TC/TDF (ANV/3TC/TDF), and that to cobicistat-boosted elvitegravir plus emtricitabine and tenofovir alafenamide (E/C/F/TAF). Main inclusion criteria included age of 18 to 65 years (inclusive), stably remaining on previous NNRTI-based ARV regimen for at least 12 months, and confirmed viral suppression at an interval of at least one month without any restriction on CD4+ cell count. Participants would be excluded if with AIDS-defining conditions, or complicating moderate, or severe liver, and/or renal impairment. Medical history, and comedications were coded using MedDRA, and WHODD, respectively.

**Results**: Finally, 762 PWH were enrolled, at a median age of 33 yr, with 97.1% being male, and on ARV regimen for a median duration of 56 months; 35.5% of participants were overweighted or obese (BMI ≥24 kg/m2). Common pre-existing cardiometabolic comorbidities or conditions included fatty liver disease (23.0%), increased LDL-C (6.3%), and increased triglyceride (5.9%). The frequencies of most cardiometabolic comorbidities, especially fatty liver disease, dyslipidemia, and hyperuricemia, were numerically lower with ANV/3TC/TDF regimen than those with E/C/F/TAF regimen through 48 weeks, but similar between ANV/3TC/TDF arm and late switch to ANV/3TC/TDF arm 48 to 96 weeks. Diagnosis of diabetes mellitus, or gout was rarely established through 96 weeks. Concomitant pharmacotherapy was also occasionally given through 96 weeks.

**Conclusions**: Cardiometabolic conditions, especially fatty liver disease, dyslipidemia, and hyperuricemia, are prevalent among virologically suppressed PWH, and mainly left unmanaged through ARV treatment. Identification and multidisciplinary care of these conditions are warranted in these PWH, even at a relatively low risk of cardiovascular disease.

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# Favorable Lipid Profile in People with HIV-1 Switching to Ainuovirine-Based Antiretroviral Regimen: Week 96 Results from SPRINT, a Randomized Phase 3 Trial

Ma P[1], Wu H[2], Zhang F[3], Cai W[4], Zhao Q[5], Wei H[6], Lu H[7], Wang H[7], He S[8], Chen Z[8], Chen Y[9], Wang M[10], Yun X[11], Fu H[11], WANG Y[11]

*[1]Tianjin Second People's Hospital, Tianjin, China, [2]Beijing Youan Hospital, Capital Medical University, Beijing, China, [3]Beijing Ditan Hospital Capital Medical University, Beijing, China, [4]Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [5]The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [6]The Second Hospital of Nanjing, Nanjing, China, [7]Shenzhen Third People's Hospital, Shenzhen, China, [8]Public Health Clinical Medical Center of Chengdu, Chengdu, China, [9]Chongqing Public Health Medical Center, Chongqing, China, [10]The First Hospital of Changsha, Changsha, China, [11]Jiangsu Aidea Pharmaceutical Co., Ltd., Yangzhou, China*

**Background**: In the SPRINT study, switch to ainuovirine, a novel non-nucleoside reverse transcriptase inhibitor, combined with lamivudine and tenofovir DF (ANV/3TC/TDF), resulted in favorable changes in body weight and serum lipids, compared to that to cobicistat-boosted elvitegravir with emtricitabine and tenofovir alafenamide (E/C/F/TAF), in virologically suppressed (VS) people with HIV-1 (PWH). In the extensional study, PWH continued or switched to ANV/3TC/TDF until 96 weeks. This secondary analysis aimed to evaluate the lipid profile of PWH switching to ANV/3TC/TDF through 48 to 96 weeks.

**Materials and Methods**: Out of 762 randomized participants, 376 participants originally assigned to ANV/3TC/TDF (376/381, 98.7%) and 371 participants originally to E/C/F/TAF (371/381, 97.4%) completed the extensional study, respectively. The safety outcomes of special interest were absolute changes from 48 wk in LDL-C, non-HDL-C, total cholesterol (TC), triglyceride (TG), and HDL-C at 72 and 96 wk. Dyslipidemia severity was evaluated using the atherosclerotic cardiovascular disease (ASCVD) risk stratification as per the primary prevention target for Chinese low-risk adults.

**Results**: Serum LDL-C remained unchanged in ANV/3TC/TDF arm from 48 wk, while a marked reduction was seen in E/C/F/TAF→ANV/3TC/TDF arm at both 72 wk (mean, -0.07 vs. -0.47 mmol/L) and 96 wk (0.01 vs. -0.30 mmol/L). Similar trends were seen in the changes in non-HDL-C, TC, and TG: 72 wk, -0.04 vs. -0.71 mmol/L, -0.06 vs. -0.87 mmol/L, and 0.06 vs. -0.79 mmol/L, respectively; 96 wk, 0.08 vs. -0.60 mmol/L, 0.05 vs. -0.77 mmol/L, and 0.09 vs. -0.85 mmol/L, respectively. Modest decline was not observed in ANV/3TC/TDF arm but E/C/F/TAF→ANV/3TC/TDF arm (72 wk, -0.02 vs. -0.16 mmol/L; 96 wk, -0.03 vs. -0.17 mmol/L). ASCVD risk-associated dyslipidemia stratifications showed comparable profiles between these two arms regarding LDL-C, non-HDL-C, TC, TG, an HDL-C. Marked improvement in LDL-C stratification was seen at both 72 wk and 96 wk for E/C/F/TAF→ANV/3TC/TDF arm.

**Conclusions**: Switch to ANV/3TC/TDF from E/C/F/TAF is favored for improved serum lipid profile and ASCVD risk-associated dyslipidemia in VS PWH.

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# Efficacy, and Safety of Ainuovirine-Based Antiretroviral Regimen in Women with HIV-1: The Pooled Analysis of the RACER, and SPRINT Studies, Two Multicenter, Randomized, Active Controlled Phase 3 Trials

Wang H[1], Wu H[2], Cai W[3], Ma P[4], Zhao Q[5], Wei H[6], Lu H[7], Zhang F[8], He S[9], Chen Z[10], Chen Y[11], Wang M[12], Yun X[13], WANG Y[13]

*[1]Department of Infectious Diseases, Shenzhen Third People's Hospital, Shenzhen, China, [2]Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, China, [3]Infectious Disease Center, Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [4]Department of Infectious Diseases, Tianjin Second People's Hospital, Tianjin, China, [5]Department of Infectious Diseases, The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [6]Department of Infectious Diseases, The Second Hospital of Nanjing, Najing, China, [7]National Clinical Research Centre for Infectious Diseases, The Third People' s Hospital of Shenzhen, The Second Affiliated Hospital of Southern University of Science and Technology., Shenzhen, China, [8]Clinical and Research Center for Infectious Diseases, Beijing Ditan Hospital Capital Medical University, Beijing, China, [9]Department of Infectious Diseases, Public Health Clinical Medical Center of Chengdu, Chengdu, China, [10]Drug Clinical Trial Institution, Public Health Clinical Medical Center of Chengdu, Chengdu, China, [11]Division of Infectious Diseases, Chongqing Public Health Medical Center, Chongqing, China, [12]Institute of HIV/AIDS, The First Hospital of Changsha, Changsha, China, [13]Clinical Research & Development, Jiangsu Aidea Pharmaceutical Co., Ltd., Yangzhou, China*

**Background**: In the RACER, and SPRINT studies, ainuovirine (ANV) plus lamivudine and tenofovir DF (ANV/3TC/TDF) demonstrated durable virological suppression non-inferior to currently approved antiretroviral regimens in people with HIV-1 (PWH). This integrated analysis aimed to evaluate the virological outcomes, and safety profile of ANV/3TC/TDF in women with HIV-1 (WWH) at week 48.

**Materials and Methods**: In the RACER study (N=34), 18, and 16 treatment-naïve WWH were randomized, and medicated with ANV/3TC/TDF, and EFV/3TC/TDF, respectively. In the SPRINT study (N=22), 11, and 11 virologically suppressed WWH were randomized, and medicated with ANV/3TC/TDF, and E/C/F/TAF, respectively. Viral suppression was defined as HIV-1 RNA titer below 50 copies/mL. Safety outcomes included common (≥10%) adverse events (AEs), with special interest in serum lipids.

**Results**: High proportion of viral suppression was achieved in either arm at 48 wk, and in both studies. Change from baseline in CD4 cell absolute count was comparable between the two arms in the treatment-naïve women at 48 wk; that was numerically higher with the ANV/3TC/TDF arm compared to that with the E/C/F/TAF arm (28.5±96.3 vs. -42.6±83.0 cells/mm3). Frequencies of treatment-emergent adverse events, and adverse drug reactions (ADRs) were also comparable between the two arms in both studies. Common AEs consisted mainly of neurological symptoms, and laboratory abnormalities. These AEs were generally similar between the two arms in both studies; however, the difference in frequency of dizziness was statistically significant for the treatment-naïve women (16.7% vs. 68.8%, P = 0.006), and those in frequencies of abnormal serum cholesterol, LDL-C, and T-wave on ECG were also statistically significant for the virologically suppressed women (all P-values <0.05). Serum LDL-C, and/or total cholesterol showed a marked divergence in change from baseline (CFB) between the two arms for either study, with a decreasing trend in the ANV/3TC/TDF arm in contrast to an increasing trend in the comparator arm, even at a statistical significance for total cholesterol in the virologically suppressed women.

**Conclusions**: Although at a small sample size, ANV/3TC/TDF regimen showed high viral suppression comparable to the comparators in both treatment-naïve, and virologically suppressed women.

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# The HCV Core Antigen Detection for Evaluation of Direct-Acting Antiviral Therapy for Patients with Chronic Hepatitis C

Matsiyeuskaya N[1], Khombak V[1]

[1]Grodno State Medical University, Grodno, Belarus

**Background**: HCV core antigen (HCV-cAg) is a serological indicator of viral replication, which allows distinguishing previous HCV infection from current infection. HCV-cAg can also be used to monitor the effectiveness of antiviral therapy and predict sustained virologic response (SVR).

**Aim of the study**: to evaluate the HCV-cAg antigen detection for assesmant of Direct-Acting antiviral therapy for patients with chronic hepatitis C (HCV).

**Materials and Methods**: The study involved 51 patients with CHC (age, Me, (Q1;Q3) - 43 (33;55); male patients - 25 (49.02%); genotype 1 CHC - 29 (58%); genotype 3 CHC - 20 (40%)) who achieved a sustained virological response on antiviral therapy with direct-acting antiviral drugs (DAA) based on the results of HCV RNA determination by PCR. Detection of core AG CHC was performed by immunochemical analysis on an ARCHITECT ABBOTT i1000SR automatic immunochemical analyzer. Detection of HCV RNA by PCR was performed on a CFX96 Touch amplifier in real time manufactured by Bio-Rad.
For statistical analysis, the R programming language version 4.1.0 with the ggplot2 extension package for graphical display of data was used. Data are presented as Me (Q1;Q3).

**Results**: All patients in the study group achieved an undetectable HCV viral load after DAA treatment. In all patients in the study group a significant decrease in HCV-cAg value was noted. The agreement between the test results by the two methods in the study group was considered if the HCV-cAg value was less than 3 fmol/l – 30 (58.8%) patients. In 21 (41.2%) patients, the HCV-cAg value was higher than 3 fmol/l, while in 19 (37.3%) of them, the values were in the range of weakly positive results - more than 3 but less than 10 fmol/l.

With the HCV-cAg value of 8 fmol/l or less, the agreement with the undetectable HCV RNA viral load according to PCR diagnostics was 96.1%. In this regard, the HCV-cAg value of 8 fmol/l or less is recommended as a criterion for SVR to DAA therapy.

**Conclusions**: The HCV-cAg indicator with cutt of point equal to 8 fmol/l could be effectivelly used for confirming SVR to DAA therapy in patients with CHC.

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# Cardiac, Liver, Renal, and Other Safety Profiles in PWH on Ainuovirine-Based Antiretroviral Regimen: Week 96 Results from SPRINT, A Randomized Phase 3 Trial

Zhao Q[1], Wu H[2], Zhang F[3], Cai W[4], Ma P[5], Wei H[6], Lu H[7], Wang H[7], He S[8], Chen Z[8], Chen Y[9], Wang M[10], Yun X[11], Fu H[11], WANG Y[11]

*[1]The Sixth People's Hospital of Zhengzhou, Zhengzhou, China, [2]Beijing Youan Hospital, Capital Medical University, Beijing, China, [3]Beijing Ditan Hospital Capital Medical University, Beijing, China, [4]Guangzhou Eighth People's Hospital, Guangzhou Medical University, Guangzhou, China, [5]Tianjin Second People's Hospital, Tianjin, China, [6]The Second Hospital of Nanjing, Nanjing, China, [7]Shenzhen Third People's Hospital, Shenzhen, China, [8]Public Health Clinical Medical Center of Chengdu, Chengdu, China, [9]Chongqing Public Health Medical Center, Chongqing, China, [10]The First Hospital of Changsha, Changsha, China, [11]Jiangsu Aidea Pharmaceutical Co., Ltd., Yangzhou, China*

**Background**: In the SPRINT study, switch to ainuovirine, a novel non-nucleoside reverse transcriptase inhibitor, combined with lamivudine and tenofovir DF (ANV/3TC/TDF), resulted in an improving trend in liver and renal function in virologically suppressed (VS) people with HIV-1 (PWH). In the extensional study, PWH continued ANV/3TC/TDF or switched to ANV/3TC/TDF from cobicistat-boosted elvitegravir with emtricitabine and tenofovir alafenamide (E/C/F/TAF) until 96 weeks. This secondary analysis aimed to evaluate the profiles of cardiac, liver, renal, musculoskeletal, and pancreatic safety in PWH switching to ANV/3TC/TDF through 48 to 96 weeks.

**Materials and Methods**: Out of 762 randomized participants, 376 participants originally assigned to ANV/3TC/TDF (376/381, 98.7%), and 371 participants originally to E/C/F/TAF (371/381, 97.4%) completed the extensional study, respectively. Changes from baseline (CFBs) in QTcF interval on electrocardiography, and clinical laboratory examinations were primary safety outcomes of interest, including blood biochemistry, as prespecified. Other prespecified secondary safety outcomes of interest included cardiac, liver, renal, musculoskeletal, and pancreatic adverse events (AEs) by MedDRA.

**Results**: The two arms showed no significant changes in QTcF interval at week 72 or 96; only one participant continuing ANV/3TC/TDF had QTcF prolongation >60 msec at week 96. Liver enzymes remained unchanged in ANV/3TC/TDF arm but increased marginally in E/C/F/TAF→ANV/3TC/TDF arm, including ALT (Figure 1, upper panel), AST, GGT, ALP, and LDH. Both total and direct bilirubin remained unchanged in both arms. Blood urea nitrogen became constant in both arms, and serum creatinine remained unchanged in ANV/3TC/TDF arm but decreased slightly in E/C/F/TAF→ANV/3TC/TDF arm at week 96 (1.0±7.7 vs. -2.7±8.1 µmol/L). This accompanied a marginal change in estimated glomerular filtration rate at week 96 (-1.1±7.4vs. 2.5±8.3mL/min/1.73m2). Serum phosphates remained constant; creatine kinase decreased slightly (-80.4±768.1 vs. -58.3±1232.3 U/L); and amylase remained constant (-0.05±16.5 vs. -0.17±17.0 U/L). Common organ-associated AEs (≥5% in either arm) included elevated AST (5.8% vs. 6.9%), and elevated GGT (4.5% vs. 6.4%).

**Conclusions**: Switch to ANV/3TC/TDF is well tolerated in VS PWHs, without clinically significant changes in organ-associated biochemical measures observed. Routine safety laboratory monitoring is generally recommended in PWHs on switching therapy.

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# From Viral Rebound to Precision Therapy: CRISPR-Cas9 and the Future of HBV Management

Resham S[1], Adnan F, Manzoor S, Khan H

[1]*ASAB, National University of Sciences and Technology, islamabad, Pakistan*

**Background**: Chronic HBV infection is a major global health issue, causing ~1 million deaths annually and affecting ~250 million people. HBV and HCV are leading causes of liver cancer and mortality, exceeding malaria and tuberculosis. Viremia often rebounds after treatment cessation, despite NAs suppressing HBV replication below detectable levels. HBV encodes HBx and integrates into the host genome. Cas9 enables precise genome editing using ~20-nucleotide guide RNAs targeting sequences near protospacer-adjacent motifs (PAMs).

**Problem**: Current antiviral therapy fails to cure chronic hepatitis B (HBV) infection, primarily because of cccDNA of HBV. Amongst other limitations of current anti-HBV treatment, failure to eliminate the viral covalently closed circular DNA (cccDNA) and emergence of resistance remain the most worrisome.

**Materials and Methods**: The HBx gene is selected and gRNA is designed using CHOPCHOP. The gRNA is synthesized and cloned into a mammalian expression vector using SnapGene. The plasmid is sent for custom DNA cloning and transformed into DH5α E. coli, with antibiotic selection ensuring uptake. Plasmid confirmation is done via gel electrophoresis. HepG2 cells are transfected using Lipofectamine, followed by puromycin selection. After 48 hours, the plasmid is extracted and quantified using Nanodrop. This workflow ensures efficient cloning, validation, and functional testing of the HBx construct for CRISPR-based applications. The CRISPR/Cas9 system is a newly developed programmable genome editing tool and allows for sequence-specific cleavage of DNA.

**Results**: The plasmid vector constructed(Anti-Hbx), was propagated in E. coli (DH5alpha) cells (ampicillin resistant), isolated, restriction digested for confirmation. Stored at -80 for further testing. Cell-line based testing on HepG2 cells in the presence of puromycin (puromycin resistance). After cells seeding 2.2x105 cells/ul per six well plate, cells were co-transfected with Hbx expressing plasmid+ CRISPR-plasmid (Anti-Hbx) through Lipofectamine mediated transfection. After 48hr incubation, using Hirt's method of extrachromosomal DNA extraction was performed transfected cells, experiment was run in triplicates. Two control wells were used (one with only HBx expression plasmid another with only CRISPR plasmid (with puromycin). Plasmid confirmation was measured using nanodrop value.

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# HCV Outreach Screening Using Quick Tests by Drug Users' Peers: First French Experience

Remy A[1]

[1]Mobile Hepatitis Team, Perpignan, France

**Background**: In 2016, the WHO set an ambitious goal to eliminate hepatitis C as a major public health threat by 2030. From 2016 French Health Ministry guidelines and French hepatology association recommendations were to treat all inmates and drug users, even fibrosis level with direct antiviral agents (DAA). Success rate of DAA was 95 to 97%. Nevertheless, access of HCV screening, care and treatment in drugs users, prisoners and homeless was low in France. The Mobile Hepatitis Team (MHT) was set up in 2013 and already cured almost 1000 HCV patients in 10 years in an outreach and test to cure approach. MHT used HCV POCT, HCV real time viral load by GENEXPERT system, liver fibrosis measure by FIBROSCAN and DAA nurse prescription. Despite all our actions, there persisted drug users not detected and/ or not treated in our 700 000 people area of operations. We have also highlighted more and more HCV recontaminated patients in our local experience (27% of total HCV treated patients by our team in 2022).

**Materials and Methods**: Our objective waso find HCV patients who are not screened or treated by a screening action performed by trained peers and refer them to a hepatitis mobile team in a test and cure approach., in the street or drug use spots.

After 2 days specific training for HCV and screening, we recruited 38 peer patients to perform HCV quick tests by fingersticks with drug users never detected or no longer knowing the result of previous screenings. All patients screened received an information leaflet and gave their consent to participate in the study. The peer patients were remunerated on the number of tests performed, regardless of the HCV result. We have planned to screen 450 drug users in 6 months.

**Results**: We contacted 38 drug users who had agreed to participate in the project but only 15 of them came to do the training. The first screening took place on February 15, 2024. As 9 months, 410 tests were performed by 7 different patients including 78 positives (19%). Twenty-two patients were HCV RNA positive (27%) and were treated by DAA. Seventeen patients were recontaminated by drug use. The typical patient of recontamination group was a man, 42 years, drug user and homeless. Twelve months results will be presented in the congress.

**Conclusions**: Our results support the hypothesis that peer HCV screening was simple and effective tool that frees up physician time, enhances nursing work and increased HCV cured patients, who were never been engaged in care process without this peer screening. There are no disadvantages highlighted after one month of operation. The strengths of this study are that the data were comprehensive and there was a large cohort of patients engaged in the screening action.

Conflicts of interest : this research was supported by GILEAD Sciences

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# A Simplified HCV Management Algorithm in Opioid Agonist Treatment Clinics in Ukraine: First Implementation Experience

Filippovych M[1], Dumchev K[1], Kiriazova T[1], Ivanchuk I[2]

[1]*Ukrainian Institute on Public Health Policy, Kyiv, Ukraine,* [2]*Public Health Center of the Ministry of Health of Ukraine, Kyiv, Ukraine*

**Background**: HCV prevalence among people receiving opioid agonist treatment (OAT) in Ukraine is 83%. Due to stigmatization from medical providers and low awareness of recent guidelines, the majority are not included in the direct-acting antiviral (DAA) treatment waiting lists, and due to low income cannot afford medications from pharmacies. An implementation study of a Simplified HCV Integrated Management (SHIM) algorithm in OAT clinics in Ukraine was piloted in 2021 and the main stage is being conducted since 2022 during complicated war conditions.

**Materials and Methods**: In eight geographically distant oblast OAT clinics, SHIM algorithm has been implemented, including 1) HCV RNA test for diagnosis confirmation; 2) Minimum of pretreatment evaluation (no genotyping, fibrosis assessment by APRI/FIB); 3) Pan-genotypic treatment regimens provided at OAT clinic; 4) On-site treatment monitoring (adherence, side effects, drug interaction assessment), counseling by OAT staff; 5) Sustained virologic response (SVR) confirmation. The costs of the laboratory tests are not covered, medications are provided for free. Quantitative and qualitative data are being collected.

**Results**: In the pilot phase, treatment completion was 83% (20/24; 2 died of COVID-19 and an accident, 2 dropouts), and SVR was achieved in 95% (19/20). 23/24 who started treatment (96%) considered the opportunity to treat HCV in the OAT clinic very valuable and the attitude was cited as “a good fortune”. The same 23 participants considered the OAT clinic the optimal place to receive HCV medications (no one preferred a specialized or primary care clinic - “we are just nothing to them”).
In the main phase, 438 patients started treatment in OAT clinics under the SHIM algorithm and 424 completed treatment (1—loss of contact due to change of residence, 1—interruption of therapy, 1—imprisonment, 4—death from causes unrelated to HCV treatment, 7—have not yet completed the treatment course). SVR was achieved in 96% (366/380). HCV and OAT treatment in the same place by one physician who “takes you by the hand and guides you professionally” was important and valuable for patients.

**Conclusions**: The availability of free HCV treatment at the OAT site significantly increases access to this life-saving treatment, improving the OAT program's attractiveness. The clinical effectiveness of HCV treatment using SHIM was high and comparable to other treatment modalities.

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# Adolescents with Confirmed Virological Failure Stand at Risk of Acquiring Resistance to Dolutegravir and Need Novel Treatment Options: Findings During the Early Stage of TLD Transition in the CIPHER-ADOLA Study

Bouba Y[1,2], Ndiang Tetang S[3], Nka A[2], Ka'e A[2], Gouissi D[2,14], Anong Beloumou G[2,6], Takou D[2], Simo Kamgaing R[2], Kamgaing N[2], Sosso S[2], Ngoufack Jagni Semengue E[2], Forgwei L[2], Ayafor C[2], Tommo Tchouaket M[2], Nguendjoung Fainguem N[2], Efakika Gabisa J[2], Ndongo Ateba F[4], Abate M[5], Wome Basseck R[2], Wiadamong A[2], Gnambi A[2], Etame N[2,6], Kengni A[2,6], Moko L[2,14], Yimga J[2], Ambe Chenwi C[2,6], Djupsa S[2], Noukayo F[7], Nga Motaze A[3], Ketchaji A[8], Njom Nlend A[9], Penda C[9], Armenia D[1], Colizzi V[10], Ndombo Koki P[4], Ndjolo A[2], Ceccherini-Silberstein F[6], Santoro MM[6], Perno C[11], Fokam J[2,12,13]

*[1]Saint Camillus International University of Health Sciences, Rome, Italy, [2]Chantal BIYA International Reference Centre for Research on HIV/AIDS Prevention and Management, Yaoundé, Cameroon, [3]Essos Hospital Centre, Yaoundé, Cameroon, [4]Mother and Child Centre, Chantal BIYA Foundation, Yaoundé, Cameroon, [5]Mbalmayo District Hospital, Mbalmayo, Cameroon, [6]Department of Experimental Medicine, University of Rome Tor Vergata, Rome, Italy, [7]Cité Verte District Hospital, Yaoundé, Cameroon, [8]Department of Disease, Epidemics and Pandemic Control, Ministry of Public Health, Yaoundé, Cameroon, [9]Department of Clinical Sciences, Higher Institute of Medical Technology, University of Douala, Douala, Cameroon, [10]UNESCO Board of Multidisciplinary Biotechnology, University of Rome Tor Vergata, Rome, Italy, [11]Bambino Gesù Children Hospital, IRCCS, Rome, Italy, [12]Central Technical Group, National AIDS Control Committee, Yaoundé, Cameroon, [13]Faculty of Health Sciences, University of Buera, Buea, Cameroon, [14]Faculty of Medicine, University of Yaoundé 1, Yaoundé, Cameroon*

**Background**: Progress in pediatric HIV programs may be jeopardized as children grow toward adolescence, especially among adolescents with perinatal HIV-infection (APHI) in low- and middle-income countries (LMICs) where limited treatment regimens could lead to poor virological response, HIV drug resistance (HIVDR) emergence and the need for newer treatment options. Thus, we sought to evaluate viral load (VL) response and HIVDR among APHI receiving tenofovir-lamivudine-dolutegravir (TLD).

**Materials and Methods**: A multi-centre cohort-study was conducted among APHI receiving TLD in the Centre-region of Cameroon. Enhanced adherence counseling (EAC) was provided during 3-months to APHI with baseline-VL≥50 copies/ml. After follow-up VL, low-level viremia (LLV) and virological failure (VF) were defined as 50-999 and ≥1000 copies/mL, respectively. Among those experiencing VF, genotypic-HIVDR was performed in protease, reverse-transcriptase and integrase regions, and interpreted using HIVdb.

**Results**: Among 252 enrollees (58.6% female, median [IQR] age: 15 [13-17]), 27.8% (n=70) had baseline-VL≥50 copies/ml including 44.3% LLV (n=31) and 55.7% VL≥1000 (n=39). The most common ART-backbones before TLD were ABC+3TC (50.0%) and TDF+3TC (41.4%) and median duration on TLD was 2-years. Following complete EAC (n=65), poor adherence reduced from 70.9% to 24.6%, and VL response showed 56.9% (n=37), 18.5% (n=12) and 24.6% (n=16) with VL<50, 50-999 and ≥1000 copies/mL respectively, indicating 93.7% (236/252) overall viral suppression. Among those experiencing VF (6.3%), genotyping was successful for 10 and 10.0% (1/10), 40.0% (4/10), 50.0% (5/10), and 11.1% (1/9) harbored HIVDR mutation(s) to protease-inhibitors (PI/r), nucleoside reverse-transcriptase inhibitors (NRTI), non-NRTI (NNRTI) and integrase strand-transfer inhibitors (INSTI), respectively. Most importantly, a non-adherent 17-year-old boy (VL=18717 copies/mL at failure) was identified with three-class drug resistance (NRTI: M41L, K70R, L74V, M184V, G190A, T215F; NNRTI: K101E, V108I, Y181C, G190A and INSTI: L74M, G118R, E138K) and in need of a regimen containing a PI/r with novel antiretrovirals (lenacapavir, fostemsavir, enfuvirtide, maraviroc, and/or ibalizumab).

**Conclusions**: Among APHI within a functional EAC program, the early stage of transition to TLD (about 2 years) shows encouraging viral suppression rate closer to the 95% expected target. However, the case of early emergence of complete TLD-resistance calls for caution on long-term treatment success and the need to rapidly design novel pediatric treatment strategies to preserve HIV elimination among adolescents in LMICs.

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# The Effect of United States Freeze on Foreign Aid on Low and Middle Income Countries in the Fight Against HIV/AIDS

Muhia J[1], Mathenge J[1], Macharia P[1], Onyango A[1]

[1]Hoymas Kenya, Nairobi, Kenya

**Background**: The US Government through USAID and PEPFAR have been a partner in implementing HIV care and treatment programs in sub-Saharan Africa that has the biggest burden of HIV globally. Kenya has been a beneficiary over the years and this has enabled Kenya to make significant steps towards the UNAIDS target of ending HIV pandemic by 2030.

Kenya has over 1.4 million Kenyans currently living with HIV. Kenya was on the verge of eliminating vertical transmission of HIV through sustained treatment of women of the reproductive age and timely initiation of prophylaxis among HIV Exposed Infants (HEI)

The freeze on USG foreign AID put the HIV programs at crossroad disrupting commodities, sample networking and testing and subsequently reversing stigma into the underrepresented and minority populations.

**Materials and Methods**: An assessment conducted in Nairobi targeting facilities previously supported by USG showed that HIV human resource for Health have been served with stop-work-orders effective 1st February, 2025. Clinics were shut down and the Kenya Medical Supplies Agency (KEMSA) issued directives to MEDS to halt any procurement and any supplies immediately. A clinician admitted that viral load samples were not processed between 24th January to 7th March 2025. Staffs operating in other non-USAID supported facilities were instructed to limit the multi-month drug dispensing for clients previously not enrolled in their clinics. A number of clients were feeling frustrated by the disruption of health services.

**Results**: Data Obtained from the USAID Fahari Ya Jamii Community Based Surveillance (CBS) dashboard showed that during the 1st quarter of FY25, the program was supporting over 54,301 Persons Living With HIV (PLHIV) in Nairobi City County. Among them 41,997 (77.34%) had a Viral Load and were reported as suppressed (<50cp/ml) During the same quarter, 672 people were newly diagnosed and enrolled into care.

The data also showed that 4,214 identified as Key and Vulnerable Population were receiving treatment during the same period. 5,632 were actively receiving Pre-Exposure Prophylaxis (PrEP).

**Conclusions**: The USG freeze on foreign order have disrupted care and treatment systems and will in turn have negative effect on the PLHIV who were relying on the system for ART and VL testing among other services.

**Recommendation**: The government of Kenya through the Ministry of Health may have to identify measures to lessen the effects of the disruption of the services.

Improve the capacity of testing laboratories for baseline and follow up laboratory investigations.

Develop sustainable and resilient funding mechanism of HIV/TB programs through budgetary allocation.

Inclusion of Key and Vulnerable population into the service delivery model.

Adopt and sustain the USAID already established systems.

Adoption of Abuja Declaratation to ensure LMIC have atleast 15% of the budget dedicated to health each year.

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# Analysis of Respiratory Pathogens and Their Association with Hospitalization Risk in Pediatric Patients with Acute Respiratory Infections (ARI): A Comparison of Inpatient and Outpatient Cases

Scutari R[1], Di Maio V[2], Mastropaolo M[1], Lucignano B[2], Cortazzo V[2], Del Carmen Pereyra Boza M[2], Onori M[2], Colagrossi L[2], Pisani M[3], D'Urbano T[2], Ferri G[2], Di Leva F[2], Villani A[3], Bernaschi P[2], Perno C[1,2]

*[1]Multimodal Research Area, Microbiology and Diagnostics of Immunology Unit, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [2]Microbiology and Diagnostic Immunology Unit, Bambino Gesù Children Hospital IRCCS, Rome, Italy, [3]General Pediatrics and ED 2nd Level, Bambino Gesù Children's Hospital IRCCS, Rome, Italy*

**Background**: This study aims to evaluate the types and prevalence of pathogens associated with acute respiratory infection (ARI) and their association with Hospitalization Risk in Paediatric Patients attending the emergency department of Bambino Gesù Children's Hospital in Rome.

**Materials and Methods**: A retrospective analysis on paediatric patients with ARI admitted to the Emergency Department, who had positive respiratory samples screened with the BioFire FilmArray Respiratory 2.1 panel (BioMérieux) between January 1, 2024, and November 30, 2024, was conducted.

**Results**: A total of 1,008 samples were collected; 568 (56.3%) from patients who did not require hospitalization, and 440 (43.7%) from those who required. Hospitalized patients were younger than non-hospitalized (0.22 [0.11-1.14] vs 1.16 [0.24-4.80] years, p<0.001), and had higher rate of lower respiratory tract infection (64.1% vs. 26.6%, p<0.001). The analysis of pathogen detection revealed a trend towards higher mono-detection in non-hospitalized patients compared to hospitalized patients (77.5% vs. 72.7%, p:0.09). Adenovirus, Influenza A, Influenza B, Parainfluenza virus 1, and Mycoplasma pneumoniae were more common in non-hospitalized patients (p<0.005), while Metapneumovirus, respiratory syncytial virus (RSV), and Bordetella pertussis were more frequent in hospitalized patients (p<0.005). The distribution of only viral and only atypical bacterial infections was similar between the two groups, but hospitalized patients had a slightly higher frequency of mixed infections (virus+atypical bacteria; 3.3% vs. 6.1%, p:0.047). Considering clinical manifestation, pneumonia in hospitalized patients was mainly associated with only viral infections (38.5% vs 76.7%, p:0.006), while non-hospitalized pneumonia patients were more likely to have infections with atypical bacteria, particularly M. pneumoniae (61.5% vs 13.3%,p<0.001). A similar trend was seen in bronchiolitis, with RSV being the predominant viral infection in hospitalized patients (18.8% vs 48.1%, p<0.001). Logistic regression analysis showed that neonates (adjusted odds ratio, AOR [95%CI]: 7.27 [2.39-22.11], p<0.001), infant (1.64 [1.01-2.66], p:0.044), respiratory failure (14.99 [1.96-114.59], p:0.009), mixed infection (2.62 [1.05-6.58], p:0.039) and RSV (2.57 [1.46-4.52], p:0.001) were positively associated with hospitalization, while a negative association was observed with M. pneumoniae (0.17 [0.06-0.0.50], p:0.001).

**Conclusions**: This study revealed significant differences in the respiratory pathogens distribution between hospitalized and non-hospitalized paediatric patients. These findings highlight the complex relationship between pathogens and clinical outcomes and provide insights to improve strategies for the diagnosis, treatment and management of paediatric respiratory infections, particularly in the hospital setting.

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# Learning Loss and Psychological Outcomes in Schools During the COVID-19 Pandemic: Evidence from the EuCARE International Cluster Randomised Trial

Bellerba F[1], Doi G[1], Tomezzoli E[1], Raimondi S[1], Cutica I[2], Mazzoni D[2], Martins Alves I[3], de Oliveira Tomás dos Santos A[3], Lopalco P[4], Gabellone V[4], Bardeck N[5], Rubio Quintanares G[6,7], Andreon C[1], Mainetti M[1], Tucci G[8], Mommo C[9], Fanti I[9], Abecasis A[3], Kaiser R[5], Incardona F[9], Gandini S[1]

*[1]European Institute of Oncology - IEO, Milan, Italy, [2]University of Milan, Milan, Italy, [3]Universidade Nova de Lisboa - UNL, Lisbon, Portugal, [4] University of Salento, Lecce, Italy, [5]University of Cologne, Cologne, Germany, [6]Paul Ehrlich Institut - PEI, Langen, Germany, [7]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran, Mexico City, Mexico, [8]Organising Bureau of European School Student Unions - OBESSU, Brussels, Belgium, [9]EuResist Network, Rome, Italy*

**Background**: The COVID-19 pandemic showed that evidence-based solutions to support the school mission during pandemics are highly needed. The Lolli-methode is a saliva PCR test well accepted by children from 3 to 18, performed in pool by class twice a week, followed, if positive, by individual saliva PCR tests for the whole class. We conducted an international cluster randomized controlled trial to assess the efficacy of the Lolli-Methode as a school surveillance tool[1] and to investigate students' psychological symptoms and learning loss linked to school closures and other preventive measures.

**Materials and Methods**: We used the Strengths and Difficulties Questionnaire[2] to measure psychological symptoms [Total Difficulties (TD), Emotional Problems (EP), Conduct Problems (CP), Hyperactivity (HY), and Peer Relationship Problems (PRP)] in students from kindergarten to high school in Italy, Portugal, and Mexico. Students' attitudes toward preventive measures were assessed through ad-hoc questionnaires. Data were collected at multiple time-points from November 2022 to March 2024. For Italian students, INVALSI provided Mathematics and Italian performance data for the school years 2018/19, 2020/21, 2021/22, 2022/23. We investigated associations among factors using linear mixed models and explored causal relationships with mediation analysis.

**Results**: We enrolled 23 schools from Italy, Portugal and Mexico and collected 685 psychological questionnaires regarding students across all grades.

Abnormal EP were observed in 17.4% of secondary students, with highest levels in high school ($p<0.01$). EP were higher in girls in middle (interaction $p=0.03$) and high school (interaction $p<0.001$), and among those reporting negative social distancing experiences ($p<0.001$).
PRP increased with age ($p<0.001$) and were higher in students with a recent distant learning experience ($p=0.02$). TD were associated with negative social distancing experiences ($p<0.001$). Girls reported a worse experience with distant learning, leading to greater TD compared to boys (Natural Indirect Effect: $p=0.03$).

In Italy, Mathematics and Italian performances declined during the pandemic compared to the pre-pandemic levels, with greater learning loss observed in students from low socio-economic backgrounds ($p=0.05$) and those whose mothers had low educational levels ($p=0.01$).
More students have reported negative emotions compared to teachers regarding the preventive measures adopted in schools. Concerning the use of the Lolli-Methode, post-intervention data collected in Italy suggest that this measure has been well-received by both students and teachers.

**Conclusions**: Pandemic-related measures significantly affected students' mental health, with distant learning being particularly detrimental. Socio-economic status and low maternal education contributed to learning loss. These findings underscore the need to prioritize students' mental health and address educational inequalities in post-pandemic recovery.

1. Raimondi S, Gandini S, Rubio Quintanares GH, et al. European Cohorts of patients and schools to Advance Response to Epidemics (EuCARE): a cluster randomised interventional and observational study protocol to investigate the relationship between schools and SARS-CoV-2 infection. BMC Infect Dis. 2023;23(1):1. doi:10.1186/s12879-022-07947-6.

2. Goodman, R. Strengths and Difficulties Questionnaire (SDQ) [Database record]. APA PsycTests.1997. doi:10.1037/t00540-000.

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# EuCARE School Project: Effectiveness of the Lolli-Methode in Managing Covid-19 Clusters and Positivity Rates in Schools

Doi G[2], Bellerba F[2], Santos A[3], Lopalco P[3], Gabellone V[4], Bardeck N[5], Rubio Quintanares G[6,8], Tucci G[7], Mainetti M[2], Andreon C[2], Mommo C[1], Fanti I[1], Abecasis A[3], Kaiser R[5], Gandini S[2], Incardona F[1]

*[1]Euresist Network, Rome, Italy, [2]European Institute of Oncology IRCCS (IEO), Milan, Italy, [3]Universidade Nova de Lisboa, Lisbon, Portugal, [4]University of Salento, Lecce, Italy, [5]University of Cologne, Cologne, Germany, [6]Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran - Mexico City, Mexico City, Mexico, [7]Organising Bureau of European School Student Unions (OBESSU), Brussels, Belgium, [8]Paul-Ehrlich-Institut (PEI), Langen, Germany*

**Background**: The COVID-19 pandemic underscored the urgent need for effective strategies to sustain school operations during health crises. As part of the European EuCARE project[1], a randomized controlled trial was conducted in schools to assess the effectiveness of the Lolli-Methode[2] in reducing infection clusters. The method was implemented in schools from kindergarten to secondary education, and was conducted in Italy, Portugal, and Mexico from November 2022 to August 2024.

**Materials and Methods**: Classes were randomised to Lolli or SoC and tested twice a week in pooled samples. Clusters were defined as two or more positive cases within a class in the same week. In Italy, we analysed differences in clusters and infection rates between arms, also exploring the association with role, time of year, and school grade. For the analysis across three countries, we focused solely on the Lolli arm.

**Results**: A total of 23 schools and 3,498 participants were enrolled. No significant differences in cluster proportions were observed between the two arms; however, the proportion of clusters among teachers was higher in the SoC arm (p=0.009). A significantly lower cluster rate was found in the Lolli arm compared to the control in secondary schools (p=0.028), whereas the trend was reversed in primary schools (p=0.058).

Regarding positivity notifications, the trial recorded 147 positive cases out of 3,949 participants. The Lolli group had nearly double the positivity rate of the control group (p=0.006). Students in the Lolli group exhibited a higher positivity rate (p<0.001), whereas teachers had a higher incidence in the SoC group (p=0.022). Across the three countries, a higher number of clusters was identified in primary schools compared to secondary schools (p=0.066). The overall SARS-CoV-2 positivity rate was 5.2%, with Portugal reporting the highest rate and Mexico the lowest (Fig.1, p<0.001).

**Conclusions**: The Lolli-Methode is a school-based screening strategy capable of reducing the risk of SARS-CoV-2 infection clusters, particularly among teachers and secondary school students. It has demonstrated adaptability across different countries and serves as an effective approach to manage COVID-19 transmission in schools.

[1]Raimondi S, et al. European Cohorts of patients and schools to Advance Response to Epidemics (EuCARE): a cluster randomised interventional and observational study protocol to investigate the relationship between schools and SARS-CoV-2 infection. BMC Infect Dis. 2023 Jan 3;23(1):1.
[2]Dewald F, et al. Effective high-throughput RT-qPCR screening for SARS-CoV-2 infections in children. Nat Commun. 2022 Jun 25;13(1):3640.

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# Impact of the COVID-19 Pandemic on the Management of HIV Infection at the Infectious Diseases Department of Casablanca

Badi H[1], Marih L[1,2], Sodqi M[1,2], Marhoum El Filali K[1,2]

*[1]Infectious Diseases Unit, University Hospital of Ibn Rochd, Casablanca, Morocco, [2]Faculty of medecine and pharmacy, Hassan II university , Casablanca, Morocco*

**Background**: The SARS-CoV-2 pandemic has led to an exceptional health crisis, potentially impacting compliance with ARV treatments in PLWH.

The objective of our study was to determine the impact of the COVID-19 pandemic on the management of PLWH followed in our department and to update the frequency of treatment failures and side effects related to the interruption of care.

**Materials and Methods**: This is a retrospective, descriptive, and analytical study including all PLWH who consulted at the day hospital of the infectious diseases department between June 1, 2020, and May 31, 2021, for viroimmunological assessment.

**Results**: 1020 PLWH were included in the study. The male gender was predominant with 50.9% and the average age was 41 years. All our patients were on ARV. During the study period, 285 patients missed their appointments at the Day Hospital. The reasons for treatment interruption and delay in consultation for these patients were diverse: travel problems during confinement (54.55%), social (31.82%), and psychological (9.09%). Even though ARV treatment was delivered by associations, 98 patients had experienced therapeutic failure under treatment (9.6%) and 17 had experienced side effects, mainly renal failure in 12 patients.

**Conclusions**: Due to different physical and social vulnerabilities, PLHIVs are at high risk of being exposed to COVID-19 and of not having access to the healthcare environment and ARVs. This study has proven that PLHIV requires special care during the coronavirus health crisis to ensure therapeutic compliance.

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# Profile and Monitoring of Pregnant Women Living with HIV in Casablanca

Badi H[1,2], Marih L[1,2], Sodqi M[1,2], Ouggane I[1,2], Ihbibane F[1,2], Marhoum El Filali K[1,2]

*[1]Infectious Diseases Unit of University Hospital of Ibn Rochd, Casablanca, Morocco, [2]Faculty of medecine and pharmacy, Hassan II university , Casablanca, Morocco*

**Background**: Pregnancy in women living with HIV is a pregnancy with a high risk of maternal-fetal complications requiring good virological and obstetric monitoring. Its management constitutes one of the priority components of the national strategic plan to combat AIDS.
The objective is to describe the epidemiological and virological characteristics of our parturients and to evaluate the rate of maternal-fetal transmission of HIV.

**Materials and Methods**: a descriptive retrospective study including women living with HIV followed at the SMI of Casablanca, and followed for pregnancy between January 2020 and December 2024.

**Results**: The study included 94 HIV-positive pregnant women, with an average age of 31 years. 78% of the parturients were married and 86% were Moroccan. Nearly half of the parturients were primigravida (52%).

HIV infection was detected during the current pregnancy in 31% of cases and during a previous pregnancy in 13%, 50% were detected during the 2nd trimester, 14% during the 3rd trimester, and 27% were at stage C. The assessment of coinfections HVC, HVB, and Syphilis was negative in all parturients, Rubella was positive at IgG in all patients while Toxoplasmosis serology was positive at IgG in five cases (5%). The initial HIV viral load was available in 73%, it was undetectable in 43%, and >1000 copies/ml in 13%. All patients were put on ARV treatment with an undetectable viral load at the end of pregnancy in 84% of cases. Cesarean section was the delivery route in 20% with a frequency of prematurity of 2.2%. All newborns were put on AZT syrup with a zero rate of maternal-fetal transmission.

**Conclusions**: Implementing a protocol for the prevention of mother-to-child transmission based on the rapid initiation of triple therapy in the mother and regular pediatric care has made it possible to lower the rate of mother-to-child transmission.

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# Visceral Leishmaniasis in a Patient Living with HIV: Diagnosis Error or Challenge?

Badi H[1], Hassan Daddy K[1], Sodqi M[1,2], Marih L[1,2], Ihbibane F[1,2], Marhoum El Filali K[1,2]

*[1]Infectious Diseases Unit, University Hospital of Ibn Rochd, Casablanca, Morocco, [2]Faculty of medecine and pharmacy, Hassan II university , Casablanca, Morocco*

**Background**: Visceral leishmaniasis, also known as kala-azar, is a vector-borne parasitic disease that can progress to a life-threatening illness in immunocompromised individuals, especially those infected with HIV.

**Materials and Methods**: We report the case of a patient with HIV-1, initially diagnosed through pulmonary tuberculosis and under antitubercular and antiretroviral (ARV) treatment including Tenofovir, Lamivudine, and Dolutegravir, with good virological control (viral load was undetectable) and a CD4 count of 72 cells/mm³, presenting with pancytopenia associated with splenomegaly.

**Results**: A 47-year-old man was treated for confirmed HIV-1 infection revealed by pulmonary tuberculosis in 2015, with an initial viro-immunological evaluation showing a viral load of 244,433 copies/ml. He was on ARV treatment with Tenofovir, Lamivudine, and Dolutegravir, which was well-managed but with persistently low CD4 counts at 72 cells/mm³. He was admitted in June 2022 to the Infectious Diseases Department for confirmed pulmonary tuberculosis associated with febrile pancytopenia, including normochromic normocytic anemia (7.6 g/dl), leukopenia (1,720) with neutropenia (640), lymphopenia (780), and thrombocytopenia (66,000), along with an inflammatory syndrome (C-reactive protein at 126) and homogeneous splenomegaly. An etiological workup was performed, including a myelogram, bone marrow biopsy (BMB), leishmaniasis serology, CMV PCR, and macrophage activation syndrome (MAS) evaluation, all of which were negative. A diagnosis of bifocal tuberculosis with confirmed pulmonary involvement and probable hematopoietic involvement was made given the clinical and biological improvement under antitubercular treatment, with anemia improving (Hb at 9.2 g/dl versus 7.6 g/dl, leukocytes 2,900 versus 1,720, and platelet count 120,000 versus 66,000).

Nine months later, the patient presented with asthenia and fever sensations. The evaluation showed pancytopenia (normochromic normocytic anemia at 7.5 g/dl, leukopenia at 1,620 with neutropenia at 840, lymphopenia at 480, and thrombocytopenia at 109,000), splenomegaly, and portal trunk dilation to 13 mm. A myelogram was performed, showing normal marrow richness with the presence of leishmania bodies (Figure 1), while leishmaniasis serology remained negative! A BMB was redone with slides for parasitological and cytopathological study, confirming the diagnosis of bone marrow leishmaniasis. The viro-immunological workup showed an undetectable viral load and CD4 count at 12 cells/mm³.

Treatment with liposomal Amphotericin B at 5 mg/kg/day via infusion was initiated with good clinical progression, fever resolution from the third day of treatment, and biological improvement from the 10th day of antifungal therapy. By day 28, the white blood cell count was 3,600 versus 1,620, neutrophils 2,440 versus 640, lymphocytes 960 versus 480, and hemoglobin level was 9 g/dl. A secondary prophylaxis course with Amphotericin B was scheduled 3 months after discharge.

**Conclusions**: The diagnosis of visceral leishmaniasis in immunocompromised individuals, especially those with HIV, requires high vigilance. Despite the specificity of serological tests, diagnosis often relies on histological examination. In cases of diagnostic delay, especially in resource-limited settings, treatment should be started based on clinical and epidemiological arguments to prevent complications and ensure effective disease management.