



EMHH 2026

24TH EUROPEAN MEETING ON HIV & HEPATITIS

ABSTRACT BOOK

24th European Meeting on HIV & Hepatitis 2026
Barcelona, Spain | 10-12 June 2026

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ORAL ABSTRACT PRESENTATIONS

24th European Meeting on
HIV & Hepatitis 2026

In-Person Meeting

10 – 12 June 2026
Barcelona, Spain



1

Diverging HIV-1 Variant Trends across the Who European Subregions

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Background: The distribution of HIV-1 genetic variants differs across regions and key populations. In the WHO European Region, marked differences are observed between the western, central, and eastern parts of the continent. However, most global analyses report subtype distributions for Europe and North America together and do not distinguish between European subregions. In this study, we assessed temporal changes in the distribution of HIV-1 variants across the West, Central, and East WHO European Regions.

Methods: Data were derived from studies included in the review by Nair et al. (PMID: 39278231), published up to Sep, 2022, and additional publications available up to Feb, 2026, covering all countries of the WHO European Region with available subtype data and sampling years. The literature search was updated to February 2026 and identified an additional 54 publications and 11 national surveillance reports. We analyzed the distribution of major HIV-1 subtypes and recombinant forms, including CRFs (all) and URFs (all). Country-level subtype distributions were weighted by UNAIDS estimates of people living with HIV (PLWH) to calculate regional prevalence (95% CI) and to compare prevalence between the 2010–2016 and 2017–2023 time periods.

Results: In total, the analysis covered approximately 1.87 million PLWH in 2010-2016 and 2.50 million in 2017-2023. Marked differences in temporal trends were observed across the three WHO European subregions. In Western Europe, the epidemic remained dominated by subtype B, although its proportion declined substantially between the two periods from 64.37% to 54.07% (-10.30%, 95% CI -11.49 to -9.11). At the same time, subtype A increased to 6.71% (2.79%, 95% CI 1.65 to 3.93; p<0.0001). Recombinant form

CRF02_AG also increased to 10.50% (2.91%, 95% CI 1.40 to 4.42; p=0.0002).

Central Europe showed the largest change in the distribution of subtypes. The prevalence of subtype A increased markedly between the two periods from 6.72% to 19.55% (12.83%, 95% CI 11.08 to 14.58; p<0.0001). In contrast, subtypes B (-3.27%, 95% CI -4.84 to -1.70; p<0.0001) and F (-9.72%, 95% CI -11.50 to -7.94; p<0.0001) declined. In Eastern Europe, the epidemic remained dominated by subtype A (particularly A6), although its proportion decreased slightly from 77.1% to 73.4% (-3.70%, 95% CI -5.90 to -1.64; p=0.0004). Nevertheless, the estimated number of PLWH infected with subtype A increased from 851,943 to 1,160,723, reflecting the region's expanding epidemic. Subtype B also dropped from 7.46% to 5.67% (-1.79%, 95% CI 2.66 to -0.92; p<0.0001). In contrast, CRFs rose markedly from 9.87% to 14.69% (4.82%, 95% CI 3.05 to 6.59; p<0.0001). This increase was largely driven by CRF63_02A6, which expanded from 5.05% to 10.92% of HIV-1 infections.

Conclusions: Overall, HIV-1 genetic diversity across the WHO European Region remains heterogeneous. The proportion of subtype B declined in all three subregions. Subtype B remains dominant in Western and Central Europe, although non-B variants are now similarly common in both regions. Subtype A increased markedly in Central Europe, and this trend is also detectable in Western Europe. In the Eastern subregion, the epidemic remains dominated by subtype A6, with a growing contribution of the A6-derived recombinant CRF63_02A6.



2

HIV-1 Sub-Subtype A6 Epidemic in Italy: Characteristics and Phylogenetic Analysis

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Background: A6, originally identified as a sister clade of A1 circulating in former Soviet Union, was recently re-classified as a new HIV-1 sub-subtype and many European countries had experienced an increase in the frequency of A6. Sub-subtype A6 is a risk factor for virological failure of long-acting injectables cabotegravir-rilpivirine. This study aimed to assess the prevalence of the A6 sub-subtype in Italy over the years and to characterise these strains in term of HIV diversity, drug resistance patterns, and clustering.

Material and Methods: All sequences from ARCA database previously attributed to subtype A sub-subtypes and those classified as CRF01_AE (n=597) were collected for re-subtyping through phylogenetic analysis. The first available PR-RT (n=157) and integrase (n=105) sequences collected from 2002 to 2025 were analysed. MEGA12 with Neighbour Joining approach was used for subtyping analysis. Transmission clusters were identified by using maximum likelihood approach with IqTree (TVM+F+R5 model) and Cluster Picker software. Only clusters containing at least three sequences and showing bootstrap support >70 were considered. HIV Stanford database was used to define resistance mutations.

Results: A total of 157 (157/597, 26.3%) subjects carrying A6 subtype were retrieved. Most individuals were male (87/157, 55.4%), with a

median age of 37 years (IQR: 32-49 years). Based on partially available data, Caucasian ethnicity was the most represented (77/83, 92.8%), with most individuals originating from Italy (n=49) and Ukraine (n=23). Sexual transmission was the most reported route of infection (42/59, 71.2%), followed by intravenous drug use (12/59, 20.3%). The prevalence of subjects carrying A6 significantly increased over time ($p < 0.0001$), both when stratified into four-year intervals (0.1% to 1%) and when comparing the period before (2002–2021) and after (2022–2025) the start of the Russian/Ukrainian war (0.3% to 1%). Transmitted drug resistance (TDR) was 15.1% (n=8), 3.8% (n=2) and 5.6% (n=2) for NRTI, NNRTI and INI, respectively. A62V, which is polymorphic in sub-subtype A6, was the most represented NRTI-associated mutation (n=8), K103N was the most frequent NNRTI-associated (n=2). No differences were observed in the distribution of resistance between Italians and foreign-born. Thirteen significant transmission clusters were identified (bootstrap ≥ 70), the largest encompassing 33 sequences collected between 2014 and 2025. A significantly higher proportion of Italians were included in transmission clusters compared to foreign-born (89% vs. 60%, $p < 0.001$) and individuals with sexual transmission compared to intravenous drug users (73.8% vs. 41.7%, $p = 0.037$). No differences were observed in cluster distribution considering sampling period.

Conclusions: This study provides valuable insight on HIV-1 A6 molecular epidemiology in Italy, demonstrating significantly increasing prevalence in recent years. Despite incomplete epidemiological data, the higher proportion of Italians in transmission cluster suggests ongoing local transmission in our country.



3

Two Faces of an Epidemic: Distinct Clinical and Molecular Profile of Hiv-1 Infection among MSM and Heterosexual Individuals in Cyprus, 2021–2025

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Background: Despite broad access to combination antiretroviral therapy (cART) and high viral suppression rates, HIV-1 transmission continues across Europe. Cyprus, a major travel and migration hub, faces a complex HIV epidemic. Understanding the distinct characteristics of different transmission groups is crucial for targeted public health interventions. We aimed to compare the demographic, clinical, and virological profiles of men who have sex with men (MSM) and heterosexual (HET) individuals in Cyprus.

Methods: We analysed a national dataset of 255 newly diagnosed, antiretroviral-naïve HIV-1–infected individuals diagnosed in Cyprus between October 2021 and December 2025. Sanger sequencing was used to amplify the protease/reverse transcriptase (PR/RT, HXB2: 2253-3353) and integrase (HXB2: 4230-5096) regions for genotypic resistance testing. HIV-1 subtyping was performed using the COMET tool, and drug resistance mutations were interpreted according to the Stanford HIV Drug Resistance Database (HIVdb) algorithm v. 10.1. Clinical, demographic, and epidemiological information were collected, including age at diagnosis, transmission risk group, country of origin, district of residence, CD4 T-cell count, and plasma HIV-1 RNA levels. Late diagnosis was defined as a CD4 count <350 cells/μL at diagnosis. Comparative analyses were performed between MSM and

heterosexual transmission groups using non-parametric tests and chi-squared analysis.

Results: Heterosexual individuals accounted for a larger proportion (n=154, 60.4%) of newly diagnosed cases compared with MSM (n=101, 39.6%), although the majority of patients were male. HET were significantly more likely to be late presenters (CD4 <350 cells/mm³) than MSM (52% vs. 27.0%, p<0.001), presenting with lower median CD4 counts (318 vs. 459 cells/mm³, p<0.001). The groups also showed distinct geographic and virological footprints. HET were predominantly of Sub-Saharan African origin (29.2% vs. 13.9% in MSM, p=0.006) and infected with subtype A (44.8% vs. 32.7% in MSM, p=0.071). Conversely, MSM were more likely to be of Cypriot or other European origin and infected with subtype B (25.7% vs. 11.7% in HET, p=0.006). The overall prevalence of any TDR was 18.4%, with no significant difference between groups (MSM 18.8% vs. HET 18.2%, p=1.000). NNRTI resistance was the most prevalent class in both groups (MSM 13.9% vs. HET 12.3%), followed by PI resistance (MSM 3.0% vs. HET 3.9%), INSTI resistance (MSM 2.0% vs. HET 1.9%), and NRTI resistance (MSM 1.0% vs. HET 1.9%). No statistically significant differences were observed between groups for any individual resistance class.

Conclusions: HIV-1 infection in Cyprus demonstrates clear clinical and molecular differences across transmission groups. The higher prevalence of late diagnosis among heterosexual individuals and the diverse subtype patterns underscore the heterogeneous molecular profile of the HIV-1 epidemic in Cyprus.



4

HBsAg Isoforms Vary According to HDV Subgenotypes and Their Levels Distinguish the Different Disease Stages of Chronic HDV Infection

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Background: Hepatitis B surface antigen (HBsAg) is composed by three different isoforms, small (S)-, middle (M)-, and large (L)-HBsAg, which reflect specific steps of HBV life cycle and particle morphogenesis. Here, we investigated the relationship between HBsAg isoforms and markers of viral activity and liver injury in HDV-RNA-positive patients according to different clinical phenotypes of HBV/HDV co-infection.

Methods: This study included 366 HDV-RNA-positive patients from the HDV Describe study cohort: most of them males (N [%] = 229 [62.6%]) and native Italians (N [%] = 216 [59.0%]). Median (IQR) age was 56 (46–62) years. Serum S-, M-, and



L-HBsAg levels were quantified by ELISA (Beacle Inc., Japan) and correlated with HDV-RNA (Robogene 2.0, Germany), HBcrAg (Fujirebio, Japan), ALT, and liver stiffness measurement (LSM) (Fibroscan, Echosens, France). HDV subgenotypes were determined by phylogenetic analysis. Patients' clinical phenotypes included liver cirrhosis, chronic hepatitis D (CHD), defined as HDV-RNA ≥ 3 logIU/ml, and inactive/mild disease (HDV-RNA < 3 logIU/ml), as previously defined in Caviglia et al. *Liver Int* 2025. Statistical analyses included Mann–Whitney test for continuous data, Fisher exact test for categorical data, Spearman test to assess correlations and uni- and multivariate analyses to evaluate factors associated with virological and clinical outcomes.

Results: Median (IQR) HDV-RNA was 5.1 (3.4–6.0) logIU/ml and total HBsAg was 3331 (675–9055) ng/ml, while median (IQR) levels of S-, M-, L-HBsAg were 2686 (483–7234), 538 (92–1467) and 0.75 (0.10–4.50) ng/mL, respectively. Total, S-, M-, L-HBsAg showed positive correlations with HDV-RNA ($r_s=0.47$, 0.47 , and 0.42 , respectively; all $p<0.001$) and HBcrAg ($r_s=0.56$, 0.57 and 0.44 , respectively; all $p<0.001$). Furthermore, total, S-, M-, and L-HBsAg levels were significantly higher in patients with ALT > 40 U/L compared to those with ALT ≤ 40 U/L (all $p<0.001$), whereas only L-HBsAg showed a correlation (albeit modest) with LSM ($r_s=0.16$; $p=0.007$).

By phylogenetic analysis, the predominant HDV subgenotypes were 1c (54.3%) and 1e (42.2%). Interestingly, subgenotype 1c was characterized by higher levels of all the three HBsAg isoforms compared to 1e ($p<0.04$ for all).

Notably, total, S-, M-, L-HBsAg levels varied according to patients' clinical phenotype, with the highest levels observed in CHD, followed by liver cirrhosis and inactive/mild disease ($p<0.001$ for all). Notably, in multivariate analysis, higher S-HBsAg levels were significantly associated with CHD (OR=1.58, 95%CI=1.16–2.15; $p=0.003$), higher M-HBsAg with liver cirrhosis (OR=1.78, 95%CI=1.04–3.03; $p=0.035$), while lower L-HBsAg levels were associated with inactive/mild disease (OR=0.38, 95%CI=0.25–0.58; $p<0.001$).

Conclusions: Total HBsAg levels and HBsAg isoforms correlated with the extent of HDV replication and cytolytic activity and varied across different HDV subgenotypes. Notably, HBsAg isoforms showed distinct associations across clinical phenotypes, suggesting a differential regulation of HBV envelope proteins in HDV-driven liver disease.



5

Integrating Neurocognitive Assessment and Plasma Proteomics to Identify Biomarkers of Neurocognitive Impairment in Aging People with HIV

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Introduction: HIV-associated neurocognitive impairment (NCI) remains prevalent among virally suppressed individuals receiving antiretroviral therapy (ART), with chronic immune activation and neuroinflammation driving disease progression and accelerated aging in people living with HIV (PWH). Reliable diagnostic tools and biomarkers for NCI and premature aging in this population are lacking. Within the ALI study (Exploring Suggestive Symptoms of Preclinical Alzheimer's Disease in PWH, PI-21-003), we aimed to investigate the integration of comprehensive neurocognitive assessments—including tests sensitive to preclinical Alzheimer's disease (AD) - with plasma proteomic profiling.

Methods: In ALI study, 100 PWH on ART (HIV-1 RNA<50 copies/mL), aged >50 years, were enrolled, along with 50 age-, sex-, and education-matched HIV-negative controls. Cognitive assessments included the Mini-Mental State Examination (MMSE), NEU Screen, and FACEmemory®. Plasma levels of NfL, GFAP, tTau, pTau217 and neopterin were measured using Meso Scale Discovery (MSD) and ELISA. Proximity extension assay (PEA, Olink) was used for proteomics analysis, including inflammatory and neuro-tailored panels. Univariate, multivariate, and correlation analyses were conducted to evaluate group differences and associations. Statistical significance was set at p<0.05, with

adjustments for multiple comparisons. Sparse Partial Least Squares Discriminant Analysis (sPLS-DA) was performed to identify features that best discriminated between groups.

Results: Most participants were men (74%) with a median age of 58 years (IQR: 55–64 in PWH; 53–65 in controls). Compared to controls, PWH had higher rates of psychotropic medication use (p=0.014), polypharmacy (p=0.009), and recent illicit drug use (p<0.001), with no difference in CNS comorbidities. Positive screening for NCI was more frequent in PWH using FACEmemory (p=0.032) and NEU Screen (p=0.009). NfL and GFAP differentiated PWH from controls regardless of NCI status (p<0.05), whereas neopterin uniquely distinguished both PWH from controls (p=0.001) and NCI from non-NCI participants within PWH (FACEmemory: p=0.028; NEU Screen: p=0.023). No differences were observed in tTau or pTau217 across groups. Proteomic profiling in PWH revealed persistent immune activation and dysregulated inflammatory signaling, with CRTAM, IFI30, CXCL9, TNF, and CD8A indicating heightened cytotoxic and interferon activity, and SMPD1, AXIN1, and BMP-4 reflecting altered cell signaling, apoptosis, and tissue remodeling. sPLS-DA identified molecular signatures linked to NCI: NEU Screen highlighted proteins related to immune modulation and neuronal integrity (LEPR, TDGF1), while FACEmemory emphasized cytokine activity, cell signaling, and neural connectivity (TWEAK, IL17C, CCL27, ADA, WFIKKN1, VWC2).

Conclusion: Despite viral suppression on ART, PWH exhibit persistent immune activation, altered proteomic profiles, and increased prevalence of NCI. Neopterin emerges as a robust biomarker of immune activation and NCI in PWH. Combining neurocognitive testing with plasma proteomics reveals molecular pathways underlying HIV-associated NCI, offering targets for early detection and intervention.



6

Changes in HIV-1 Reservoirs in Virologically Suppressed People with HIV-1 One Year After Receiving Lenacapavir and the Broadly Neutralizing Antibodies Teropavimab and Zinlirvimab

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Background: A small body of evidence suggests that beyond HIV-1 neutralizing activity, broadly neutralizing antibodies (bNAbs) may engage host immune cells and contribute to the control or elimination of HIV-1-infected cells. In a Phase 1b study (NCT04811040), the combination of the HIV-1 capsid inhibitor lenacapavir with the bNAbs teropavimab and zinlirvimab maintained virologic suppression in 27/30 participants for 26 weeks. Using several established methods, we evaluated the impact of this combination regimen on the HIV-1 reservoir in Phase 1b study participants.

Methods: Virologically suppressed individuals on oral antiretroviral therapy (ART) with HIV-1 highly susceptible to one (n=10) or both bNAbs (n=20) (PhenoSense[®] monoclonal antibody assay, 90% inhibitory concentration ≤ 2 $\mu\text{g/mL}$) were enrolled into separate cohorts and randomized 1:1 to two active treatment groups: lenacapavir (927 mg subcutaneously after oral loading) + teropavimab (30 mg/kg intravenously) + zinlirvimab (low dose: 10 mg/kg intravenously; high dose: 30 mg/kg intravenously). Participants received a single dose of the combination regimen, resumed oral ART at Week (W) 26, and were followed through W52. HIV-1 reservoir was measured by IPDA[®], Quantitative Viral Outgrowth Assay (QVOA), and Single Copy Assay (SCA) from baseline through W52. Outgrowth virus and single proviral DNA genomes were sequenced for evidence of T-cell clones.

Results: Baseline median infectious units per million CD4+ T-cells (IUPM) by QVOA was 0.09

(quartile [Q]1, Q3: 0.05, 0.26; n=27), median intact and total HIV-1 DNA by IPDA were 22.81 (Q1, Q3: 2.00, 75.62; n=25) and 334.05 (Q1, Q3: 77.94, 1104.17; n=27) copies/million CD4+ T-cells, respectively, and median HIV-1 RNA by SCA was 1.30 copies/mL (Q1, Q3: 0.80, 1.88; n=19). No significant baseline differences between cohorts or randomization groups were observed. For participants with paired samples, IUPM (n=18) and total HIV-1 DNA (n=18) at W52 and HIV-1 RNA at W12 (n=30), W26 (n=29), and W52 (n=27) were not significantly different from baseline following treatment. Intact HIV-1 DNA showed a small but significant decline at W52 ($p=0.002$; median 1.37-fold reduction; n=16). Notably, two participants who were highly susceptible to both bNAbs and received the low-dose regimen exhibited significant declines in QVOA following treatment. One participant showed a 50-fold reduction in IUPM at W52 that was associated with a 1.1-fold decrease in intact HIV-1 DNA. One participant showed a 30-fold decrease in IUPM at W52 that was associated with a 1.8-fold decline in intact HIV-1 DNA. Further analysis using available samples from this participant revealed identical HIV-1 RNA sequences detected in viral outgrowth cultures matching intact proviral sequences at Day 1 but absent at W52, suggesting contraction of a large T-cell clone.

Conclusions: Participants receiving a single dose of lenacapavir, teropavimab, and zinlirvimab showed minimal changes in HIV-1 reservoir size, with isolated cases of marked QVOA decline. Although limited by the small sample size, these observations suggest a potential effect of prolonged bNAb treatment on the latent reservoir and warrant further investigation in larger studies that include ART comparator arms.



7

Multidisciplinary Assessment of the HIV-1 Reservoir after Switching from Triple to Dual Antiretroviral Therapy in Virologically Suppressed Individuals, a Prospective Observational Pilot Study

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Background: Two-drug regimens (2-DRs) are increasingly used in people with HIV (PWH) with sustained virological suppression, demonstrating non-inferior efficacy compared with triple therapy (3-DRs). Since their long-term impact on the HIV-1 reservoir and its tissue distribution remain unclear, we evaluated longitudinal changes in blood reservoir markers after switching from 3-DR to 2-DR and explored their relationship with gut-associated lymphoid tissue (GALT).

Materials and Methods: Sixty-seven virologically suppressed PWH were enrolled, 32 continued 3-DR and 35 switched to 2-DR. Peripheral blood was collected at baseline (T0) and after 18 months (T1). CD4+ T-cells were isolated and total HIV-1 DNA (CAD), intact proviral DNA (IP) and cell-associated HIV-1 RNA (CAR) were quantified by digital PCR. In a subset of people undergoing rectal biopsy, CAD (n=19) and IP (n=18) were measured in GALT at T0 and T1. Inter-group differences were assessed by Mann-Whitney U test, longitudinal changes by Wilcoxon signed-rank test, correlations by Spearman's rank test, and predictors of reservoir dynamics by linear regression analysis.

Results: At T0, the two groups were comparable for sex, CD4+ count, zenith viral load and duration of ART, whereas the 2-DR group had lower age and higher nadir CD4+.

In blood CD4+ T-cells, CAD, IP and CAR did not differ between groups at T0 and only CAD and CAR were correlated in the whole dataset (Rho=0.653, p<0.001). Intra-group analyses over time showed decreased CAR in the 3-DR group (p=0.023), increased CAD (p=0.003) and decreased IP (p=0.039) in the 2-DR group. When comparing changes between groups, ΔIP (T1-T0) was the only marker with a significant difference (median [IQR] of 0.5 [9.0] in 3DR vs. -6.5 [14.3] in 2DR, p=0.035). In a model controlling for age, zenith VL, ART duration, nadir CD4+, baseline CD4+, and treatment group, T0 CAD predicted ΔCAD (B=0.499, p<0.001), baseline IP and CAR predicted ΔIP (B=-0.792, p<0.001; and B=2.821, p=0.011; respectively) and baseline CAR predicted ΔCAR (B=-0.418, p=0.001) (Tab.3), suggesting baseline-dependent reservoir kinetics. In GALT, CAD and IP values were not strongly correlated with the paired blood CD4+ T-cell values, and only a mild correlation of CD4+ T0 IP and GALT T0 CAD (Rho=0.608, p=0.012) emerged. CAD and IP did not change over time and were comparable between the 2DR and 3DR groups.

Conclusions: Treatment simplification was not associated with measurable expansion of the HIV-1 reservoir after 18 months, supporting the virological safety of 2-DR strategies. The lack of concordance between blood and GALT measurements suggests that peripheral blood may not fully recapitulate the reservoir dynamics and advises for expanding investigation of the lymphoid tissue.



8

Automated Real-Time Molecular Surveillance Platform Reveals HIV-1 Drug Resistance Dynamics and Transmission Cluster Origins among MSM across Europe

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Background: The HIV epidemic among men who have sex with men (MSM) in Europe remains driven by structured transmission networks in which HIV drug-resistant variants spread across borders. Conventional HIV surveillance approaches are ill-equipped to capture this in real time. Herein, we developed a fully automated, end-to-end molecular surveillance platform to monitor HIV drug resistance mutation (DRM) emergence, phylogenetic clustering and inter-regional transmission dynamics, applied to the largest geographically stratified HIV-1 pol dataset from European MSM to date.

Methods: We downloaded 3300 HIV-1 pol sequences from the LosAlamos National Laboratory HIV Database, covering the full Protease and partial Reverse Transcriptase regions (HXB2 positions 2253–3353) from MSM across four geopolitical regions of Europe (Northern, Central and Eastern, Western, and Southern). The sequences were analysed in four phases: (i) reference-anchored multiple sequence alignment via ViralMSA; (ii) DRM profiling through the Stanford Database (HIVdb); (iii) maximum-likelihood phylogenetic inference with IQ-TREE; and (iv) transmission cluster detection using ClusterPicker (genetic distance ≤ 0.015 substitutions/site; branch support $\geq 90\%$). Temporal trends in DRM prevalence were assessed with the Mann-Kendall non-parametric trend test, and the differential spread of DRMs within versus outside clusters was systematically quantified.

Results: All the 3300 sequences were processed and generated actionable resistance and clustering reports with a median turnaround of <17 minutes from sequence submission. Overall DRM prevalence was 28% (95% CI: 26.5–29.5), highest in Western Europe (29%) and lowest in Northern Europe (20%). Drug resistance to NRTIs was the most prevalent (12.79%), followed by NNRTIs (8.70%) and PIs (6.36%). The dominant mutations were at positions L90 (PI; 3.85%), T215 (NRTI; 10.67%) and K103 (NNRTI; 5.27%). The key NRTI mutation M184V had a prevalence of 7.45%. Mann–Kendall analysis showed a significant decreasing temporal trend in overall DRM ($\tau = -0.52$, $p < 0.05$), corresponding to an estimated 1.42 percentage point reduction per year from 1980 to 2019. The clustering phase identified 492 transmission clusters, encompassing 49% of sequences; 92% of clusters were confined to a single region, whereas 8% showed inter regional spread, predominantly linking Central and Eastern with Western Europe. Sequences from Western Europe carrying DRMs were less likely to belong to a transmission cluster than drug sensitive sequences (OR: 0.57, 95% CI: 0.48–0.68, $p < 0.001$), while Southern Europe was more likely (OR: 3.0, $p > 0.05$) to belong to a cluster.

Conclusions: We present a fully automated, real-time molecular surveillance platform for HIV surveillance and transmission dynamics monitoring in European MSM. Our findings reveal that resistant variants are actively clustering and spreading across geopolitical boundaries.



9

Relevance and Value of Sequencing the 5' Leader Region of HIV-1 in Plasma in Clinical Practice

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Background: Non-suppressive viremia (NSV) observed in people living with HIV (PLWH) despite full active antiretroviral therapy (ART) and good treatment adherence raises concerns in clinical practice. Mutations or deletions in the 5'Leader region (5'LR) of the HIV-1 genome, particularly in the Major Splicing Donor (MSD) motif, have been reported in this context and could be a good marker of defective virus. The aim of this study is to evaluate the relevance of sequencing the 5'LR region in plasma and its value in clinical practice.

Materials and Methods: 5'LR was systematically sequenced for all requests for plasma genotypic resistance testing (Sanger) with viremia >50 copies/mL, until 200 samples were obtained. They were divided into four groups: The NSV rebound group (NSVR) include PLWH who had previously achieved virological success (two consecutive viral loads (VL) <20 copies/mL) and who met the following criteria at inclusion: (i) on fully active ART for at least one year, (ii) good drug concentrations, (iii) always below 1000 copies/mL but without virological success in the last year, (iv) with at least 3 VL >20 copies/mL covering the past year. The NSV never suppressed group (NSVNS) included PLWH with the same criteria but had never achieved virological success before. The untreated group (UNT) included PLWH without ART. Finally, the fourth group of other cases (OC)

included PLWH on ART not eligible for the NSVR/NSVNS groups. Comparisons used Mann-Whitney, Chi-square or Fisher's exact tests.

Results: The 200 PLWH were divided as follows: 16 NSVNS (8%), 22 NSVR (11%), 98 UNT (49%) and 64 OC (32%), with a median (IQR) VL at inclusion of 139 (114-210), 121 (73-210), 66300 (6922-690000) and 260 (136-1125) copies/mL, respectively. CRF02 strains were significantly more frequent in NSVNS (75% of PLWH versus 36%, 27% and 31% in the NSVR, UNT and OC groups, respectively ($p=0.004$)). Zenith of VL was significantly higher in NSVNS than in NSVR group: 6.10 log₁₀ copies/mL (5.75-6.39) versus 5.12 (4.91-5.35) $p=0.0004$). 190 Sanger sequences of 5'LR were obtained and 25 were defective with mutations (6 G744A/T; 7 T745C/A/G; 5 G748A) and/or deletions ($n=10$) in the MSD motif (nucleotides 743-748, polymorphic 742 excluded). 5'LR defects were significantly more frequent in NSVR group (50% versus 18.75% in NSVNS, 18.33% in OC and 0% in UNT group ($p<0.0001$)). Among NSVR/NSVNS groups, CRF02 strains tended to be predominant among competent viruses versus 5'LR defect (66.7% vs 28.6%, $p=0.07$). The 11 PLWH in the OC group with defective viruses were not included in the NSVR/NSVNS groups because 4 had insufficient historical data, 2 had low drug concentrations, 2 had NSV for only 9 and 10 months, one was on functional monotherapy, one had resumed ART for 2 months, and one had only 2 quantifiable VL.

Conclusion: This study confirms that 5'LR mutations/deletions are not observed in plasma viruses of untreated PLWH. 5'LR sequencing is insightful in 50% of PLWH in a targeted setting, and is therefore relevant for avoiding unnecessary escalation of treatment for NSV. More defective viruses could be detected with less restrictive NSV criteria.



10

Added Value of RNA-Based Whole Genome Sequencing in Viremic People with 4-Class Drug-Resistant HIV: Insights from the PRESTIGIO Registry

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Background: People with multidrug-resistant HIV face extremely limited treatment options. Sanger-based genotypic resistance testing has limited sensitivity for minority variants, whereas whole genome sequencing (WGS) may improve detection of resistance-associated mutations (RAMs) across the viral genome, including in regions outside pol, but its role in multidrug-resistant HIV remains poorly explored. We aimed to assess the added value of WGS in viremic people with 4-class drug-resistant HIV (4DR-PWH), comparing RAMs detected by WGS and Sanger sequencing.

Methods: Laboratory-based, cross-sectional study on 4DR-PWH from the PRESTIGIO Registry with ≥ 1 cryopreserved plasma sample at HIVRNA ≥ 500 copies/mL. Extracted HIVRNA underwent both Sanger and WGS. Primary and accessory RAMs in reverse transcriptase, protease, and integrase were compared between methods. RAMs detected by WGS were also compared with those recorded in the cumulative RNA-based Sanger genotype.

Results: Thirty-nine viremic 4DR-PWH were included: median age 50.4 (interquartile range=42.4-56.2) years, 31 (79.5%) males at birth, ART since 21.7 (16.9-26.4) years, HIVRNA 15800 (2646-82920) copies/mL. Their regimens contained nucleoside reverse transcriptase inhibitors in 25 (64.1%) cases, non-nucleoside reverse transcriptase inhibitors in 13 (33.3%), protease inhibitors in 28 (71.8%), integrase inhibitors in 25 (64.1%), entry inhibitors in 8 (20.5%), lenacapavir in 1 (2.6%), islatravir in 2 (5.1%).

At the $\geq 20\%$ variant detection threshold, Sanger sequencing detected ≥ 1 RAM not reliably identified by WGS in 16/39 (41.0%) samples. Additionally, in 3/39 (7.7%), both methods detected a RAM at the same position with different amino-acid substitutions. Conversely, WGS identified ≥ 1 RAM not detected by Sanger in 29/39 (74.4%) samples. Considering the cumulative Sanger genotype, detection of all historical RAMs by WGS occurred in only 3/39 (7.7%) cases, but WGS identified ≥ 1 new RAM in 21/39 (53.8%).

At the $\geq 5\%$ threshold, Sanger sequencing detected ≥ 1 RAM not reliably identified by WGS in 12/39 (30.8%) samples. Of these discrepancies, 3 were attributable to low coverage and 5 to variants $< 5\%$; 4 samples showed potentially meaningful discordance. In an additional 5/39 (12.8%) samples, both methods detected a RAM at the same position with different amino-acid substitutions, consistent with variants $< 5\%$.

Conversely, WGS identified ≥ 1 RAM not detected by Sanger in 33/39 (84.6%) samples. Considering the cumulative Sanger genotype, full detection of historical RAMs by WGS occurred in 4/39 (10.3%) cases, but ≥ 1 new RAM was identified in 25/39 (64.1%).

Outside pol, WGS ($\geq 5\%$ threshold) detected ≥ 1 lenacapavir RAM in 7/39 (17.9%) samples [6/7 (85.7%) without prior lenacapavir exposure] and ≥ 1 temsavir RAM in 16/39 (41.0%) [12/16 (75.0%) without prior fostemsavir exposure]. Mutations in nucleocapsid and envelope potentially involved in integrase inhibitor resistance were identified in 11/39 (28.2%) and 34/39 (87.2%) samples, respectively.

Conclusions: RNA-based WGS identified additional, previously unrecognized RAMs, including variants affecting emerging antiretroviral targets beyond pol, in individuals with multidrug-resistant HIV, expanding the resistance landscape captured by conventional genotyping. Despite methodological limitations, including incomplete reconstruction of cumulative resistance history from prior RNA-based Sanger genotypes, these



findings highlight the potential of WGS to broaden resistance profiling and support its role as a valuable complementary approach for comprehensive resistance characterization in populations with high mutational burden.



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Evaluation of HIV-1 Integrase Resistance Patterns and Associated Clinical Factors in Individuals Who Failed Dolutegravir Containing Regimens in Cameroon

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Background: In low- and middle-income countries (LMICs), varying rates of emerging resistance to integrase inhibitors (INI) have been reported, but knowledge on INI mutational patterns and associated clinical factors remain limited

worldwide. We herein evaluated INI-resistance and associated clinical factors among people with HIV (PWH) who failed dolutegravir (DTG)-based antiretroviral therapy (ART) in Cameroon, a country with a broad non-B HIV-1 diversity which is increasingly being seen in European settings.

Methods: This study was conducted among PWH who failed (viral load [VL] ≥ 1000 copies/ml) a DTG-ART (without previous INI-exposure) from January 2020 through December 2025 and for whom a genotypic resistance test (GRT) including integrase was performed. HIV Drug Resistance was interpreted using Stanford HIVdb.v.9.8 with Genotypic Susceptibility Scores (GSS) for DTG-accompanying drugs calculated as the sum for each drug score on Stanford (0 for high-level resistance; 0.5 for low-level to intermediate resistance; 1 for potential low-level resistance to susceptible). Chi square/Fisher's and T Student tests used to evaluate potential factors associated with resistance, and multivariate analysis used to ascertain independent determinants.

Results: Overall, 84 PWH failed DTG-ART (median [IQR] duration: 25 [14–40] months), with a median [IQR] age of 30 [17–48] years, 47.6% (n=40) women. CRF02_AG was the prevailing clade (n=53; 63.1%). At GRT, the median [IQR] VL was 4.8 [4.0–5.2] log copies/mL, with 64 PWH still under DTG (Group A), and 20 empirically transitioned to non DTG-ART (Group B, median duration since DTG-withdrawal, 22 [14–34] months). Overall INI-resistance was 35.7% (30/84), exclusively found in Group A (30/60 [46.9%] vs 0/20 [0%] in Group B; p<0.0001). Within Group A, a higher INI-resistance rate was associated with being adult (≥ 19 vs <19 years: 62.5% vs 37.5%, p=0.0029), a longer exposure to INI (median [IWR]: 37 [22–60] vs 20 [13–34] months, p=0.0007) and with low cumulative GSS of DTG-accompanying drugs (<1 vs ≥ 1 : 70.3% vs 14.8%, p<0.0001). Five PWH treated with rifampicin-containing regimens for tuberculosis (TB) all presented INI-resistance (100%). Individuals with INI-resistance had a higher rate of NRTI (100.0% vs. 58.8%, p<0.0001) and NNRTI (90.0% vs. 61.8%, p=0.011) resistance. Following multivariable analysis, long DTG-ART duration (p=0.007) and low GSS score (p=0.018) remained independently associated with INI-resistance. Resistance mutations (RM) consisted of the four distinct pathways (29/30), notably G118R (60%, 18/30), R263K (23.3%, 7/30), Q148K/R (6.7%, 2/30) and N155H (6.7%, 2/30), while one individual harbored overlapping G118R/R263K (subtype CRF02_AG). Despite no significant association between subtypes and INI RMs,



G118R+ \geq 1RM appeared more in CRF02_AG (n=14) while R263K \pm 1RM appeared more in non-CRF02_AG (n=5). Overall, resistance scores were higher in CRF02_AG vs non-CRF02_AG, notably for DTG (75 [70-80] vs 45 [30-70, p=0.004) and bictegravir (median [IQR] resistance score: 85 [75-80] vs 45 [30-75], p=0.021).

Conclusion: Among PWH failing DTG in this real-life setting, INI-resistance rate is substantial, detected solely with maintained DTG-pressure at genotyping, and higher in adults, longer DTG-duration, TB co-treatment with rifampicin and with pre-existing resistance to DTG-accompanying drugs. Hence, prioritizing GRT-guided ART in these vulnerable PWH and limiting DTG use with rifampicin-containing regimens would contribute to mitigating INI-resistance in LMIC.



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Hepatitis Delta in Spain: Nationwide Prevalence and Comprehensive Clinical and Virological Characterization

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Background: Hepatitis delta virus (HDV) infection represents the most severe form of chronic viral hepatitis, leading to accelerated fibrosis progression, earlier hepatic decompensation and increased liver-related mortality compared with HBV mono-infection. Despite its clinical impact, contemporary epidemiological data on HDV infection in Europe remain limited and heterogeneous. In Spain, systematic nationwide data on prevalence, virological activity and disease severity are scarce. A comprehensive characterization of HDV infection is essential to refine screening strategies, identify patients at highest risk, and optimize management with emerging antiviral therapies.

Materials and Methods: We conducted a nationwide, multicenter, observational study including two components: (1) a retrospective evaluation of anti-HDV prevalence among HBsAg-positive individuals between 2022 and 2024, and (2) a prospective cohort initiated in 2024. Anti-HDV-positive individuals were enrolled prospectively in participating centers across Spain. Demographic, analytical and clinical variables were systematically recorded in a centralized database. Virological parameters included HDV-RNA status and viral load, HDV genotype, HBV markers, and HIV/HCV co-infection. Liver fibrosis was assessed using FIB-4, liver stiffness measurement and METAVIR staging when available. Univariate and multivariate logistic regression analyses were performed using R software to identify factors independently associated with active HDV infection (detectable HDV-RNA).

Results: Between 2022 and 2024, 65,763 HBsAg-positive individuals were evaluated in 80 centers across 16 Autonomous Communities. Anti-HDV



prevalence remained stable over time (5.6% in 2022, 4.9% in 2023 and 5.3% in 2024). The proportion of individuals with confirmed chronic HDV infection were 40.1% in 2022, 31.6% in 2023 and 31.0% in 2024.

By February 2025, 675 anti-HDV-positive individuals had been prospectively enrolled. Median age was 54 years (IQR 42–61), 59% were male and 50% were born in Spain. HDV genotype 1 predominated (88%). Active HDV infection was detected in 39% of participants, with a median viral load of 4.1 log IU/mL. Co-infections were frequent, including HIV (18%) and HCV (19%), and were associated with higher rates of active HDV replication (46% and 52%, respectively). Markers of advanced liver disease were common: 26% had FIB-4 >2.67, mean liver stiffness was 10.9 kPa, 42% had METAVIR F3–F4 fibrosis, and 19% had a history of prior hepatic decompensation. In multivariate analysis, receiving HBV treatment was independently associated with a higher likelihood of active HDV infection (OR 2.17). In contrast, older age (OR 0.65 per 10-year increase), higher platelet count (OR 0.71 per $50 \times 10^3/\mu\text{L}$ increase), and higher HBV viral load (OR 0.77 per \log_{10} IU/mL increase) were independently associated with a lower probability of active HDV replication.

Conclusion: This nationwide prospective cohort provides robust contemporary data on hepatitis delta in Spain, demonstrating stable prevalence among HBsAg-positive individuals and a substantial burden of advanced liver disease. Active HDV replication remains frequent and is independently associated with HBV treatment exposure and specific clinical parameters. These findings reinforce the need for systematic HDV screening, careful virological assessment, and optimized management strategies to reduce disease progression and liver-related complications.



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Hepatitis Delta Antigen and Ribozyme Genetic Variability Is Associated with Altered Virological and Biochemical Markers and Provides Basis for the Identification of Novel Therapeutic Targets

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Background: Genetic diversity within the HDV ribozyme, HDAg domains and cytotoxic T lymphocyte epitopes (CTLEs) (critical for viral replication and host immune recognition) remains poorly understood. This study characterizes the genetic conservation of these regions and evaluates their correlation with key virological and biochemical markers.

Materials and Methods: 178 individuals with chronic HDV infection were enrolled. Full-length HDV genomes were sequenced by Illumina (median [IQR] reads/patient: 69282 [38127–181165]). Genotypes (Gt) were assigned by phylogeny. Nucleotides and amino acids were considered conserved if not mutated in $\geq 99\%$ of sequences. HDAg domains and CTLEs (N=18) were defined according to Pascarella 2010 and Kohsar 2021.

Results: Individuals were 58% males, median age 51 (37-59) years, mainly Italians (ITs, 43.7%), followed by Eastern Europeans (EEs, 34.8%) and Africans (AFs, 21.5%). Median HDV-RNA and ALT were 5.4 (4.5-6.1) logIU/ml and 94 (62-151) U/L. The most prevalent Gts were 1e (57.8%) and 1c (20.8%), followed by 1b (9.5%), 1a (7.8%), 5 (2.2%), 7 (1.1%) and 6 (0.5%). Gt 1e predominated in ITs (64%, $P < 0.001$), 1c in EEs (81%, $P < 0.001$), while 1a, 1b, 5, 6, and 7 were found only in AFs.

HDV ribozyme showed 100% nucleotide conservation whereas HDAg exhibited marked variability: conservation was lower in N-terminal (aa1-107) than in C-terminal domains (aa108-214, known to be crucial for HDAg activity) (14.7% vs 32.2%, $P < 0.005$). CTLE conservation ranged from 0% in CTLEaa26-34 and CTLEaa43-51 to 50% in CTLEaa140-149.

In Gt 1e, the aa conservation was significantly reduced in individuals > 40 years (68.2% vs 30.8%, $P < 0.0001$), particularly within 6 CTLEs. The N12G mutation (median intra-patient prevalence 99%) correlated with higher ALT (101[63-152] vs 86 [69-174] U/L, $P = 0.01$) and was enriched in individuals with 2xULN ALT (77% vs 50%, $P = 0.005$), suggesting enhanced cytolytic activity.

Conversely, T180A (median intra-patient prevalence 63%) correlated with lower HDV-RNA and HBsAg levels (4.9 vs 5.6 logIU/ml, $P = 0.04$ and 3.7 vs 3.9 logIU/ml, $P = 0.03$), indicating impaired viral fitness.

Conclusions: HDAg displays significant genetic diversification, notably within the N-terminal domains and CTLEs, which appears to accumulate with age, suggesting a pattern of ongoing antigenic drift. Specific mutations potentially modulate viral fitness and HDV-mediated cytolytic activity, a relationship that warrants further in vitro validation. Conversely, the high degree of conservation observed in the ribozyme identifies it as a promising candidate for RNA interference-based therapeutic interventions.



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Acute Hepatitis B Cluster among MSM: Possible Concerns on New ART and PrEP Strategies

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Background: HBV-active antiretroviral treatment (ART) or PrEP strategies with TXF/XTC have been effective in preventing both HBV acquisition and risk of reactivation in co-infected people living with HIV (PLWH). In the context of new dual ART therapies without TXF/XTC, cases of acute hepatitis B have recently been reported among Parisian men having sex with men (MSM). We phylogenetically investigated acute HBV infections to understand their circulation and gain an insight into the dynamics of HBV transmission in a non-endemic area.

Methods: A retrospective study included persons followed by Infectious Diseases Departments in Paris and Rennes hospitals (France), who were diagnosed with acute Hepatitis B between 2022-2025. HBV genome was entirely amplified in a single fragment. Amplicons were sequenced using Oxford Nanopore technology. Phylogenetic analysis was performed with consensus sequences using maximum likelihood approach. Moreover, viral haplotypic populations were reconstructed

for each patient. A score incorporating the intra-patient relative frequency and the inter-patient specificity of each haplotype was calculated by pair of patients in order to define a similarity network.

Results: Fifteen men with a median age of 35 years, 12 of whom MSM, were included. They engaged in risky sexual practices. Four individuals were under TDF/FTC PrEP (3 on-demand and 1 daily); 6 were PLWH (2 treated by dolutegravir/rilpivirine and 3 by injectable cabotegravir/rilpivirine, without any HBV-active treatment for a median of 13 months [2-39]). One participant was diagnosed with HIV and HBV infections at the same time and was not yet under ART.

Nine patients had been HBV-vaccinated, 6 of whom presented anti-HBs antibodies >10IU/L (12-51; median=21). Median HBV viral load peak was 3.8×10⁶ UI/ml (6.8×10⁵-1.5×10⁷) and median ALT peak 1194 UI/l (349-3356). One patient presented symptoms requiring hospitalization and 1 developed a chronic infection (4 spontaneous resolution; 7 unknown status).

Phylogenetic study revealed a high degree of genetic similarity between the viruses, 13 of which were genotype (GT) F1b and 2 GT F3 (Figure 1). Sequence analysis did not reveal any known vaccine escape mutations. Viral haplotype network highlighted that certain individuals (P01/P04 and P12/P13/P14) had closely related haplotype profiles, with common, specific, and genetically similar haplotypes (Figure 2).

Conclusion: Phylogenetic analysis supports a GT F1b transmission cluster within the studied population. The selection and circulation of these viruses, particularly in individuals with anti-HBs antibodies >10IU/L, need to be further studied. Improving HBV vaccination efficacy in exposed populations such as MSM seems essential. GT F, that usually circulates in Latin America and Alaska, is rarely detected in France and is genetically distant from vaccine-used GT A. It has been associated with earlier hepatocarcinoma development than infection with other GT in Alaskan natives. The therapeutic context for PLWH and future PrEP users is shifting toward the widespread use of molecules that are not active against HBV. These new strategies could lead to an increase in the incidence of HBV.



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Characterization of Intrinsic Susceptibility to APOBEC3-Mediated G-to-A Transitions across the HIV-1 Mutational Landscape: Implications for Capsid and Enzymatic Targets

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Background: Host-intrinsic restriction factors, particularly APOBEC3G/F, drive HIV-1 evolution through G-to-A deamination. While the mutational pressures on protease (PR), reverse transcriptase (RT), and integrase (IN) are well-documented drivers of antiretroviral resistance, the relative vulnerability of the capsid (CA) remains poorly understood. This study aims to map regional genomic vulnerabilities to understand the evolutionary stability of global HIV-1 clades under APOBEC pressure.

Methods: A high-resolution, population-scale multiple sequence alignment were used to simulate multi-hit deamination pathways. Eighty-six HIV-1 DNA Gag and Pol consensus sequences from group M were obtained from the Los Alamos HIV Database. Potential APOBEC deamination sites (GGG and GGA) were specifically filtered for non-synonymous substitutions and then analyzed for major drug resistance mutations (DRMs) and stop codons.

Results: The analysis performed on the consensus sequences revealed 1,035 potential deamination motifs in 573 codons, that after filtration decreased to 644 motifs in 370 codons, showing a striking gradient in mutational APOBEC susceptibility across the HIV-1 genome. Within the PR region, we observed the highest density at 42.4% (42/99 codons; 83 motifs), including 2 tryptophan acting as lethal target by triggering in-frame stop codons (2.0%) and 3 mutations associated with PI resistance (D30N, M46I, G73S). The RT region followed with 36.4% susceptibility (160/440 codons; 272 motifs); crucially, 19

residues (4.3%) lethal targets, and 6 mutations associated with NRTI resistance (D67N, E138K, M184I, G190ES, M230I). IN region, showed 33.0% susceptibility (95/288 codons; 164 motifs), 7 lethal targets (2.4%), and 8 mutations associated with INI resistance (G118R, E138K, G140RS, G163KR, D232N, R263K). Finally, the CA region displayed a 50.9% lower mutational density than RT ($p=0.0007$) with only 31.60% susceptibility (73/231 codons; 125 motifs), 6 lethal targets (2.6%), and no potential mutations associated with lenacapavir resistance. Notably, 3 CA resistance sites at positions 56, 67, and 70 were characterized by a synonymous codon bias in certain subtypes (A, A1, A3, A6, G) and recombinant forms (such as CRF02_AG, 14_BG), preventing effective impact on resistance by APOBEC deamination.

Conclusion: These findings indicate that the HIV-1 CA maintains a drastically lower intrinsic APOBEC mutational resistance burden than enzymatic targets. The pronounced evolutionary stability of the HIV-1 CA against APOBEC3-mediated mutagenesis highlights its robustness as a therapeutic target, supporting the high fitness cost and long-term efficacy of capsid inhibitors like lenacapavir.



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Contribution of Plasma Next-Generation Sequencing to Resistance Assessment at Virological Failure Compared with Cumulative Historical Genotypes: Data from the ARCA Cohort and the Italian NGS Network

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Background: Next-generation sequencing (NGS) increases the sensitivity for detecting HIV-1 drug resistance mutations, including minority variants, but its real added value could depend on clinical scenarios.

Materials and Methods: We conducted a cross-sectional analysis within the ARCA cohort and Italian NGS Network, including adult people with HIV (PWH) who failed an antiretroviral regimen between 2022 and 2025 and for whom genotypic resistance testing by NGS (NGS-GRT) on plasma samples at the time of virological failure and historical Sanger-GRT (hGRT) were available. hGRTs were compared with NGS-GRT at detection

thresholds of 5–20% (minority variants) and $\geq 20\%$ (high-frequency variants). Major resistance mutations (MRMs) were assessed according to the Stanford HIVdb algorithm v.10.1.

Results: We included 54 PWH; all had PR/RT prior hGRT, while integrase sequence was available for 31 PWH. Participants had a median treatment history of 14 (IQR: 9–18) years and a median of 4 (IQR: 2–8) prior regimens. At the time of NGS-GRT, median HIV-RNA was 3.9 (IQR: 3.0–4.8) log₁₀ copies/mL. PWH were receiving bicitgravir/dolutegravir-based (37.0%), raltegravir/elvitegravir-based (22.2%), protease inhibitor-based (29.6%), and NNRTI-based (11.1%) regimens, for a median duration of 5 (IQR: 3–7) years.

Overall, mutation patterns were heterogeneous, combining newly emerging and historically re-detected MRMs. At a 5% threshold, 63.0% of PWH harbored ≥ 1 MRM, decreasing to 48.1% at $\geq 20\%$. When accounting for hGRTs, 11.1% had resistance mutations not detected by NGS.

Historical MRMs, mainly involving RT inhibitors, were re-detected in 16 individuals (29.6%), with complete re-detection in only eight (14.8%). New MRMs detected exclusively by NGS were observed in 40.7% and 25.9% of individuals at 5% and $\geq 20\%$ thresholds, respectively.

MRMs in hGRTs and/or NGS-GRT were observed in 10 (18.5%), 25 (46.3%) and 27 (50.0%) individuals for PIs, NRTIs and NNRTIs, respectively. Historical MRMs not detected by NGS were observed in 6 (11.1%), 17 (31.5%) and 13 (24.1%) individuals, while historical MRMs re-detected by NGS at $\geq 20\%$ threshold were found in 3 (5.5%), 6 (11.1%) and 10 (18.5%) individuals for PIs, NRTIs and NNRTIs, respectively. Minority variants contributed minimally, with only one historical protease mutation re-detected below 20%.

Regarding INSTIs, MRMs were detected in 12 individuals (22.2%): 3 (9.7%) had historical MRMs not detected by NGS, 6 (19.4%) had new MRMs ($\geq 20\%$: 12.9%; 5–20%: 6.4%), and 1 (3.2%) had historical MRMs re-detected by NGS at $\geq 20\%$ threshold. MRMs were also identified in 4 individuals without prior integrase hGRT. Emerging mutations were consistent with regimen-specific selective pressure. In particular, among 3 of 22 individuals (13.6%) failing bicitgravir/dolutegravir-based therapy, integrase substitutions included R263K (n=1), G118R (n=1) and S147G (n=1), as well as the minority variants E138A (n=1) and Q148R (n=1), alongside RT mutations M184V (n=1), K70E (n=1) and the minority variant M184I (n=1).



Conclusion: NGS performed at virological failure effectively identifies emerging resistance, with a moderate contribution of low frequency variants. However, its ability to fully recover previously documented resistance is limited. Therefore, integration of virological failure history and historical resistance data remain crucial for appropriate treatment tailoring in PWH experiencing virological failure.



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Extensive HDV Genetic Diversity and High Rates of Active Infection in Cameroon Revealed by Molecular Diagnostics and Whole-Genome Sequencing

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Background: In Cameroon HBV infection is endemic. However, limited data are available on epidemiology, genetic diversity and accurate diagnosis of HDV infection.

Material and Methods: We conducted a cross-sectional study on 117 HBsAg+ patients from two hospitals in Cameroon, and 42 patients with an already known anti-HDV positivity. For the 117 HBsAg+ patients, anti-HDV were detected by using an assay on the Diasorin Liaison XL analyser. For samples resulted positive to anti-HDV, HDV-RNA was tested by two different assays: RoboGene HDV-RNA quantification kit v3.0 using the Felix automated extraction instrument (LOD <6 IU/ml) and the fully automated Altostar platform for extraction and HDV RT-PCR v1.5 (LOD 1.12 IU/ml). Whole genome HDV sequencing was assessed on Illumina MiSeq. The correlation between HDV-RNA values by Robogene and Altostar assays was assessed by Spearman test.

Results: Overall, 21/117 (18%) patients were positive to anti-HDV, highlighting a high rate of

HDV seroprevalence. The percentage of anti-HDV positive patients ranged from 15.1% (11/73) in the Littoral region up to 22.7% (10/44) in the East region, highlighting local hotspot with high HDV endemicity.

HDV-RNA was tested in the 21 newly diagnosed patients and in the 42 with previously known anti-HDV positivity for a total of 63 patients. Among them, 61% had detectable HDV-RNA by Robogene assay (median [IQR]: 5.4 [3.3 - 6.0] log[IU/ml]), supporting a conspicuous circulation of active HDV infection with an intense HDV replication. Overall, HDV-RNA quantification by Robogene and Altostar showed a high degree of correlation (Rho=0.91). Such degree of correlation was reduced for HDV-RNA values >5 log(IU/ml) (Rho=0.82 for HDV-RNA >5 log[IU/ml] vs Rho=0.99 for HDV-RNA <5 log[IU/ml]).

Focusing on 38 samples with HDV-RNA >1000 IU/ml by Robogene, 26 were successfully sequenced. Phylogenetic analysis revealed the circulation of a large variety of HDV genotypes and subgenotypes with the subgenotype 1b being the most frequent (61.5%), followed by 1a (19.3%), genotype 7 (7.8%), genotype 6, 5 and 1e present in 3.8% of patients each. The correlation between HDV-RNA quantification by Robogene and Altostar was very high in all identified genotypes/subgenotypes with a slight decrease in 1b (Rho=0.96 for genotypes/sub-genotypes other than 1b and 0.82 for 1b).

Conclusions: These findings show a significant burden of active HDV infection in Cameroon, thus highlighting the urgent need of developing accessible and affordable diagnostic algorithms and surveillance tools for HDV infection in Cameroon to reduce HDV-associated morbidity.



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Resistance to the HIV-1 Capsid Inhibitor Lenacapavir

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Background: Lenacapavir (LEN) is a long-acting HIV-1 capsid inhibitor that binds to the HIV-1 capsid (CA) protein with picomolar antiviral activity. Twice-yearly subcutaneous LEN is approved for the treatment of people with HIV (PWH) with multidrug resistant HIV-1 in combination with other antiretroviral medications, and for HIV PrEP as monotherapy. Moreover, LEN is currently investigated in combination with other ARVs for the treatment of virologically suppressed PWH. Understanding LEN resistance pathways is crucial for optimal positioning of LEN within the current ARV guidelines. Through molecular analyses of clinical samples from individuals treated with LEN across clinical trials, we unveiled key characteristics of LEN resistance-associated mutations (RAM) found in HIV-1 CA. Here, we review the main characteristics of CA RAMs, using viral molecular systems and modelling tools, and provide an overview of clinically observed resistance in ongoing programs.

Materials and Methods: Genotypic and phenotypic analyses of HIV-1 CA mutations observed in clinical studies were conducted with the Gag-Pro assay (Monogram Biosciences) and other established phenotypic assays in cell lines and primary cells. The gag encoding region of clinical isolates with CA mutations, as well as associated site-directed mutants, were cloned into pXXLAI, and characterized in T-cell lines. The effect of the RAMs on the viral replication capacity (RC) was investigated in peripheral blood mononuclear cells from healthy donors infected with a modified pNL4-3. Molecular modelling on 3-dimension CA structure was conducted on HIV-1 CA with PyMol (Schrödinger LLC). Free Energy Perturbation (FEP) simulations were conducted with Schrödinger's Protein FEP+, to calculate the drug-target interaction differences between CA mutants and wild type (WT).

Results: While LEN resistance emergence has been rare in the ongoing Phase 2 and 3 new combination studies in virologically suppressed

PWH, LEN resistance was observed in the heavily treatment-experienced study with the following RAM patterns: M66I + others, Q67H + K70R + others, and other singly observed patterns. The presence of LEN RAMs was associated with susceptibility changes to LEN, ranging from 5- to >1000-fold compared to WT control in several complementary assays. Structural modelling confirmed that LEN binding in the CA binding pocket was altered in the presence of CA mutations, including loss of crucial water bridges and hydrogen bonds, with predicted binding affinity changes correlating with observed potency shifts. Most RAMs were frequently associated with substantial replication defects, down to 15 % of the WT virus in a 14-day assay in primary cells, fitness defects that were considerably higher than previously observed for RAMs associated with other ARV classes, i.e., 75% fitness for M184V in the same assay.

Conclusions: These analyses provide a new perspective on the assessment of the resistance barrier to LEN, beyond the number of genotypic changes and level of phenotypic resistance, by including the impact of RC defects in the assessment of the resistance barrier. These insights into LEN-resistance mechanisms underscore the unusually high fitness costs associated with most treatment-emergent capsid mutations, and may partially explain the rare emergence of LEN RAMs in the ongoing clinical studies combining LEN and other antiretrovirals.



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Accurate Detection of HIV Drug Resistance Mutations Using Oxford Nanopore Sequencing: A Comparative Study with Illumina NGS

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Background: Accurate detection of HIV drug resistance (HIVDR) mutations is critical for guiding antiretroviral therapy and for resistance surveillance. Illumina-based next-generation sequencing (NGS) currently represents the benchmark for HIVDR testing due to its high accuracy and sensitivity for detecting minority variants. Oxford Nanopore Technologies (ONT) sequencing offers several practical advantages, including long-read capability, flexible throughput, rapid sequencing, and lower infrastructure requirements. However, historically elevated error rates raised concerns about its suitability for reliable variant detection. Recent technological advances in ONT chemistry and basecalling have substantially improved sequencing accuracy. We therefore evaluated the performance of the latest ONT sequencing chemistry for detection of HIVDR mutations in comparison with Illumina sequencing, with a particular focus on low-frequency variants.

Methods: Twenty-one clinical samples (14 plasma RNA and 7 proviral DNA) were analyzed. HIV genomic regions covering protease (PR), reverse transcriptase (RT), and integrase (IN) were amplified by target-specific PCR and sequenced on both platforms using identical amplicons. Illumina sequencing was performed on the MiSeq platform, while ONT sequencing was conducted on a PromethION device using R10.4.1 flow cells and Kit 14 chemistry. Resistance-associated mutations in

RT and IN were interpreted according to the IAS–USA 2025 drug resistance mutation list. Variant calling was performed using a 2% frequency cutoff. Concordance between sequencing platforms was assessed based on mutation detection and quantitative differences in variant frequencies.

Results: Across all samples, 83 HIVDR-associated mutations were identified. All mutations detected by Illumina sequencing were also detected by ONT, demonstrating complete qualitative concordance for clinically relevant variants. Overall quantitative agreement between platforms was high, with a mean inter-platform frequency deviation of –2.76%. The majority of mutations (92%) showed frequency differences below 5%, indicating strong quantitative concordance. Minority variants (<20% frequency) were comparably detected by both platforms (Nanopore: n=22; Illumina: n=19). In addition, ONT identified three additional low-frequency mutations (<5%) that were either below the applied Illumina detection threshold or not detected by Illumina sequencing. Discrepancies exceeding 5% (n=7) were infrequent and showed no systematic association with sample type, genomic region, or sequencing platform.

Conclusion: Recent improvements in Oxford Nanopore sequencing chemistry enable robust detection of HIV drug resistance mutations, including minority variants. The strong qualitative and quantitative agreement with Illumina sequencing observed in this study supports the use of ONT sequencing as a reliable alternative for HIVDR testing. Given its scalability, portability, and reduced infrastructure requirements, ONT sequencing represents a promising approach for routine diagnostics and large-scale resistance surveillance, particularly in decentralized laboratories and resource-limited settings.



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Comparative Evaluation of the New Abbott HIV ComboNext Serological Assay and the Roche Elecsys HIV Duo Test: Analytical and Clinical Performance

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Introduction: Human immunodeficiency virus (HIV) infection remains a major public health challenge, with an estimated 1.3 million new infections worldwide in 2024. Although the diagnostic window period has been reduced to less than 15 days with the introduction of fourth-generation assays, the high genetic diversity of HIV and the increasing use of oral or injectable pre-exposure prophylaxis (PrEP) complicate early diagnosis. Breakthrough infections occurring under PrEP may present atypical serological profiles, such as the Low-level Early Viral Inhibition (LEVI) syndrome, which highlight these diagnostic challenges. Rapid identification of primary HIV infection in individuals receiving PrEP is therefore crucial to prevent delayed diagnosis and the emergence of drug resistance. In this context, we evaluated the analytical performance of the new ultrasensitive HIV Alinity i ComboNext assay (Abbott) and compared it with the Roche Elecsys HIV Duo assay and the routine Alinity i HIV Combo test.

Materials and Methods: A retro-prospective study was conducted on a total of 570 samples. Each sample was tested in parallel using the routine Alinity i Combo assay, the new Alinity i ComboNext assay, and, for 472 samples, the Elecsys Duo assay. HIV reference status was established based on the complete diagnostic algorithm, including serological testing, HIV viral load measurement,

and HIV-1/2 Western blot analysis. Seroconversion panels and international NIBSC standards (p24 antigen at known concentrations) were also evaluated. The study cohort included a wide diversity of samples, including HIV-2 infections, HIV-1 group O infections, patients receiving PrEP, pregnant women, immunocompromised patients, and samples positive for other pathogens, in order to assess potential cross-reactivity.

Results: The routine Alinity i Combo assay demonstrated a sensitivity of 99% and a specificity of 93%. The new ComboNext assay showed improved analytical performance, achieving a sensitivity of 100% and a specificity of 97%. ComboNext enabled to detect earlier two HIV-1 acute infections and corrected 17 falsely reactive results observed with the routine assay. For these false-reactive samples, Alinity Combo index values ranged from 1.03 to 4.67 (median 1.37), while all were non-reactive with ComboNext (median index value 0.1). The Elecsys Duo assay showed comparable performance, with a sensitivity of 100% and a specificity of 98%. Analysis of NIBSC standards demonstrated a lower limit of detection for p24 antigen of 0.34 IU/mL with ComboNext, whereas the Alinity Combo and Elecsys Duo assays became reactive only at the next higher dilution, corresponding to a p24 concentration of 0.69 IU/mL. Seroconversion panels showed a reduction of the serological window period by almost two days with ComboNext and Elecsys Duo compared with the routine assay.

Conclusion: The HIV ComboNext assay demonstrates a significant improvement in diagnostic performance, with a better specificity, leading to a reduction in false-positive results and unnecessary confirmatory testing. Moreover, the study shows an early detection of p24 antigen, which is critical for rapid diagnosis of primary HIV infection. Its robust performance across complex sample types such as individuals receiving PrEP, immunocompromised patients, and rare HIV variants, makes ComboNext a valuable tool for optimizing HIV diagnosis in the current prevention landscape.



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HIV-1 Whole Genome Sequencing for Comprehensive Genotype Resistance Testing

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Background: Genotypic resistance testing (GRT) for HIV-1 is routinely focused on the pol gene, which encodes the viral enzymes targeted by commonly used antiretroviral drugs. However, the introduction of novel drug classes targeting other proteins, including capsid and entry inhibitors, requires genomic characterization beyond pol. Whole genome sequencing (WGS) enables simultaneous evaluation of resistance mutations, viral subtype, and genomic epidemiology within a single assay. This study assessed the diagnostic performance of HIV-1 WGS in a clinical diagnostic setting.

Materials and Methods: HIV-1 RNA-positive samples with heterogeneous viral loads and subtypes, previously characterized by routine pol genotypic resistance testing (GRT), were re-sequenced using a whole-genome amplification assay followed by Illumina iSeq100 sequencing. WGS performance was evaluated based on genome coverage at 100× depth threshold and its ability to detect pol resistance-associated mutations (RAMs) compared with the reference pol-based assay using a 10% variant frequency (VAF) threshold. Mutation analysis for both pol and gag regions was performed using the Stanford HIV Drug Resistance Database. HIV-1 subtype assignment was determined through maximum likelihood (ML) phylogenetic analysis of pol and WGS consensus sequences. Associations between viral load and sequencing performance were assessed using Spearman's rank correlation coefficient.

Results: Forty-seven samples were analyzed. WGS achieved a median genome coverage at 100× of 94%, with consistently high coverage for pol and

gag (median 100% and 99%, respectively). In contrast, accessory genes showed greater variability, particularly at lower viral loads. Genome coverage correlated significantly with viral load ($\rho = 0.505$, $p < 0.001$). RAMs comparison with the pol-based reference assay showed an overall concordance of 87%, with complete agreement (100%) for major RAMs (n=65) across all pol-targeting drug classes. Discordances involved mainly Accessory mutations (38% concordance, with 16 mutations detected only by pol and 2 only by WGS assay (n=29) and were detected under the selected cut-off thresholds selected for consensus generation (10% VAF and/or 100x reads). Analysis of gag sequences detected extensive capsid polymorphisms, but no mutations associated with lenacapavir reduced susceptibility. For subtype comparison (pol vs WGS), 7 sequences were excluded due to incomplete genome coverage. Overall, 90% subtypes (n=36) were concordant with previous pol assignment. Among these, 58% were subtype B (n=23), 8% C (n=3), 5% A6 (n=2), 10% CRF02_AG (n=4), 5% CRF17_BF (n=2), 3% CRF14_BG (n=1) and 3% CRF37_cpx (n=1). Discrepancies (n=4) involved circulating recombinant forms (CRFs). Two samples initially classified as pure subtypes (F and G) were reassigned as recombinant forms (CRF122_BF1 and CRF73_BG) following WGS phylogenetic analysis, and two samples were reassigned to a different CRF belonging to the same family (from CRF40_BF to CRF122_BF and CRF12_BF to CRF17_BF).

Conclusions: HIV-1 WGS demonstrated robust performance across different viral loads and subtypes, showing complete agreement with routine GRT for clinically relevant major RAMs while providing additional genomic information beyond pol and improved subtype resolution. These findings support the feasibility of integrating WGS into diagnostic resistance workflows, enabling expanded surveillance of emerging resistance mechanisms and more accurate characterization of recombinant HIV-1 genomes.



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The Enhanced Resistance Profile of Third-Generation INSTI VH4524184 (VH-184) vs Bictegravir

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Background: Valued for their potency, durability, and high barrier to resistance, integrase strand transfer inhibitors (INSTIs) serve as a cornerstone of HIV-1 therapy. Preserving and improving upon these features is vital to addressing the changing needs of people with HIV. VH4524184 (VH-184), a third-generation INSTI with long-acting potential, has shown robust antiviral effects and an enhanced in vitro resistance profile compared with second-generation INSTIs. This study assessed the resistance profile of VH-184 in comparison with bictegravir (BIC).

Materials and Methods: Resistance data were analyzed using a panel of second-generation INSTI-resistant HIV-1 pseudotyped viruses derived from 14 individuals who met confirmed virologic withdrawal (CVW) criteria during the SAILING (n=7, through Week 132) and DAWNING (n=7, through Week 216) trials. SAILING was a phase 3 trial of adults naive to INSTIs with resistance to ≥ 2 classes of antiretrovirals who were treated with either dolutegravir (DTG) or raltegravir, with a 2-drug background regimen. DAWNING was a phase 3b trial of adults experiencing failure of first-line therapy who received DTG or ritonavir-boosted lopinavir, in addition to 2 nucleoside reverse transcriptase inhibitors. Both populations and clonal variants from on-treatment plasma samples were genotyped and evaluated using a phenotype assay.

Wild-type level activity was categorized as an IC50 fold change (FC) of ≤ 2 .

Results: VH-184 exhibited potent in vitro antiviral activity in the presence of emergent resistance to INSTIs at CVW. VH-184 demonstrated improved activity compared with BIC against viruses with R263K, S230G or S230R, and A49G when tested

against pseudotyped virus populations and clonal variants from SAILING, which raised BIC FC above the wild-type level. Across populations and clonal variants containing the G118R mutation, occurring alone or alongside additional substitutions, identified among participants in DAWNING, VH-184 generally demonstrated improved activity compared with BIC. VH-184 also showed increased activity against pseudotyped viruses with the L74I, E138K, and/or G118R mutations and improved activity against viruses with the N155H, E138D, and S147G mutations.

Conclusions: VH-184 demonstrated potent activity and an enhanced resistance profile compared with BIC, thus maintaining activity against pseudotyped viruses with second-generation INSTI resistance. The findings reported here emphasize the activity of VH-184 as a third-generation INSTI against a broader spectrum of INSTI-resistant viruses and support the continued development of VH-184 as a long-acting injectable for HIV.



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No Cross-Resistance and Effective in Vitro Coverage of HIV-1 with Primary Reverse Transcriptase Resistance by a Combination of Islatravir and Lenacapavir

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Background: Islatravir (ISL) is a nucleoside reverse transcriptase translocation inhibitor (NRTTI) that blocks HIV-1 reverse transcription through both immediate and delayed chain termination. Lenacapavir (LEN), a first-in-class long-acting HIV-1 capsid inhibitor, interferes with capsid-mediated nuclear import of HIV-1 DNA, virus assembly and release, and proper capsid core formation. The potent antiviral activities, non-overlapping mechanisms of action, and long-acting pharmacokinetic profiles of ISL and LEN make them an attractive combination for the treatment of HIV-1. Two Phase 3 studies (ISLEND-1, NCT06630286; ISLEND-2, NCT06630299) are investigating a fixed-dose once-weekly oral ISL/LEN combination in virologically suppressed people with HIV-1.

Methods: In-vitro experiments were conducted to characterize the antiviral activity of ISL and LEN against HIV-1 variants with reverse transcriptase substitutions identified in previously published ISL resistance selection experiments. Site-directed mutants were cloned into the pXXLAI HIV molecular clone and transfected into 293T cells to produce infectious viral supernatants. Viral replication was assessed in the RevLucGFP reporter cell line across a range of inhibitor concentrations, and fold-change in susceptibility was calculated relative to the wild-type control virus. Additionally, the frequency of virologic breakthrough by HIV-1 variants harboring common RT resistance mutation patterns was assessed using clinically relevant concentrations of ISL and LEN, alone or in combination. Protease-RT fragments from clinical isolates with known resistance-associated mutations were subcloned into the pXXLAI HIV molecular clone, and the

resulting viruses were assayed in static resistance selection experiments in MT-2 cells.

Results: Phenotypic profiling with ISL revealed a low-to-moderate shift in half-maximal effective concentration (EC₅₀) values (6- to 35-fold) for ISL-selected HIV-1 variants containing M184I or M184V, either alone or in combination with A114S (with or without M41L), S162Y, H221Y, or T165A, consistent with prior reports. In comparison, LEN inhibited all variants without loss of potency. Similarly, no loss in potency was observed with the negative controls tenofovir alafenamide, doravirine, and dolutegravir from the nucleoside reverse transcriptase inhibitor (NRTI), non-NRTI and integrase strand-transfer inhibitor classes, respectively. The positive control NRTI emtricitabine lost activity (>100-fold EC₅₀ shift) against M184I/V-containing variants. Clinically-derived viral isolates representing the following RT resistance mutation patterns were generated and phenotyped: M184V± K65R or Thymidine Analogue Mutations (TAMs), and TAMs only. None-to-low levels of breakthrough were observed with LEN monotherapy at clinical target exposure concentrations across all viruses tested over the course of a 35-day assay (n=3). Low level of breakthrough occurred at clinical target exposure concentrations of ISL among mutants harboring M184V+TAMs at baseline, but not among the other viruses tested. Breakthrough with LEN was associated with the emergence of previously characterized capsid inhibitor resistance mutations L56I and M66I, while ISL breakthrough occurred in the context of M184V+TAMs without additional emergent mutations. Importantly, ISL+LEN combination treatment prevented any breakthrough, including among variants encoding M184V±TAMs at baseline.

Conclusions: The non-overlapping resistance profile between ISL and LEN, along with potent coverage of common RT resistance mutations by LEN, supports the ongoing clinical evaluation of weekly oral ISL/LEN for the treatment of HIV-1.



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Europe Is Still Characterized by a Relevant Burden of HDV Infection and Double Reflex Testing Can Be Valuable to Increase HDV Diagnosis at an Earlier Stage of Liver Disease

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Background and Aims: Hepatitis D virus (HDV) causes the most severe viral hepatitis, however often remains underdiagnosed. Here, we investigate HDV screening and seroprevalence in a large set of HBsAg+ subjects and we report the characteristics of active HDV infection in Europe and the Middle-East before (2021-2023) and after (2024-2025) the implementation of double reflex testing (DRT, anti-HDV testing in all HBsAg+ subjects with unknown HDV serology and HDV-RNA if anti-HDV+ as per EASL guidelines).

Method: This multicenter study included 23927 HBsAg+ subjects from 21 hospitals in Europe (Great Britain, Switzerland, Luxembourg, Germany, Belgium, Sweden, Lithuania, Spain, Italy [N=2], Slovenia, Croatia, Romania [N=2], Serbia [N=2], Slovakia, Bulgaria) and the Middle East (Israel, Turkey [N=2]). The retrospective phase (2021-2023) evaluated 15170 HBsAg+ subjects while the prospective phase (2024-2025) implemented DRT in 8757.

Results: Subjects were 57.6% males, 92.3% HBeAg-negative, with median (IQR) age of 49 (39-61) years and only 29% under NUC-treatment. The rate of HDV screening strongly increased after DRT implementation from 58% to 94.5% (P<0.001) (100% in 13 hospitals). HDV seroprevalence decreased after DRT implementation from 5.9% to 3.7% (P<0.001). Notably, HDV-RNA testing raised from 80.7% to 94.7% (P<0.001) while HDV-RNA positivity remained substantial (47% and 36.6%; P=0.04), supporting a relevant circulation of active HDV infection. Overall, DRT increased anti-HDV detection by 36.5% (P<0.001) and reduced the proportion of untested HDV-RNA cases to 5.4% compared to 19.3% (P<0.01). Compared to 2021-2023, anti-HDV+ subjects diagnosed by DRT more frequently had ALT<40 U/l (66% vs 33.3%, P<0.001) and a less advanced liver fibrosis (F0/F1 in 94% vs 33.8%, P<0.001),



supporting HDV detection in milder liver disease. In the 304 subjects with active infection, median (IQR) HDV-RNA was 5.5 (4.4-6.5) logIU/ml. Notably, compared to 2021-2023, subjects with active HDV infection detected by DRT had significantly lower HBsAg (2323 [1034-6109] vs 5158 [1740-11031] IU/ml), HBV-DNA (30 [0-2300] vs 131[16-2172] IU/ml), and ALT (46 [37-70] vs 79 [53-144] U/L) ($P < 0.001$ for all), supporting DRT as a valuable approach for an early HDV diagnosis, with lower cytolytic activity.

Conclusion: This study reveals a still relevant burden of HDV infection with an intense HDV replication across Europe. Implementing double reflex testing increases HDV case identification and enables diagnosis at earlier stages of liver disease, a critical step to strengthen linkage to care and reduce HDV-related morbidity and mortality.



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Characterization of HIV-1 Non Canonical Mutations in Cameroonians Who Failed a DTG-Based Regimen

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Background: Virological failure (VF) to Dolutegravir (DTG) may occur without the selection of resistance in the integrase (IN) region, suggesting other mechanisms of resistance. Some in vitro and in vivo studies, mainly in subtypes B and C, have reported the selection of non-canonical (NC) mutations outside the IN region associated with IN inhibitor (INI) resistance, though clinical evidence remains limited. Our study aimed to investigate potential patterns of NC mutations among people with HIV-1 who failed a DTG-containing regimen in Cameroon.

Methods: A cross-sectional study was conducted on individuals with DTG-VF at the Chantal Biya International Reference Centre, Cameroon. HIV-1 whole-genome sequencing (WGS) was performed using ABL DeepCheck Assay on Illumina MiSeq, covering gag (p1, p7, p17, p24), env (gp120, gp41), and 3'polypurin tract (3'PPT) to assess the prevalence of NC mutations (using the NGS cutoff of 5%) in relation to major INI resistance. Fisher's and covariation analysis were used to evaluate

potential factors associated with NC mutations (with significance $p < 0.05$).

Results: WGS was successful from 37 individuals (median HIV-RNA 61,502 cps/ml [IQR 14,281–149,492] and duration of INI exposure 20 months [IQR 15–29]). More than half 21/37 (56.8%) had HIV-1 vertical transmission, 25/37 (67.6%) experienced poor adherence, and CRF02_AG recombinant 22/37 (59.5%) was the prevailing clade. Overall, DTG resistance rate was 12/37 (32.4%). At least one NC mutation in gag, env and 3'PPT was found in 25.9%, 100% and 8.1% of individuals, respectively. NC mutations in CRF02_AG vs non CRF02_AG were similarly distributed.

Particularly, we found 12 known NC mutations in gag (N=5) and in env (N=7), but none had a significant association with the presence/absence of INI major resistance. Two known mutations (V13I in p7 and V181I in gp41) were highly polymorphic (>90%). Notably, we identified 17 novel NC mutations (3 in gag, 14 in env) prevailing in individuals harboring INI resistance, and 2 (H9S in gag, S144N in env) rather prevailing in those without. Thirty-four/37 individuals had at least one NC novel mutation. None of the novel NC mutations were associated with GRT viral load and specific INI major mutations (particularly the most observed G118R and R263K) and the known NC mutations. Only one statistically significant association was detected between two novel NC mutations (gp120_T232N and gp120_I320T, Rho 0.63, p-value = 0.042 after Benjamini-Hochberg correction). In the 3'PPT, no NC mutations associated with INI resistance were observed.

Conclusions: In this non-B HIV-1 setting, NC mutations were highly present in DTG-VF cases. Several known NC mutations were found despite no significant association with major INI resistance, while novel NC mutations in both gag and env were significantly associated with INI major resistance. The covariation analyses suggests potential co-evolutionary patterns between NC mutations in env, and a role for compensatory fitness under DTG pressure. These findings underscore the value of WGS for a broader characterization of DTG resistance outside of the integrase region. Overall, further studies with a larger population of DTG treated individuals are needed to better delineate the clinical relevance of the NC mutations.



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Optimising HIV-1 5' Leader Region Sequencing to Detect Non-Suppressible Viraemia

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Background: The HIV-1 5' leader region is a highly conserved sequence regulating essential steps of the viral life cycle. It contains key functional elements, including the dimerisation initiation site (DIS), the major splice donor site (MSD), the packaging signal (Ψ) and the primer binding site (PBS).

Mutations or deletions within this region, particularly affecting the MSD, have been associated with persistent non-suppressible viraemia (NSV) in patients receiving optimal antiretroviral therapy. These alterations enable transcription of defective proviruses and the release of RNA-containing but non-infectious viral particles lacking envelope proteins, which may explain persistent residual viraemia despite treatment.

Sequencing of the 5' leader region may help identify these mutations and clarify the origin of NSV, avoiding unnecessary treatment intensification and improving clinical management. The aim of this study was to optimise NGS protocol for analysing this region and detecting mutations potentially associated with NSV.

Materials and Methods: Seven plasma samples from viraemic patients with viral loads ranging from 10 million to 100 copies/mL were analysed to optimise amplification and sequencing protocol for the target region.

RNA extraction was performed using the Maelstrom 4800 system (TanBead). For samples with viral loads below 500 copies/mL, 3 mL of plasma were ultracentrifuged prior to extraction to concentrate viral genetic material. The HIV-1 5' leader region was amplified using specifically designed primers (Nouhin et al. 2023) and the Q5® Hot Start High-Fidelity PCR kit.

Amplicons were sequenced on an Illumina NextSeq 1000 platform. FASTQ files were aligned and assembled against an HIV-1 reference

sequence from Los Alamos database (<https://www.hiv.lanl.gov>) using CLC Genomics Workbench. Consensus sequences were analysed using BioEdit to identify mutations within the region of interest.

Results: Sequencing of the 5' leader region was successfully achieved in six plasma samples from viraemic patients, with the lowest successfully analysed viral load being 100 copies/mL.

Amplification was unsuccessful in one plasma sample with a viral load of 300 copies/mL.

Preliminary analysis of the obtained sequences revealed multiple nucleotide changes within the 5' leader region, including A758T in two samples, A760T in two samples and A757G in one sample. Small deletions at positions 756 (n=4) and 760 (n=4) were identified, as well as a deletion at position 734 in one sample. Additionally, small insertions at position 759 were observed in two samples.

However, none of the sequences showed the previously described deletions d22 (positions 727–748) or d21 (positions 740–760), nor the nucleotide substitutions T745A and T745C, which have been reported to affect the MSD and the packaging signal (Ψ) and to potentially generate defective proviruses. This finding is consistent with the fact that samples from patients with active viraemia were analysed to optimise the protocol.

Conclusions: A next-generation sequencing-based strategy for analysing the HIV-1 5' leader region was successfully optimised. Our results demonstrate the feasibility of studying this region in plasma samples with low-level viraemia and also in proviral DNA, enabling the identification of mutations potentially associated with NSV. This approach may help clarify the origin of persistent viraemia in patients receiving antiretroviral therapy.



POSTER ABSTRACT PRESENTATIONS

24th European Meeting on
HIV & Hepatitis 2026

In-Person Meeting

10 – 12 June 2026
Barcelona, Spain



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MPXV-Positive Samples in Berlin 2022 to 2026: NGS Sequencing and Phylogenetic Analyses

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Background: In May 2022, there was a major outbreak of MPXV, the West African Clade II variant. The epidemic quickly subsided in Europe after vaccination programs in the summer of 2022, but sporadic positive MPXV detections remain to this day, even in vaccinated individuals. This raises the question of genetic evolution of the virus and phylogenetic development.

Methods: For this study, 43 samples from 2022 that tested positive for MPXV and 77 samples from 2023 to 2026 were sequenced. The used xGen Monkeypox Virus Amplicon Panel provides a single-tube, two-step PCR amplification workflow with primer sets designed to create amplicons across the entire genome. Library prep was performed with PCR-based barcoding (MiSeq) or using the QIAseq FX DNA Library Kit, or for the Nanopore using rapid- and native barcoding. Sequencing was performed with the MiSeq (Illumina®) and with the MinION (Oxford Nanopore®). Bioinformatic processing was carried out using DRAGEN (Illumina®) or a self-generated pipeline, followed by phylogenetic analysis using Nextclade (Nextstrain®).

Results: The comparison between the sequences based on the four different workflows showed substantial differences in e.g. coverage or frame shifts. The sequencing of the IDT-panel worked better with the MiSeq, as this MPXV Panel provides many short sequence segments that are less suitable for Nanopore Rapid Barcoding. However, the Nanopore Native Barcoding showed similarly good results as the sequencing with MiSeq. Subtype identification showed that there was a diversification of subtypes during the course of the pandemic. While in 2022 97.2% were closely related in the B lineage, far more diverse lineages were assigned in 2023 to 2026. All patient samples until the 3rd quarter of 2025 were found to belong

to MPXV clade IIb. In the 4th quarter of 2025, the first sample clade Ib appeared and in the end of the 1st quarter 2026 all positive MPXV sample belonged to clade Ib. Some clusters can be recognized within the patient samples, which are probably due to the repeated introduction of different viruses into the Berlin MSM community.

Conclusions: In persons infected with clade IIb, lineages diversified over the course of the pandemic. While 97.2% of lineages belonged to lineage B in 2022, additional lineages D, E and F could be identified during 2023, 2024 and 2025. We could show since December 2025 an increasing number of clade Ib infections. These observations point to multiple underlying infection events that have led to independent transmission clusters. No development in MPXV regarding vaccine evasion has been clearly identified to date.



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Baseline Genetic Diversity of the HIV-1 Capsid in Benguela, Angola: Implications for Future Lenacapavir Use

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Background: In 2024, Western and Central Africa accounted for 19% of all AIDS-related deaths despite representing about 13% of people living with HIV globally. Additionally, Central Africa, as the epicentre of the global HIV-1 pandemic, exhibits substantial HIV-1 genetic diversity. Angola is no exception, co-circulation of multiple subtypes and recombinant forms in the country promotes viral diversification. While most regional studies focus on the pol gene given its relevance for antiretroviral resistance, gag remains underexplored despite its growing therapeutic importance. In 2022, capsid inhibitor lenacapavir (LEN) was approved by the European Medicines Agency and U.S. FDA for multidrug-resistant HIV-1 treatment, and in 2025, for use as PrEP. LEN, the first long-acting antiretroviral targeting the viral capsid, provides new options for treatment and prevention. However, several capsid mutations reducing LEN susceptibility have been reported, underscoring the need for baseline resistance profiling in regions of pronounced genetic diversity. Here, we aimed to characterise HIV-1 molecular epidemiology in Benguela, Angola, via analysis of the capsid region and to evaluate circulating mutations associated with reduced LEN susceptibility.

Materials and Methods: A cross-sectional study was conducted that included 243 treatment-naïve individuals recently diagnosed with HIV-1 at the General Hospital of Benguela, Angola, between August 2016 and January 2017. Dried blood spots were preserved on Whatman™ Indicating FTA® Elute Micro Cards (Cytiva, Wilmington, DE, USA), and proviral DNA was extracted. The capsid-coding region was amplified using nested PCR and

sequenced via Sanger methodology. Maximum-likelihood phylogenetic analyses were conducted using IQ-TREE, which identified the Transition model (TIM) as the best-fit nucleotide substitution model. Node support was assessed by ultrafast bootstrapping with 1000 replicates. Recombinant forms were characterised by bootscanning in SimPlot v.3.5.1, employing 120–200 nucleotide sliding windows and 10–20 nucleotide step sizes when genetic divergence suggested recombination. Capsid mutations reducing LEN susceptibility were scanned using Stanford HIVdb, ANRS-MIE, IAS-USA and previous mutation reports in the literature.

Results: High-quality capsid sequences were obtained from a subset of samples and revealed extensive HIV-1 genetic diversity. Several pure subtypes were identified, with subtype C being the most prevalent (31.25%), followed by G (6.25%), sub-subtype A1 (5.0%), F1 (3.75%), A2 (1.25%), A6 (1.25%), and subtype H (1.25%). Recombinant viruses represented half of the total infections, with unique recombinant forms (URFs) accounting for 37.5% of sequences. Among circulating recombinant forms, CRF02_AG, CRF14_BG, CRF18_cpx, CRF45_cpx, and CRF124_cpx were each detected at low frequencies (2.5%). Analysis of capsid resistance-associated mutations did not reveal any that conferred phenotypic resistance to LEN, though T107A and T107S, detected in seven sequences (9.7%), are accessory mutations with unknown impact on resistance to LEN.

Conclusions: These findings position Angola as a transitional region between highly diverse Central Africa and subtype C-dominated Southern Africa. The high proportion of URFs underscores the importance of continued molecular surveillance of HIV-1 diversification and recombination. Importantly, the lack of circulating capsid resistance-associated mutations suggests that LEN-based therapeutics are likely to be highly effective. Establishing this baseline of capsid genetic diversity provides valuable information for future monitoring as capsid-targeting therapies become widely implemented.



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HIV-1 Genetic Diversity in Benguela, Angola: Insights into Transmitted Resistance to Integrase Strand Transfer Inhibitors

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Background: Angola harbours extensive HIV-1 genetic diversity, reflecting its central role in the early evolution and ongoing diversification of the global HIV-1 pandemic. Nearly all major subtypes and multiple circulating recombinant forms (CRFs) co-circulate in the country, creating favourable conditions for viral recombination and genetic diversification. Integrase strand transfer inhibitors (INSTIs) are now a cornerstone of antiretroviral therapy due to their high potency, favourable tolerability, and elevated genetic barrier to resistance. Following the adoption of dolutegravir (DTG) as first-line therapy in Angola in 2021, establishing a baseline of transmitted drug resistance (TDR) to INSTIs prior to widespread exposure is critical. This need has become increasingly urgent in the context of limited pre-treatment resistance screening capacity in low- and middle-income settings and recent disruptions to international HIV funding. In January 2025, the temporary halt of PEPFAR highlighted the vulnerability of HIV treatment and prevention programmes dependent on external support. Angola is among the top ten countries with the highest reliance on USAID funding, underscoring the importance of locally generated molecular surveillance data to inform sustainable treatment strategies.

Materials and Methods: We conducted a cross-sectional study of 243 treatment-naïve individuals recently diagnosed with HIV-1 infection attending the General Hospital of Benguela, Angola. Proviral DNA was extracted from FTA™ cards (Cytiva,

Wilmington, DE, USA), and the integrase-coding region was amplified by PCR and sequenced using the Sanger method. Phylogenetic analyses were performed using maximum-likelihood methods implemented in IQ-TREE, which identified the best-fit nucleotide substitution model as the General Time Reversible (GTR) model. Branch support was assessed by ultrafast bootstrapping with 1000 replicates. Recombinant forms were characterized by bootscanning through SimPlot v.3.5.1, employing a sliding window of 100-200 nucleotides and step sizes of 10-20 nucleotides. Drug-resistance mutations (DRMs) associated with INSTIs were identified using the Stanford HIVdb, ANRS-MIE, and IAS-USA algorithms.

Results: High-quality integrase sequences were obtained from 92 samples, representing 16 distinct genetic forms. Unique recombinant forms (URFs) accounted for 50.0% of sequences, followed by subtype C (10.9%) and sub-subtype F1 (8.7%). Phylogenetic analyses revealed high subtype diversity and complex recombinant patterns, consistent with Angola's role as a hotspot of HIV-1 diversification. Among the 81 sequences analysed for resistance, six INSTI-associated DRMs were identified in 23 sequences (28.4%), including five accessory mutations (L74I, L74M, Q95K, T97A, E157Q) and one major mutation (E92G). Most mutations were individually predicted to confer low-level or potential low-level resistance, and no sequence showed resistance to DTG or bictegravir. The accessory mutation L74I was detected in 17.3% of sequences, underscoring the importance of continued surveillance due to its potential independent and synergistic role in resistance development, particularly to cabotegravir.

Conclusions: These findings provide a pre-DTG baseline of HIV-1 INSTI resistance in Benguela, Angola, and support the continued use of DTG as first-line therapy. The high prevalence of URFs and extensive subtype diversity highlight the need for continued molecular surveillance to guide treatment strategies and inform future resistance monitoring in a region central to global HIV-1 diversification.



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Inconsistent Hepatitis Delta Virus Subgenotype Classification Limits Epidemiological Comparability

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Background: Hepatitis delta virus (HDV) is classified into eight genotypes, with genotype 1 being the most prevalent worldwide. Although genotype assignment is well established, the subgenotype classification of HDV-1 remains inconsistent across phylogenetic frameworks. Different reference sequence sets and clustering criteria have resulted in non-equivalent subtype definitions, limiting comparability between studies and potentially affecting epidemiological analyses and genotype–phenotype correlations. We therefore evaluated the level of agreement between two published HDV-1 subgenotype classification systems and assessed how reference selection influences subgenotype assignment in clinical isolates.

Materials and Methods: A comparative phylogenetic analysis was performed using 357 near full-length HDV genome sequences obtained from individuals with detectable HDV-RNA. Sequences were aligned using MAFFT v7. Phylogenetic trees were constructed in MEGA v6 with the Neighbor-Joining method under the Tamura 92 model with gamma distribution (T92+G), and branch support was assessed by 1,000 bootstrap replicates. Subgenotype assignment was conducted independently

according to the reference sequences proposed by Le Gal et al. (subgenotypes 1a–1d) and Karimzadeh et al. (subgenotypes 1a–1e). Concordance was evaluated at both genotype and subgenotype levels. Recombination was screened using a sliding-window similarity analysis (250 nt window, 10 nt step) against reference clusters, identifying significant breakpoints only when spanning at least 150 nt. Breakpoint uncertainty was estimated at ± 125 nt, and all preliminary assignments will be further validated with RDP.

Results: Genotype-level clustering was fully concordant across both reference systems. HDV genotype 1 was the most frequent (329/357, 92.2%), followed by genotype 5 (23/357, 6.4%), three cases of genotype 7 and single cases of genotypes 3 and 6. Among genotype 5 sequences, subgenotypes 5a (n=7) and 5b (n=16) were identified. Among genotype 1 isolates, 30 potential 1c/1d recombinant strains were initially identified.

Excluding these putative recombinant sequences from the comparison between classification systems, 299 genotype 1 isolates were retained for subgenotype assignment. Marked discrepancies were observed in HDV-1 subgenotype assignment between classification systems. Among genotype 1 sequences (n=299), only 10 (3.34%) showed concordant subgenotype classification using both reference frameworks. Using the Le Gal classification, subgenotype 1d predominated (245/299, 81.9%), whereas 1a, 1b and 1c accounted for smaller proportions (8%, 6.4% and 3.7%, respectively). When the Karimzadeh references were applied, the distribution shifted markedly: subgenotype 1e became predominant (172/299, 57.5%), followed by 1c (90/299, 30.1%), while 1a, 1b and 1d were infrequent (5.7%, 6.4% and 0.3%, respectively).

Conclusion: HDV genotype determination appears robust and reproducible across classification systems. However, HDV-1 subgenotype assignment is highly sensitive to the choice of reference sequences, resulting in marked differences in subtype distribution. These findings underscore the need for a harmonized and consensus-based phylogenetic framework to ensure reliable epidemiological interpretation and to support future clinical and translational research in HDV infection.



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Lower Risk of Hepatocellular Carcinoma in Patients with Chronic Hepatitis B, Co-infected or Not with HIV, Using Tenofovir than Using Entecavir

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Primary liver cancer is the sixth most frequently diagnosed malignancy worldwide and the second leading cause of cancer-related mortality. Hepatocellular carcinoma (HCC) is the predominant histological subtype of primary liver cancer, accounting for the majority of cases, and continues to carry a poor prognosis, particularly when diagnosed at advanced stages (1). Cirrhosis and hepatocellular carcinoma are closely related comorbidities and remain a major clinical concern, even in countries with broad access to antiviral therapy and specialized care. Furthermore, chronic liver diseases continue to represent significant causes of hospitalization and mortality among people living with HIV (2,3,4).

This study aimed to compare the use of tenofovir disoproxil fumarate (TDF) and entecavir (ETV) in patients with chronic hepatitis B virus (HBV) infection, with or without HIV coinfection, assessing the risk of progression to hepatocellular carcinoma.

The study population consisted of patients receiving antiviral therapy for HBV, and the incidence of HCC was analyzed according to the antiviral regimen used. The results demonstrated a significant protective effect associated with TDF use. The estimated relative risk (RR) for progression to HCC among patients receiving tenofovir was 0.08, corresponding to an approximate 90% reduction in the risk of developing hepatocellular carcinoma compared with the alternative treatment group. Notably, none of the HIV-coinfected patients—who represented 18.67% of the total sample—developed HCC. Among these coinfecting individuals, 96% were receiving TDF-based

therapy. Among patients with HBV infection overall, those treated with TDF also showed a lower incidence of HCC compared with those treated with ETV. Of the 26 patients who developed hepatocellular carcinoma during follow-up, only 1.2% were receiving TDF, whereas 17.1% were on ETV therapy ($p < 0.05$), indicating a statistically significant difference between treatment groups. Regarding mortality directly attributable to HCC complications, nine patients died during the study period. The mortality proportion was lower in the TDF group (6.4%) compared with the ETV group (9.8%), further supporting the potential protective role of tenofovir not only in reducing HCC incidence but also in influencing clinical outcomes among patients who developed malignancy.

Overall, these findings suggest that tenofovir use is associated with a substantial reduction in the risk of progression from chronic hepatitis B to hepatocellular carcinoma, including in patients coinfecting with HIV. The results underscore the importance of antiviral treatment selection in modifying the natural history of chronic HBV infection and potentially improving long-term liver-related outcomes. Careful therapeutic decision-making may therefore play a critical role in reducing the burden of hepatocellular carcinoma in this high-risk population.



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Advancing HIV Care in Resource-Limited Settings: Integrating Hepatitis C Treatment with ART for Improved Health Outcomes in People Living with HIV

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Background: Co-infection with hepatitis C (HCV) remains a significant threat to the health of people living with HIV (PLWH), particularly in resource-limited settings where access to both diagnostics and treatment is often severely restricted. Untreated HCV accelerates liver damage, complicates HIV management, and increases morbidity and mortality. At CIHEB-Kenya, we observed that HCV treatment was often overlooked, despite its critical impact on patient health. This study presents an innovative integrated care model that combines HCV treatment with antiretroviral therapy (ART) in Nairobi, using direct-acting antivirals (DAAs) to simultaneously address both infections. By integrating these treatments, we aim to enhance overall health outcomes for PLWH and reduce the dual burden of HIV and HCV.

Materials and Methods: We screened 1,200 PLWH across five major healthcare facilities in Nairobi for HCV. Those diagnosed with HCV were immediately started on a 12-week course of DAAs, alongside their ART regimen. The integrated care model provided adherence support, liver function monitoring, and counseling throughout the treatment process. Data were collected on HCV diagnosis, ART adherence, liver function, and HIV viral suppression during and after treatment to assess the effectiveness of this integrated approach.

Results: HCV Diagnosis: 25% of PLWH tested positive for HCV, and 90% of these individuals were successfully linked to care ($p < 0.001$), showing that timely diagnosis and linkage to care can significantly improve treatment outcomes.

Treatment Adherence: An impressive 95% of patients completed the full 12-week course of DAAs ($p < 0.001$), demonstrating the high adherence rates fostered by the patient-centered care approach.

Sustained Virologic Response (SVR): 92% achieved SVR, indicating successful eradication of HCV ($p < 0.001$), confirming the effectiveness of DAAs in treating HCV.

Liver Function: 80% of participants showed significant improvement in liver function ($p = 0.003$), reducing the long-term impact of HCV-related liver damage.

HIV Viral Suppression: Among those treated for HCV, 85% achieved HIV viral suppression ($p = 0.004$), demonstrating the synergistic effect of combined treatment for both infections.

Conclusions: Integrating HCV treatment with ART in a patient-centered model has proven to be a highly effective approach to managing co-infections in PLWH. The results clearly show that addressing HCV alongside HIV leads to significant improvements in adherence, viral suppression, and liver health, ultimately enhancing long-term health outcomes. This integrated approach is cost-effective, scalable, and should be implemented across resource-limited settings to reduce the burden of co-infections and improve patient care. By combining HCV and HIV treatment, we are not only providing better care but also laying the foundation for sustainable healthcare models in regions with high co-infection rates.



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A Cross-Sectional Analysis of Prevalence and Risk Factors for HIV, HBV and HCV among Hemodialysis Patients from Buea, Cameroon

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Background: Hemodialysis patients are at increased risk of acquiring blood-borne infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) infections because of their susceptibility to invasive procedures and their immunosuppressive state. This study aimed to determine the prevalence of HIV, HBV, and HCV among hemodialysis patients at the Buea Regional Hospital (BRH) and identify potential risk factors for transmission.

Methods: A facility-based cross-sectional study was conducted between April and June 2024 among patients receiving maintenance hemodialysis at the BRH, Cameroon. A minimum sample size of 88 participants was calculated; 100 eligible patients were enrolled. Data were collected using a structured questionnaire to assess sociodemographic characteristics, risk factors, and knowledge of HIV, HBV, and HCV. Serological testing for HIV, HBV, and HCV was performed using rapid diagnostic test kits according to manufacturers' instructions. Infection prevention practices were assessed through direct observation of hemodialysis sessions. Data were analyzed using SPSS Statistics version 22.0, with associations assessed at $p < 0.05$.

Results: The prevalence rates of HIV, HBV, and HCV were 17.0%, 9.0%, and 3.0%, respectively. HIV positivity was significantly associated with lower education and a history of sexually transmitted infections. Most participants demonstrated moderate to good knowledge of these infections; however, only 38% recognized HBV as vaccinepreventable, and just 5% reported receiving the vaccine. Observations revealed gaps

in infection control, including inadequate hand hygiene and absence of dedicated areas for patients with known infections

Conclusion: Hemodialysis patients at BRH carry a high burden of bloodborne infections. Strengthened vaccination programs, improved infection control, and larger multicenter studies are needed to reduce transmission risks among patients with chronic kidney disease in Cameroon.



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The Risk of Hepatitis B Reactivation during Immunosuppressive Therapy in B-Cell Cancers: The Role of Antiviral Prevention

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Background: Patients receiving immunosuppressive therapy for B-Cell cancers such as lymphoma and leukemia, may be at an increased risk for reactivation of Hepatitis B infections. Immune Checkpoint Inhibitors, (ICIs), weakens immune responses that control latent viruses. Reactivation of Hepatitis B leads to disruption of necessary cancer treatment, liver complications, increased morbidity. This qualitative study assesses clinical data to observe whether antiviral medications may reduce the likelihood of the reactivation of Hepatitis B in patients due to treatments for B-cell cancers, and to examine clinical strategies that can be used to manage these issues.

Materials and Methods: A qualitative review was carried out to evaluate the risk of Hepatitis B reactivation in patients with B-Cell malignancies who are currently receiving immunosuppressive Treatments. Literature included viral infection reactivation rates, antiviral treatments, and treatment outcomes. Specific data sets were reviewed comprising of 1,057 patients with chronic Hepatitis B infection receiving ICIs, while another study reviewed 633 patients with Hepatitis B infection and cancer who are also undergoing ICI therapy. Across these trials, reactivation incidents were evaluated and statistical comparisons were made between patients receiving antiviral treatments and those who weren't. These clinical trials revealed that reactivation was statistically higher for patients with chronic Hepatitis B infection compared to those without. Additionally, a multinational survey of 56 oncology centers throughout Europe including Italy, France, and Spain assessed the clinical practices needed in order to manage

patients suffering from Hepatitis B, allowing them to receive immunotherapy.

Results: Across examined studies, reactivation was statistically higher in those with chronic infection compared to those with a past infection. It was evident that the absence of antiviral treatments was associated with increased rates of reactivation risk. Antiviral prophylaxis significantly reduced the risk of viral reactivation. HBV prophylaxis is recommended by European and American medical societies, for managing HBV infection in patients receiving ICIs. It is clear that screening cancer patients for HBV before beginning ICIs is crucial. By monitoring HBV biomarkers, it reduces the likelihood of complications and inhibits potential mortality amongst these patients.

Conclusions: Our qualitative study concluded that cancer treatment with ICIs has been associated with the reactivation of the Hepatitis B virus, particularly among patients with chronic infection. When antiviral prophylaxis is not administered to patients, studies show the clinical studies we reviewed reflected that there is a significantly higher rate of reactivation without prophylaxis compared to minimum rates of reactivation for patients receiving prophylaxis treatment. Across examined studies, cancer patients receiving immunotherapies reflected relatively low reactivation rates, yet almost all cases occurred in patients with chronic HBV infection. HBV screening before initiation of Immunotherapy or ICI treatment is crucial. Consequently, expansion of screening and antiviral therapy may reduce hepatitis reactivation incidents during Immunosuppressive treatment in patients with B cell cancers. Therefore the conclusion of our qualitative study emphasizes that patients who are receiving antiviral treatments are not likely to have reactivation of Hepatitis B. Strategically, European Oncologists are in the process of developing methodical and systematic treatment improvements aiming at optimum treatment outcomes.



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Tenofovir vs. Entecavir in Chronic Hepatitis B: A Retrospective Cohort Study Of Hepatocellular Carcinoma Risk in a Tertiary Hospital in Southern Brazil

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Background: Chronic Hepatitis B Virus (HBV) infection remains a major global health burden, affecting approximately 296 million individuals worldwide and leading to significant morbidity and mortality due to cirrhosis and Hepatocellular Carcinoma (HCC). Although Nucleos(t)ide Analogues (NAs) such as Tenofovir Disoproxil Fumarate (TDF) and Entecavir (ETV) effectively suppress HBV replication, their comparative efficacy in reducing HCC risk remains controversial.

Methods: This retrospective cohort study analyzed HBV-monoinfected patients treated with either TDF or ETV at a tertiary hospital in southern Brazil between 2014 and 2021. Patients with co-infections (HIV, HCV), prior HCC diagnosis, liver transplantation, or others antiviral treatments, like lamivudine e/ou alfainterferon, were excluded. Data on demographics, treatment regimens, disease progression, and HCC incidence were extracted from institutional databases. Statistical analyses included Fisher's exact test and Poisson regression to determine Relative Risk (RR) and 95 % Confidence Intervals (95 % CIs).

Results: Of the 127 included patients, 66 (52 %) received TDF and 61 (48 %) received ETV. Over a 7-year follow-up period, 10 patients developed HCC – 8 in the ETV group (13.1 %) and 2 in the TDF group (3 %). In the raw analysis, TDF use was associated with a significantly lower risk of HCC progression (RR = 0.23, p = 0.057, 95 % CI: 0.05–1.046). After adjusting for the variable's cirrhosis/advanced fibrosis and age in the multivariate analysis, this association lost statistical significance (RR = 0.33, p = 0.18, 95 % CI:

0.068–1.685). This indicates that the apparent protective effect of tenofovir may have been influenced by these variables and may have limited the statistical power of the adjusted model. Patients receiving ETV had a higher prevalence of advanced liver disease, including cirrhosis (64.7 % vs. 35.3 %, p < 0.05), hepatic encephalopathy (7 % vs. 2.4 %, p < 0.05), and portal hypertension (12.5 % vs. 3.4 %, p < 0.05). The incidence rate of HCC was 1.12 per 100 person-years (to be interpreted with caution due to limited follow-up data).

Conclusion: Treatment with TDF was associated with a lower risk of HCC compared to ETV in the bivariate analysis, but this association lost significance in the multivariate analysis. These findings suggest that the initially observed protective effect of TDF against hepatic carcinogenesis may have been partially explained by con- founding factors (cirrhosis/advanced fibrosis and age), as well as reflecting the limited sample size. Further studies are warranted to provide a more robust comparative evaluation of antiviral therapies.



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Bictegravir/Emtricitabine/Tenofovir Alafenamide (B/F/TAF) in ART Treatment-Naïve (TN) People with HIV (PWH) by Time since Diagnosis (Rapid, Intermediate, and Late Start) in the Pooled BICSTaR Cohorts

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Background: Starting 2014, rapid start of antiretroviral therapy (ART) after HIV diagnosis established slowly and became more pronounced with the introduction of bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF). We assessed the outcomes of starting rapidly, intermediate or late with B/F/TAF in BICSTaR.

Methods: Data from the final dataset (2024) were pooled from BICSTaR cohort studies (Asia/Canada/EU/Israel), representing participants from 14 countries. Treatment-naïve PWH newly initiating ART with B/F/TAF were divided into three groups according to time between diagnosis and ART start, rapid (≤ 7 days), intermediate (8-182 days) and late (>182 days), and were followed for 24 months (M). Parameters assessed were baseline characteristics, viral suppression (HIV-1 RNA <50 copies/mL; missing=excluded [M=E] and discontinuation=failure [D=F]), median (Q1, Q3)

change in CD4 cell count, CD4/CD8 ratio, and drug-related (serious) adverse events (DR[S]AEs).

Results: We included n=137 participants with rapid start (median [Q1, Q3] time to start: 2 days [0, 5]), n=345 with intermediate start (28 days [15, 49]) and n=59 with late start 1303 days [577, 3993]). Sex at ART-start was n=131 (96%) males in rapid; n=317 (92%) males in intermediate; n=55 (93%) males in late starters. Median age (Q1, Q3) was 32 (27, 40), 35 (28, 45), and 39 years (30, 47) respectively. Median HIV-1 RNA viral load (VL, log₁₀ copies/mL) (Q1, Q3) was 5.16 (4.54, 5.79) in rapid, 4.91 (4.19, 5.46) in intermediate and 4.53 (3.81, 5.14) in late starters (Kruskal-Wallis $p < 0.001$). Median CD4 cell counts (cells/ μ L) (Q1, Q3) at baseline were 298 (143, 424) in rapid, 353 (214, 496) in intermediate, and 356 (255, 517) in late starters (Kruskal-Wallis $p = 0.199$). Reasons for initiating B/F/TAF were early treatment according to guidelines (93%, 88%, 58%), treatment as prevention (20%, 10%, 25%), patient's wish (31%, 17%, 31%), and others (1%, 5%, 14%).

At 24M, viral suppression (M=E/D=F) was 92%/87% (rapid), 96%/91% (intermediate) and 98%/91% (late) with no reported treatment emergent resistance to B/F/TAF. Median absolute CD4 cell counts and CD4/CD8 ratios improved from baseline to 24M (rapid: +268 [143, 443] CD4 cells/ μ L, ratio +0.40 [0.20, 0.60]; intermediate: +236 [113, 415] cells/ μ L, ratio +0.41 [0.27, 0.70] and late: +220 [154, 334] cells/ μ L, ratio +0.30 [0.18, 0.60]). DRAEs occurred in 12.4% [rapid], 11.3% [intermediate], and 16.9% [late] participants; Most frequent ($\geq 1\%$ in total population) DRAEs were weight increase (n=10 [7.3%]/ 10 [2.9%]/ 3 [5.1%]) and headache (n=1 [5.1%]/ 4 [1.2%]/ 1 [1.7%]). N=6 (33.3%) with rapid, n=12 (20.7%) with intermediate and n=0 participants with late start discontinued B/F/TAF due to DRAEs. DRSAEs occurred in n=1 participant (intermediate group; immune reconstitution inflammatory syndrome). Persistence on B/F/TAF was high and similar in groups (96% in rapid, 95% in intermediate and 98% in late starters).

Conclusions: In this cohort, most participants started ART >7 days after diagnosis. More rapid initiation was associated with higher baseline viral load (significant) and lower CD4 cell count (not significant). Treatment with B/F/TAF was highly effective and generally well tolerated irrespective of the timing of treatment initiation. Of note, we observed similar high persistence in the rapid start group compared to the intermediate and late group.



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Switching to Doravirine/Islatravir (100 mg/0.25 mg) Once Daily Maintains Viral Suppression Through Week 48 in the Presence of Archived NNRTI Resistance-Associated Mutations or M184I/V in Proviral DNA

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Background: Doravirine/islatravir [DOR/ISL (100/0.25mg)] is an investigational once-daily single tablet regimen of DOR, an approved non-nucleotide reverse transcriptase inhibitor (NNRTI), and ISL, a novel nucleoside reverse transcriptase translocation inhibitor (NRTTI), being evaluated in 3 Phase 3 studies (P051: NCT05631093; P052: NCT05630755; P054: NCT05766501) in adults living with HIV-1. Participants with HIV-1 RNA <50 copies/mL and no known treatment failure or documented DOR resistance were randomized (2:1) to switch to DOR/ISL (100/0.25mg) once-daily or to continue baseline antiretroviral therapy (bART) in P051 or BIC/FTC/TAF in P052. In P054, participants who previously received DOR/ISL (100/0.75mg) were switched to DOR/ISL (100/0.25mg) in a single-arm study. This analysis examined the prevalence and impact of preexisting resistance-associated mutations (RAMs) at baseline in proviral DNA on virologic outcomes among participants who received DOR/ISL through Week 48 in P051, P052, and P054.

Methods: Preexisting RAMs present in proviral DNA were identified retrospectively using the GenoSure Archive assay (Monogram Biosciences). The impact of NNRTI RAMs and M184I/V was evaluated in participants with HIV-1 RNA ≥50 copies/mL at any time through Week 48 using the

following virologic outcome categories: discontinued with confirmed HIV-1 RNA ≥200 copies/mL (DISC); low-level viremia (LLV, confirmed HIV-1 RNA ≥50 copies/mL without meeting discontinuation criteria); transient viremia (TV, HIV-1 RNA ≥50 copies/mL at any on-treatment visit preceded and followed by HIV-1 RNA <50 copies/mL).

Results: Of the 1227 participants with baseline resistance data who received DOR/ISL, 6 (0.5%), 23 (1.9%), and 71 (5.8%) met the criteria for DISC, LLV, or TV, respectively (Table 1). Among participants who received DOR/ISL, 26.3% had preexisting NNRTI RAMs and 5.7% had preexisting M184I/V. The percentage of participants in each virologic outcome category was similar between all participants and those with NNRTI RAMs or M184I/V.

Conclusions: Preexisting NNRTI RAMs or M184I/V in proviral DNA did not impact virologic suppression through Week 48 in participants switching to DOR/ISL (100/0.25mg) in Phase 3 clinical studies.



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Baseline and Week 96 Resistance Analysis in Participants Receiving Bictegravir + Lenacapavir in the Phase 2 Portion of Study ARTISTRY-1

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Background: ARTISTRY-1 is a Phase 2/3 study evaluating once-daily bictegravir (BIC) plus lenacapavir (LEN) versus a Stable Baseline Regimen (SBR) in virologically suppressed (VS) adults with HIV-1 receiving complex multi-tablet regimens. In the Phase 2 portion (N=128), participants were randomized 2:2:1 to BIC 75 mg+LEN 25 mg, BIC 75 mg+LEN 50 mg, or SBR.

Methods: Baseline HIV-1 genotypes were derived from cumulative historical genotypic reports and/or proviral DNA genotyping of Day 1 samples. The impact of baseline resistance-associated mutations (RAMs) on Week 96 efficacy was evaluated. Post-baseline genotypic and phenotypic resistance testing was performed in participants with confirmed virologic rebound (HIV-1 RNA \geq 50 copies/mL; analyzed if \geq 200 copies/mL) or at last visit with \geq 200 copies/mL using Monogram or Institute for Immunology and Genetics assays. To phenotypically characterize observed polymorphisms, mutations were introduced by PCR-based site-directed mutagenesis in an HIV-1 molecular clone. Viruses were evaluated in a standardized multi-cycle phenotypic assays to quantify LEN fold-change in susceptibility versus wild-type. For structural context, amino acid side-chain rotamers were evaluated for potential steric clashes for HIV-1 capsid mutations of interest in the published HIV-1 capsid LEN-bound model using ChimeraX. Codon analysis estimated the genetic barrier for substitutions at the positions of interest.

Results: Baseline genotypes were available for 96.9% of participants (124/128). Primary RAMs were frequent: nucleoside reverse transcriptase

inhibitor (NRTI) RAMs in 81.5%, non-nucleoside reverse transcriptase inhibitor (NNRTI) RAMs in 65.3%, protease inhibitor (PI) RAMs in 46.0%, and integrase strand-transfer inhibitor (INSTI) RAMs in 10.6% (proviral DNA genotyping only). The presence of baseline RAMs did not affect efficacy at Week 96. One participant receiving BIC (75 mg)+LEN (50 mg) met the criteria for post-baseline resistance testing and was included in the Final Resistance Analysis Population. This participant had no emergent resistance to either BIC or LEN and had preexisting NRTI, NNRTI and PI RAMs. Sequencing at last visit identified the N74T polymorphism in capsid with wild-type susceptibility to LEN (LEN fold-change of 1.2 of wild-type) in comparison to the known LEN RAM N74D (fold-change of 18.1 of wild-type). After discontinuation of BIC+LEN, the regimen was changed to include subcutaneous LEN + dolutegravir bid + maraviroc, resulting in HIV-1 RNA suppression $<$ 50 copies/mL. Genetically, N74T (ACN) is \geq 2 nucleotides away from N74D (GAT/GAC), indicating a higher genetic barrier and no obvious genetic linkage between N74T and N74D. Structural modeling showed multiple rotamers for N74T/N74D, none of which exhibited steric clashes between N74T and LEN, in contrast to N74D and LEN.

Conclusions: Despite high frequencies of baseline resistance, high rates of viral suppression were maintained through 96 weeks in PWH receiving daily BIC+LEN. No on-treatment resistance to study drugs was detected through $>$ 96 weeks. The capsid polymorphism N74T had no impact on LEN susceptibility. Codon analyses indicated N74T was unlikely to favor transition to N74D, supporting the limited clinical relevance of N74T. These findings highlight the durability of once-daily BIC+LEN and support the development of the BIC+LEN single-tablet regimen to optimize treatment options in VS PWH.



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Dolutegravir + Lamivudine in People with HIV Who Are Naive to Antiretroviral Therapy with Very High Viral Load: Systematic Literature Review of Real-World Evidence and Randomized Controlled Trials

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Background: Dolutegravir + lamivudine (DTG + 3TC) is approved for initial antiretroviral therapy (ART) in people with HIV-1 irrespective of viral load (VL), and DTG + 3TC labeling does not have restrictions based on VLs; however, major HIV guidelines do not recommend the use of DTG + 3TC for initial ART in those with VL $\geq 500,000$ copies/mL. Because it can be challenging in clinical trials to recruit individuals with very high baseline VL, they are often underrepresented, and robust evidence for this population is lacking. Herein, we summarize real-world evidence (RWE) and randomized controlled trial (RCT) data on first-line DTG + 3TC use among people naive to ART with baseline VL $\geq 500,000$ copies/mL.

Materials and Methods: A systematic literature review (SLR) was conducted using PRISMA guidelines to identify evidence on the use of DTG + 3TC in people with HIV and high baseline VL. Real-world evidence studies published between January 2013 and July 2025 reporting DTG + 3TC use in ≥ 10 people with HIV were identified from structured searches of Ovid MEDLINE, Embase, PubMed, Cochrane Library, and relevant international conference proceedings. In addition, data from GEMINI-1/-2, STAT, DOLCE, and D2ARLING phase 3/4 RCTs were identified from previously compiled clinical trial evidence. Across RWE and RCT sources, virologic suppression outcomes were summarized for individuals naive to ART initiating DTG + 3TC with baseline VL $\geq 500,000$ copies/mL. Reported proportions of participants who

achieved VL < 50 copies/mL were extracted at Weeks 24, 48, and 96 for RWE studies and at Week 48 (DOLCE, D2ARLING, STAT) or Week 144 (GEMINI-1/2) for RCTs.

Results: The SLR resulted in 382 real-world publications that reported DTG + 3TC use in ≥ 10 people with HIV, representing 111 cohorts and 68,870 individuals. Of these, 9 studies reported effectiveness data in people naive to ART at baseline with VL $\geq 500,000$ copies/mL (n=363), 6 of which reported data at applicable time points (n=300). Across these 6 studies, 76% (197/260), 87% (202/231), and 96% (91/95) of individuals achieved VL < 50 copies/mL at Weeks 24, 48, and 96, respectively. Across 5 RCTs including 1107 people naive to ART and initiating DTG + 3TC, 75 (7%) had baseline VL $\geq 500,000$ copies/mL. Among these individuals, 81% (61/75) achieved VL < 50 copies/mL at Week 48 or 144, demonstrating comparable virologic efficacy to RWE studies.

Conclusions: This SLR shows that RWE demonstrates high virologic suppression rates among individuals naive to ART with very high baseline VL initiating DTG + 3TC, consistent with findings from RCTs. These data, in addition to the indication of DTG + 3TC as initial ART in people with HIV-1 without any restriction to VL at baseline, support the inclusion of DTG + 3TC for individuals with high VL at baseline in treatment guidelines.



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Less Pills, Lasting Control: Real-World Experience with Long-Acting Cabotegravir plus Rilpivirine in Portugal

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Background: Long-acting injectable antiretroviral therapy with cabotegravir plus rilpivirine (CAB+RPV LA) has emerged as an effective alternative to daily oral antiretroviral therapy (ART) for people living with HIV-1. While potential drug-drug interactions and comorbidity warrant careful consideration, the reduction in pill burden and the possibility of improved adherence represent meaningful advantages. International post-approval studies have consistently demonstrated high levels of virological efficacy and patient satisfaction. Nevertheless, real-world data from the Portuguese population remains limited.

Materials and Methods: A retrospective observational study was conducted including all patients who initiated CAB+RPV LA at a single Portuguese HIV treatment centre between September 2024 and December 2025. Demographic, clinical, and laboratory information was collected from medical records. One patient was excluded from the sample due to off label use of CAB+RPV LA; all other patients were included without further exclusion criteria.

Results: Of 282 patients included, 210 were male (74.5%) and 58.5% were under 50 years of age. The majority were Portuguese (61.4%) or Brazilian (21.6%). The median body mass index (BMI) was 26.4 kg/m², with 21.6% presenting BMI > 30 kg/m². Immunisation against hepatitis B virus was documented in 73.8% of patients. Overall, 60.9% had more than one comorbidity and, among those, 38.1% were receiving more than two chronic medications, 12.1% met criteria for polypharmacy (concomitant use of ≥5 chronic medications). HIV acquisition was mostly sexual, namely in heterosexual patients (46.5%) and men who have sex with men (43.4%). The median

duration of infection was 10.9 years, with a median of 9 years on ART – 35.6% had received two previous regimens and 46.6% had received three or more. About 36.5% had prior exposure to non-nucleoside reverse transcriptase inhibitors. Prior to switching, 86.9% were receiving an integrase inhibitor-based regimen and 42.9% had undergone previous genotypic resistance testing without clinically significant mutations. All patients had virological suppression at baseline, except for five with detectable HIV-1 RNA ranging from 72 to 230 copies/mL. The median CD4+ T-lymphocyte count was 637.5 cells/mm³.

The main reasons for switching were greater flexibility (56.5%) and concern about missed doses (21.0%). Following the switch, all patients maintained virological suppression (HIV-1 RNA < 50 copies/mL), except for two cases with low-level viraemia at 1–3 months, one at 6–9 months, and one at 12 months of follow-up. Only one case of confirmed virological failure was observed at six months of therapy.

Treatment discontinuation occurred in 8.5% of patients, mostly due to injection-site pain (33.3%). One patient discontinued treatment because of severe toxic epidermal necrolysis (Lyell's syndrome).

Conclusions: In this real-world heterogeneous Portuguese cohort, long-acting injectable antiretroviral therapy with CAB+RPV demonstrated high rates of sustained virological suppression and a favourable safety profile. Discontinuation rates were low and mainly related to injection-site reactions. These findings support its effectiveness and feasibility across a clinically diverse population.



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WG-am Combinations Restore Antiviral Potency against Fostemsavir and Lenacapavir-Resistant HIV-1 in Salvage Therapy

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Background: Resistance to fostemsavir (FTR) and lenacapavir (LEN) threatens heavily treatment-experienced (HTE) patients with limited therapeutic alternatives. Together with ibalizumab (IBA), these agents constitute main salvage therapy options, yet resistance development can leave patients with no effective treatments. Tryptophylglycine-amide (WG-am), a naturally occurring metabolite from elite controllers, demonstrated dual inhibition of HIV-1 entry and reverse transcription. We evaluated WG-am combinations with seven antiretrovirals against clinically relevant FTR- and LEN-resistant isolates to assess its potential role in salvage therapy.

Materials and Methods: Nine HIV-1 isolates were studied: four FTR-resistant (gp120 mutations M426P, M426L, S375T, S375N) and five LEN-resistant (capsid mutations Q67H, N74D, T107N), exhibiting 2.9- to 50.9-fold resistance levels. Antiviral combination assays were performed in TZM-bl cells using 7x7 dose-matrix format with WG-am combined with FTR, IBA, LEN, darunavir (DRV), raltegravir (RAL), efavirenz (EFV), or tenofovir (TDF) at predefined ratios optimized for each antiretroviral. After 48h, viral inhibition was assessed by luciferase assay. Synergy was analyzed using SynergyFinder software using the Zero Interaction Potency (ZIP) model. Combinatorial activity was classified as synergistic (>10), additive (-10 to 10), antagonistic (<-10) according to ZIP synergy scores.

Results: Across 63 WG-am combinations tested against FTR- and LEN-resistant isolates, ZIP synergy scores ranged from -0.236 to 15.576. In FTR-resistant isolates, the most potent synergistic interactions were observed with FTR itself, achieving high ZIP scores against S375T (15.576) and M426P (12.595) isolates, with an overall mean ZIP of 8.06 (± 3.81) across all FTR-resistant viruses. IBA combinations demonstrated the most consistent and robust additive or synergistic profile, with mean ZIP scores of 10.03 (± 1.31) overall, showing uniformly high synergistic activity across both FTR-resistant (range 8.509-11.123) and LEN-resistant (range 8.480-11.873) isolates. DRV also exhibited synergistic potential, particularly against S375T (13.983) and several LEN-resistant isolates (up to 11.436). In contrast, TDF, RAL, and EFV combinations showed more variable responses, with TDF demonstrating synergy in some LEN-resistant isolates but limited additive activity in FTR-resistant viruses, while RAL and EFV generally produced additive rather than synergistic effects. Importantly, even isolates exhibiting high-level phenotypic resistance retained strong synergistic responses to WG-am combinations. When analyzed at the clinically relevant threshold of $\geq 90\%$ viral inhibition, 55 of 63 combinations (87.3%) demonstrated synergistic activity, underscoring the robustness of these interactions at therapeutically meaningful inhibition levels.

Conclusions: WG-am combinations demonstrated predominantly additive or, in some cases, synergistic antiviral activity against both FTR- and LEN-resistant HIV-1 isolates. The exceptional and consistent synergy observed with IBA (mean ZIP 10.03 ± 1.31) and FTR (mean ZIP 8.06 ± 3.81) is particularly relevant for HTE populations, where these agents serve as critical salvage therapy options. With 87.3% of combinations achieving synergistic interactions at $\geq 90\%$ viral inhibition, these findings support further evaluation of WG-am as a potential salvage therapy component, offering mechanistically distinct combination options for patients with limited therapeutic alternatives. The preserved synergy despite high-level resistance suggests that WG-am dual mechanism of action may establish complementary inhibitory barriers, potentially increasing the genetic barrier to resistance development in salvage therapy settings.



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Switch to DOR/ISL (100/0.25 mg) QD from BIC/FTC/TAF: 96-Week Update from a Blinded Phase 3 Study

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Background: The NNRTI doravirine (DOR) and the investigational nucleoside reverse transcriptase translocation inhibitor (NRTTI) islatravir (ISL) have complementary mechanisms of action and resistance profiles. The primary analysis of the MK8591A-052 study in people living with HIV-1 demonstrated that switching to DOR/ISL 100 mg/0.25 mg was noninferior to continuing bicitgravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF) in maintaining virologic suppression at Week 48. Here we present Week 96 efficacy and safety data from this study.

Methods: Phase 3, double-blind clinical trial (NCT05630755) in adults with HIV-1 RNA <50 copies/mL for at least 3 months on BIC/FTC/TAF, CD4+ T-cell count ≥50 cells/mm³, and no prior therapy failure or known DOR resistance. Participants were randomized 2:1 to switch to DOR/ISL (100/0.25 mg) or continue BIC/FTC/TAF. Discontinuation was required for confirmed decline in total lymphocytes (≥30% and <1.0 x10⁹/L) or CD4+ T-cells (≥30% and either <350 cells/mm³ if ≥350 at baseline or <200 cells/mm³ if ≤349 at baseline). The proportion of participants with HIV-1 RNA ≥50 copies/mL (FDA snapshot) at Week 96 was a secondary endpoint. Other virologic outcomes (HIV-1 RNA <50 and <200 copies/mL), safety, and tolerability through Week 96 were also assessed.

Results: Overall, 342 participants were switched to DOR/ISL and 171 continued BIC/FTC/TAF. At Week 96, 5 (1.5%) participants on DOR/ISL vs 2 (1.2%) on BIC/FTC/TAF had confirmed HIV-1 RNA ≥50 copies/mL (difference: 0.3%, 95% confidence interval: -2.8, 2.4); 2 (0.6%) vs 0, respectively, had discontinued due to lack of efficacy; and 3 (0.9%) vs 1 (0.6%), respectively, had discontinued for other reasons with HIV-1 RNA ≥50 copies/mL at discontinuation. Proportions of participants with adverse events (AEs) through Week 96 were similar between groups. The Week 96 DOR/ISL AE profile was similar to Week 48 with no new safety findings. There were no differences between arms in CD4+ T-cell or total lymphocyte count (TLC) changes through Week 96. In both groups, 0.6% (DOR/ISL n=2, BIC/FTC/TAF n=1) discontinued due to protocol-specified decreases in CD4+ T-cell and/or TLC counts.

Conclusions: DOR/ISL (100/0.25 mg) maintained a high rate of virologic suppression and had similar efficacy to BIC/FTC/TAF at Week 96. DOR/ISL demonstrated an AE profile comparable to BIC/FTC/TAF and did not adversely affect lymphocytes. These findings are consistent with the study's primary Week 48 results.



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Accessing HIV Care to Irregular Migrants in Israel, 2019-2024

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Background: In Israel, irregular migrants (IM) living with human immunodeficiency virus (HIV-1) that have no access to regular health insurance are provided with HIV-1 related health coverage under a public-private partnership (PPP) program initiated by the Ministry of Health in 2014.

Materials and Methods: Here we characterized IM referred to the PPP in 2019-2024 and used a linear mixed-effects model to follow-up their CD4 and HIV-1 viral load (VL) counts for a median period of 16 months. Subtypes, resistance mutations and phylogenetic relationships were determined in all cases with viral failure and in selected cases with available blood remains.

Results: A total of 231 of 238 referred to the PPP, initiated antiretroviral treatment (ART) with nucleoside reverse transcriptase inhibitors (NRTIs) and either non-nucleoside reverse transcriptase inhibitors (NNRTIs, 61.5%, 142/231) or protease inhibitors (PIs, 38.5%, 89/231). Irrespective of the treatment regimen, all these individuals increased their CD4 and decreased their VL trajectories over time ($p < 0.001$). However, mixed model analysis revealed two classes of CD4 trajectory patterns. Comparison between these two patterns revealed that Class-1 individuals started with lower initial

CD4 counts compared to Class-2 (median of 115 cells/mm³, IQR 70-171 compared to median of 312 cells/mm³, IQR 104-510, $p < 0.001$) and experienced slower recovery compared to Class-2. Most Class-1 individuals originated from Africa (78% vs. 52%, $p = 0.016$). Treatment failure was observed in 5.6% of all individuals receiving treatment under the program. Sequencing analysis, enabled in 23% of the treated cohort, revealed that the reverse transcriptase (RT) M184V (13%) and K103N (7.4%) were the most prevalent mutations.

Conclusions: While treatment was not consistent with current recommendations for first-line therapy, the virological and immunological response of most patients was favorable and the prevalence of cases with resistance mutations was not higher than that identified in people living with HIV-1 who are covered by the national health insurance. Despite the limitations of the PPP, this program may provide a unique therapeutic opportunity for IM with HIV-1.



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Antiretroviral Therapy as a Predictor of Frailty and Cardiovascular Risk in an Aging Cohort in Houston, Texas, USA

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Background: Frailty and cardiovascular disease (CVD) risk are increasingly prevalent among people living with HIV (PLWH) aged 50 years and older. The contribution of contemporary antiretroviral therapy (ART) regimens to frailty phenotypes and cardiometabolic risk remains incompletely understood, particularly in real-world clinical settings.

Methods: We conducted a cross-sectional analysis of 132 PLWH aged ≥50 years receiving care at a single urban HIV clinic in Houston, Texas. Frailty status was classified as robust, pre-frail, or frail using a standardized frailty scale. ART regimens were categorized by commonly prescribed regimens, and body composition was assessed using waist-hip ratio (WHR). Cardiovascular risk was estimated using pooled atherosclerotic cardiovascular disease (ASCVD) risk scores. Associations between ART regimens, frailty status, WHR, and ASCVD risk were evaluated using chi-square tests and bivariate comparisons.

Results: The median age was 60.0 years (IQR, 55.0–65.5); 53.0% identified as non-Hispanic Black and 75.0% as male. Overall, 41.7% of participants were pre-frail or frail. Polypharmacy was common, with 76.5% taking ≥5 non-HIV medications. Among commonly prescribed ART regimens, no statistically significant association was observed between regimen and frailty status when frailty categories were analyzed separately ($p=0.10$). However, when pre-frail and frail participants were combined, a statistically significant association emerged, suggesting possible power limitations in stratified analyses. Mean WHR differed significantly across ART regimens ($p=0.04$), with higher WHR observed among participants receiving dolutegravir/lamivudine–

based therapy (Dovato). No significant differences in ASCVD risk scores were observed across treatment regimens.

Conclusions: In this aging cohort of PLWH receiving care within a single clinical environment, ART regimen was associated with differences in body fat distribution but not with estimated ASCVD risk. The observed association between ART and frailty when pre-frail and frail states were combined suggests that ART-related vulnerability may emerge earlier in the frailty continuum. Longitudinal studies are needed to clarify causal pathways linking ART, metabolic changes, and frailty progression.



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Switch to DOR/ISL (100/0.25 mg) QD from Oral ART: Week 96 Update from an Open-Label Phase 3 Study

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Background: In an open-label, phase 3, randomized clinical study (MK-8591A-051; NCT05631093) in virologically suppressed adults with HIV-1, switching to the fixed combination of doravirine with islatravir (DOR/ISL 100/0.25 mg) once daily showed non-inferior efficacy to continuing oral antiretroviral therapy (ART) and was well tolerated at Week 48. We report on efficacy and safety results through Week 96.

Methods: After Week 48 of the comparative portion of the study, all participants receive open-label DOR/ISL (100/0.25 mg) through Week 144. Discontinuation is required for participants with clinically significant confirmed viremia (CSCV; 2 consecutive HIV-1 RNA ≥ 200 copies/mL 4 [± 1] weeks apart) or confirmed decline from baseline in total lymphocytes ($\geq 30\%$ and $< 1.0 \times 10^9/L$) or CD4+ T-cells ($\geq 30\%$ and either < 350 cells/mm³ if ≥ 350 at baseline, or < 200 cells/mm³ if < 350 at baseline).

Results: On Day 1, 366 participants switched to DOR/ISL (Group 1) and 185 continued their baseline ART (bART; Group 2). Mean age was 49.8 (± 12.3) yrs; 39.7% were female at birth, 45.4% Black, and 14.5% Hispanic or Latino/a; median time since HIV diagnosis 13.3 (IQR 7.2-20.4) yrs. bART was InSTI-based (64.2%), NNRTI-based (30.3%), or PI-based (5.4%) with median duration 3.8 (IQR 2.0-6.3) yrs. At Week 48, 177 participants

(95.7%) in Group 2 switched from bART to DOR/ISL, for a total of 543 on DOR/ISL. At Week 96, HIV-1 RNA was ≥ 50 copies/mL in 9 participants: 7 (1.9%) from Group 1 and two (1.1%) from Group 2, while virologic suppression was maintained in 92.6% and 96.6%, respectively. One participant in Group 1 (and none in Group 2) had CSCV after Week 48 and was discontinued. No participant developed treatment-emergent resistance to either DOR or ISL through Week 96. Adverse event rates in Group 2 (Weeks 48-96) were similar to those in Group 1 (Weeks 0-48). Mean % change (from start of DOR/ISL) in CD4+ T-cell and total lymphocyte counts were comparable across the groups at Week 96, with no discontinuations due to decreased CD4+ T-cell or total lymphocyte count. Mean weight change after starting DOR/ISL was similar across the treatment groups, with a greater change seen in participants who switched from weight-suppressive bART containing efavirenz and/or tenofovir disoproxil fumarate.

Conclusions: Switching to DOR/ISL (100/0.25 mg) maintained a high rate of viral suppression over 96 weeks with no emergent resistance to DOR or ISL. DOR/ISL was well tolerated and did not adversely affect lymphocyte counts.



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Adoption of Cabotegravir + Rilpivirine in Clinical Practice

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Background: Long-acting injectable antiretroviral regimen cabotegravir + rilpivirine offers a treatment option for people living with HIV who are virologically suppressed and may improve treatment experience by reducing daily pill burden and HIV-related stigma. Data from Research Partnership's Therapy Watch HIV EU5 study is used to evaluate uptake of cabotegravir + rilpivirine among people switching antiretroviral therapy following European Commission marketing authorisation in December 2020.

Materials and Methods: HIV specialists across the EU5 completed quarterly online surveys in 2021, and bi-annual online surveys between 2022 and 2025. Each clinician submitted 10 anonymised records of people living with HIV who had either newly initiated or switched antiretroviral therapy within the previous 12 weeks. Data captured included demographics, disease status, treatment history, and prescribing rationale. Temporal trends were assessed using z-tests to determine statistical significance ($P \leq 0.05$).

Results: The proportion of people switching to cabotegravir + rilpivirine in the prior 12 weeks increased, growing progressively from 0.9% in 2021 (n=4893) to 17.9% in 2025 (n=2799) with the steepest growth observed between 2022 and 2023 ($P \leq 0.05$).

Among people switching to cabotegravir + rilpivirine, regimen selection was driven by patient request (range 30.2%-58.7%) and lifestyle related factors including convenience (range 7.1%-14.1%). Clinical drivers such as efficacy/potency and new mechanism of action were reported less frequently and declined over time.

Conclusions: This study demonstrates the sustained growth of long-acting injectable cabotegravir + rilpivirine within EU5 clinical practice, supporting its transition from a newly authorised treatment option to an established component of HIV care. Drivers for regimen selection reflect a broader shift in HIV

management, with optimisation of the treatment experience and shared decision-making becoming increasingly central to regimen choice alongside durable viral suppression. Variation across EU5 markets also indicate local differences in adoption of this regimen and prescribing rationale. Collectively, these trends highlight an evolving treatment landscape in which patient-centred factors are playing an increasingly prominent role in shaping therapeutic strategies.



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Quantitative Profile of Proviral HIV-DNA during Low-Level Viremia among Adolescents with Perinatally Acquired HIV Receiving Dolutegravir-Based Regimen in Cameroon

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Background: Antiretroviral therapy (ART) has led to unprecedented control of viral replication, enabling children living with HIV to reach adolescence and adulthood. However, adolescents living with perinatally-acquired HIV (ALPHIV) face numerous treatment challenges including persistence of immune dysfunction, poor ART adherence, and adverse effects. These challenges may lead to low-level viremia (LLV) and expand the size and diversity of viral reservoirs. Considering the limited data on the characteristics of viral reservoirs during LLV, this study aims to ascertain the quantitative profile of total HIV-DNA during LLV in ALPHIV.

Materials and Methods: A cross-sectional study was conducted among ART-experienced ALPHIV receiving TDF/3TC/DTG (TLD) in Cameroon in the years 2020-2025, with at least 2-years of follow-up after starting TLD. Socio-demographic plus clinical data were collected; viral load (VL) and CD4 T-cell count were measured. LLV was defined as having VL 50-999 copies/ml (stratified into two groups: 50-199 and 200-999 copies/ml) at the final time point. Total HIV-DNA levels were quantified

through a commercial kit (HIV-1 DNA Test PRO, Diatheva, using Altona extraction platform), normalized per 10⁶ blood-cells. Statistical associations were evaluated by Spearman's correlation and Mann-Whitney U test, as appropriate; statistical significance was set $p < 0.05$.

Results: Overall, 219 ALPHIV were enrolled and 21.0% (n=46) experienced LLV. Among them, 60.9% were females, 36.9% were from rural areas, median [IQR] age was 14 [11-17] years, ART-duration was 6 [4-11] years, and the median TLD-duration was 25 [16-31] months. The median [IQR] VL was 145 [64-390] copies/ml, with 58.7% (n=27) between 50-199 copies/ml and 41.3% (n=19) between 200-999 copies/ml. Contextual CD4 T-cell count was available for 16 ALPHIV, with a median value of 855 [581-1066] cells/mm³, with 87.5% having a CD4+ count ≥ 500 cells/mm³. The median [IQR] total HIV-DNA was 94.9 copies/10⁶ blood-cells. No significant correlation was found between total HIV-DNA levels and VL ($r = -0.02$; $p = 0.89$), ART-duration ($r = -0.25$; $p = 0.10$) and TLD-duration ($r = 0.13$; $p = 0.40$). Similarly, no significant difference in total HIV-DNA levels was found between participants with VL 50-199 and 200-999 copies/ml (100.3 vs 81.93, $p = 0.38$). However, a moderate and positive correlation was found between CD4 T-cell count and total HIV-DNA levels ($r = 0.5$; $p = 0.03$). The Kruskal-Wallis H test indicated no statistically significant difference in HIV-DNA levels between HIV-RNA levels in the previous years of follow-up (VL ≥ 1000 at least once, $n = 13$; between 50-999 at least once, $n = 13$; always < 50 , $n = 20$; $p = 0.137$). Finally, participants residing in rural areas presented higher total HIV-DNA levels compared to those residing in urban areas (Median [IQR]: 175 [81-270] vs 74 [54-117] copies/10⁶ blood-cells, $p = 0.007$).

Conclusion: This study shows a moderate level of total HIV-DNA among ALPHIV with LLV receiving TLD. No significant correlation was found between total HIV-DNA levels and LLV. However, total HIV-DNA was positively associated with CD4 count and residing rural areas, highlighting potential contextual factors that may influence HIV reservoir dynamics in adolescents. These findings contribute to the limited data on viral reservoir characteristics during LLV and highlight the importance of considering both biological and contextual determinants when developing strategies to limit the persistence of HIV reservoirs in adolescents.



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Transmitted Drug Resistance in New HIV Diagnoses in Spain: Evidence from 18 Years of Monitoring in CoRIS (2007–2025)

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Background: The evaluation of drug resistance in new HIV diagnoses in Spain has been conducted by CoRIS since 2007, with annual updates through 2023. In the present work, we extend this

surveillance by analyzing data from 2024 and 2025. In this study, we present the year-over-year trend (2007–2025) of transmitted drug resistance (TDR) in new HIV diagnoses, as well as primary resistance to first-line antiretroviral drugs.

Methods: New diagnoses from CoRIS centers were included. Following quality control of the sequences, mutations in RT/Pro/INI associated with TDR were investigated. Additionally, we evaluated clinically relevant resistance to drugs recommended as first-line treatment in the GESIDA guidelines across the different periods analyzed. Stanford University's tool and phylogenetic analysis with Mega were used for subtyping.

Results: Between 2007 and 2025, 9,839 people living with HIV (PLWH) with new diagnoses were analyzed. TDR shows a stable trend during this period, with the highest levels observed for NNRTIs. Primary resistance to first-line antiretrovirals (ARV) showed a notable decrease in 2014, when NNRTIs were no longer recommended as preferred starting regimens.

During the 2024–2025 study update, a total of 827 people with HIV (PWH) were evaluated, featuring high sequencing success rates with available data for RT (n=815), Protease (n=813), and Integrase (n=613). The analysis revealed a 16.1% overall prevalence of Transmitted Drug Resistance (TDR), primarily driven by NNRTIs at 10.4% and NRTIs at 4.7% (specifically M184V at 1.5%), while resistance to PIs and INIs remained minimal at 0.3% and 0.3%, respectively. A key clinical finding was the identification of the M184V mutation in 12 individuals, with a noteworthy two of these cases occurring in patients with a history of PrEP use, alongside a 8% detection rate of Rilpivirine-associated Resistance-Associated Mutations (RAMs). Resistance levels peaked for efavirenz (9.9%), contrasting with the high susceptibility maintained for bictegravir and dolutegravir, which both showed resistance rates of only 0.2%. The molecular landscape was dominated by subtype B (78.4%), while non-B variants were characterized by a 6.9% prevalence of CRF02_AG, followed by subtypes A6 (1.7%) and A1 (1.2%).

Conclusions: The 2024–2025 period showed a TDR prevalence of 16.1%, predominantly linked to NNRTI resistance (10.4%). Clinically, the data confirms the exceptional durability of Bictegravir and Dolutegravir, with resistance rates remaining negligible at 0.2%. Furthermore, the M184V mutation was found in only 12 cases and showed a minimal association with PrEP use. These findings



validate the continued efficacy of current first-line regimens as the primary therapeutic strategy in Spain.



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Emerging Patterns and Public Health Impacts of Drug Resistance in HIV and Viral Hepatitis: An Integrated Global Analysis

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Background: The ongoing global scale-up of antiretroviral therapy (ART) and antiviral regimens for HIV, hepatitis B (HBV) and hepatitis C (HCV) has dramatically reduced morbidity and mortality. However, the emergence and spread of drug-resistant viral strains threaten to undermine treatment successes and compromise elimination targets. Drug resistance arises from viral mutations that diminish drug efficacy, propagated through treatment failure, sub-optimal adherence, and transmission of resistant variants. Recent WHO surveillance indicates early yet widening resistance to integrase strand transfer inhibitors, including dolutegravir (DTG), especially in populations with prior ART exposure, raising concerns for durable global control strategies.

Materials and Methods: This study synthesizes data from global drug resistance surveillance systems (WHO HIV and hepatitis drug resistance surveys), published cohort studies, and meta-analyses, focusing on viral genetic resistance markers and phenotype treatment failure. Resistance prevalence was quantified using standardized thresholds (e.g., $\geq 10\%$ prevalence of resistance mutations prompting regimen review). Logistic regression and trend analyses were applied to assess changes over time across regions. Key genetic resistance mutations were identified via genotype sequencing in national sentinel surveillance and research cohorts. Comparative analyses examined resistance in treatment-experienced versus treatment-naive populations. Statistical significance was defined at $p < 0.05$ and 95% confidence intervals (CIs) computed where applicable.

Results: Global ART coverage reached 31.6 million individuals in 2024, reflecting dramatic treatment

scale-up. Nevertheless, increasing levels of HIV drug resistance have been documented, with dolutegravir resistance observed up to 4.8% in persons without viral suppression and up to 19.6% among extensively treated individuals, far exceeding thresholds that prompt public health action. These resistance trends were corroborated by regional cohort data showing high resistance to NRTIs and NNRTIs in treatment failures, with M184V and K103N mutations predominant. Evidence from systematic analyses indicates that in some high-burden settings, prevalence of resistance in treatment-failing patients can exceed 70%, emphasizing urgent need for regimen optimization and resistance testing. Additionally, resistance arising in pre-exposure prophylaxis failure underscores the necessity for timely viral load monitoring to preserve future treatment options. Parallel challenges exist for HBV and HCV, where antiviral resistance mutations are emerging, although surveillance remains less comprehensive.

Conclusions: The spread of drug resistance in HIV and viral hepatitis represents a critical threat to public health progress and elimination goals. Integrated surveillance data reveal significant levels of resistance in key populations, especially among individuals with prior heavy treatment exposure, suggesting that current ART scale-up alone is insufficient without robust resistance mitigation strategies. Investment in routine resistance testing, enhanced viral load monitoring, adherence support, and timely therapy switches are essential. The WHO's Integrated Drug Resistance Action Framework (2026–2030) provides a unified road map emphasizing prevention, surveillance, research, laboratory capacity, and governance to control resistance spread and safeguard antiviral efficacy.



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Baseline HIV-1 Capsid (p24) Mutations and Polymorphisms in Newly Diagnosed Infections in Greece: Early Signals for Lenacapavir Resistance Surveillance

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Background: Transmitted drug resistance remains a global concern, with up to 26% of individuals initiating antiretroviral therapy harboring resistance to first-line agents. Lenacapavir (LEN), a first-in-class long-acting injectable capsid (p24) inhibitor (CAI), has demonstrated sustained viral suppression in heavily treatment-experienced individuals and is expanding into prevention strategies. Resistance to LEN has been linked to specific p24 codons, including L56, M66, Q67, K70, N74, and T107, according to the 2025 IAS–USA and Stanford HIVdb updates (Figure 1). However, baseline p24 variability in treatment-naïve populations remains poorly characterized.

Methods: We analyzed 262 newly diagnosed, antiretroviral-naïve HIV-1 subjects. An in-house Sanger assay covering near full-length p24 was applied to all samples. Approximately 30% also underwent in-house NGS using a limited 40-cycle amplification strategy (20 RT-PCR + 20 nested cycles) to minimize amplification bias. NGS achieved >3000 reads per nucleotide. HIV-1 subtyping was performed using COMET, and mutations were interpreted according to IAS–USA and Stanford HIVdb criteria; polymorphic substitutions were systematically recorded.

Results: Median age was 42 years (IQR: 33–50), median HIV-1 RNA 4.9 log₁₀ copies/mL (IQR: 4.63–5.75), and 79.4% were male. Transmission categories included 45.4% MSM, 28.3% heterosexual individuals, and 26.3% PWID. No major or accessory LEN resistance-associated mutations (LEN-RAMs) were detected by either Sanger or NGS. Polymorphic substitutions were identified in 81/231 (35.1%) p24 codons (Table 2),

including positions associated with LEN resistance. The polymorphic mutations T107A (8/262; 3.1%) and T107S (9/262; 3.4%) were observed; neither co-occurred with Q67H. All detected variants were confirmed by NGS (>98%). Subtype distribution was heterogeneous, with 36.3% A, 17.9% B, and 27.1% CRFs, predominantly CRF02_AG and CRF35_AD.

Conclusions: Baseline capsid resistance to lenacapavir was not observed in this cohort, despite substantial viral genetic diversity and polymorphic variability across p24 codons. Although substitutions at resistance-associated positions, including L56C, N74X, T107A and T107S (the latter two polymorphic), were detected, none corresponded to established LEN-RAMs. These findings suggest preserved baseline susceptibility to LEN capsid inhibitor in Greece. Ongoing molecular surveillance of the capsid (p24) gene remains essential as long-acting capsid inhibitors are increasingly implemented in clinical practice and prevention settings.



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HIV-1 Transmitted and Acquired Drug Resistance across National Surveillance, Multicentre and Community-Based Studies in Portugal: A Scoping Review

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Background: HIV-1 drug resistance threatens the effectiveness of antiretroviral therapy and the global targets for HIV elimination. Portugal has experienced dynamic changes in its HIV-1 epidemic, with evolving patterns of transmitted drug resistance (TDR) and acquired drug resistance (ADR).

This scoping review aimed to map the available evidence on HIV-1 TDR and ADR in Portugal, identify temporal trends and resistance patterns, and highlight research gaps to inform future surveillance and public health strategies.

Methods: This scoping review followed the PRISMA-ScR guidelines. We searched PubMed, Scopus, and Web of Science from 2016 and 2026. Eight studies were eligible for inclusion, covering the period from 2001 to 2024. Data were extracted for TDR and ADR prevalence by drug class, resistance mutations, temporal trends, and transmission subgroups. Evidence was synthesised narratively with thematic analysis.

Results: The overall TDR prevalence across studies ranged from 9.4% (2001-2017) to 11% (2014-2019). NRTI resistance ranged from 3.9% (2014-2019) to 4% (2001-2017), with M41L being the most frequent mutation (1.4% and 1.6%, respectively), followed by M184V/I (1% and 1.3%, respectively). NNRTI resistance was 5% in both periods (2001-2017 and 2014-2019), with K103N/S as the most prevalent mutation (3.2% and 3.1%, respectively). PI resistance ranged from

2.8% (2001-2017) to 3.9% (2014-2019), with L90M as the most frequent mutation (1.2% and 2.2%, respectively). INSTI resistance was not assessed in most periods, however one study reported a prevalence of 0.3% (2022-2023), while another among MSM (2023-2024) reported no INSTI resistance.

Temporal analysis spanning 2001-2017 showed an increasing trend in TDR, from 7.9% in 2003 to 13.1% in 2017. In the 2014-2019 study, TDR showed a decreasing trend from 16.7% in 2014 to 9.2% in 2016, followed by an increase from 8.9% in 2017 to 21.9% in 2019. TDR was associated with subtype B and C, heterosexual transmission group and higher viral load.

Overall, the ADR prevalence ranged from 41.1% (2022-2023) to 69% (2001-2017). For 2001-2017 period, NRTI resistance was 57.8%, mainly driven by M184I/V (45.3%), T215Y/F (17.4%), and M41L (16%). NNRTI resistance was 45.8%, predominantly associated with K103N/S (26%). PI resistance was 23.6%, with L90M most frequent mutation (11.3%). A 2022-2023 study reported a 7% prevalence of acquired INSTI resistance. Another study (2022-2024) assessing INSTI resistance in cases of virological failure found a prevalence of 5.5%, with N155H (32.6%) and R263K (30.4%) as the most frequent mutations.

ADR showed a consistent declining trend from 2001 to 2017. Overall ADR decreased from 86.6% to 51.0%, with reductions for NRTIs (80.8% to 33.3%), NNRTIs (46.4% to 40.2%), and PIs (52.7% to 10.8%).

These findings highlight the urgent need for larger studies with post-2019 data. Identification of INSTI resistance remains limited in Portugal and requires systematic screening alongside with other ARV classes.

Conclusions: This scoping review synthesizes evidence on HIV-1 TDR and ADR in Portugal (2001-2024), identifying trends relevant to treatment guidelines and public health. The concerning increase of TDR and the encouraging decline of ADR reflect changes in treatment access, regimen efficacy, and transmission dynamics.



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Species-Level Virome Profiling Reveals Gut Prokaryotic Virome Dysbiosis in People with HIV-1 on Antiretroviral Therapy

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Background: Alterations in the gut bacterial communities are well documented in people with HIV-1 (PWH). Less is known about the gut viral communities (termed as virome), largely due to technical challenges in studying virome. Recent methodological advancements in the knowledge of the virome provide new evidence for a critical role of the gut virome in human health by shaping microbial community profiles, modulating host immunity and influencing metabolic processes. Herein, we performed an exploratory study on the gut DNA virome in PWH on antiretroviral therapy (PWH-ART), using species-level virome profiling approach.

Materials and Methods: This study included 13 PWH-ART and 11 HIV-1 negative controls, shotgun metagenome sequencing was performed on the faecal samples, virome profiling was performed using two complementary viral taxa identification pipelines, MetaPhlan4 and Phanta, providing high-resolution taxonomic profiles at species level. Viral alpha and beta diversity were measured and compared between PWH-ART and controls. Differential abundance analysis (DAA) was performed to identify group-specific viral taxa. Spearman's rank correlation analyses were performed between clinical variables and abundance of significant viral taxa identified by DAA.

Results: Most of the gut DNA viruses were yet-to-be-identified prokaryotic viral species, and bacteriophages constitute the majority of the identifiable viral populations, with most belonging to the order Caudovirales (mainly Siphoviridae, Myoviridae, Podoviridae families). A significantly lower gut viral alpha diversity and higher variation in overall viral composition (beta diversity) was

observed in PWH-ART compared to HIV-1 negative controls. Remarkably, multiple undescribed viral species were differentially abundant between PWH-ART and controls. Correlations were found between certain undescribed viral species and clinical variables, including CD4+ T cell counts and age.

Conclusions: Our findings suggest the presence of gut prokaryotic virome dysbiosis among PWH-ART. Further studies are warranted to validate our findings and to elucidate whether the observed gut virome dysbiosis is mediated by HIV-1 infection itself, or ART, or both.



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Improving CMV Surveillance in Allogeneic HSCT during and after Letermovir Prophylaxis: CMV-RNA as a Marker of Active Replication

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Background: Cytomegalovirus (CMV) infection continues to be a significant contributor to morbidity and mortality among recipients of allogeneic hematopoietic stem cell transplantation (allo HSCT). The introduction of Letermovir (LTV) prophylaxis, which blocks the viral terminase complex and consequently prevents the processing of CMV genomic concatemers, has markedly lowered the incidence of CMV infection and reactivation. Despite this progress, the most appropriate strategy for virological surveillance remains uncertain. CMV DNA has been so far the only virological marker, even if there is increasing evidence that its quantification in plasma mainly reflects CMV genomic fragments or concatemers. CMV RNA, targeting UL21.5 mRNA packaged within virions, has recently emerged as a potential tool to better reflect the real burden of virions. On this basis, the present study investigates the kinetics of CMV RNA and CMV DNA during and following LTV prophylaxis.

Methods and Materials: We enrolled 42 onco-hematological patients (pts) undergoing allo-HSCT and receiving LTV prophylaxis for at least 2 months: 30 (71.4%) were monitored after LTV for a median time of 87 [IQR 71–134] days. In total, 464 plasma samples were collected longitudinally and analyzed for CMV DNA and CMV RNA levels using the Elitech assays, with limits of quantification of 112 IU/mL and 30 IU/mL, respectively.

Results: During LTV prophylaxis, 5 pts (11.9%) exhibited CMV DNA >112 IU/mL (median [IQR]: 296 [194–1258]), mostly presenting as single or recurrent blips. None of these individuals showed detectable CMV RNA, suggesting absence of virion production and the release of CMV-DNA concatemers. After discontinuation of LTV, 16 of 30 pts (53.3%) developed CMV DNA >112 IU/mL (median [IQR]: 536 [314–3908]); CMV RNA was positive in 5/16 (31.2%). CMV-RNA positivity was associated with higher levels of plasma CMV-DNA (median [IQR]: 12196 [9194–29729] IU/mL in pts with CMV-RNA+ vs 787 [493–2392] IU/mL in pts with CMV-RNA-, $p=0.0005$) and also with a markedly prolonged duration of CMV DNA detectability (median [IQR]: 129 [53–159] days in CMV RNA+ vs 10 [7–20] days in CMV RNA-, $p<0.0001$). This reinforces that transient and low level rises of CMV-DNA do not reflect virion production. Notably, CMV-RNA predicted CMV-related symptoms better than CMV-DNA (PPV 100%, NPV 68% vs 80% and 48%). Kinetic evaluation during anti CMV treatment revealed a more rapid decline and earlier clearance of CMV RNA, consistent with ongoing release of CMV DNA fragments despite effective suppression of viral particle production.

Conclusions: CMV RNA offers a more refined interpretation of virological monitoring by differentiating active viral replication from residual genomic material. Its incorporation into clinical practice may support more responsible anti CMV treatment stewardship, reducing overtreatment and mitigating the burden of drug associated toxicity.



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External Quality Assessment Data Analysis as Part of Development and Post-Market Surveillance of the AltoStar® HDV RT-PCR Kit 1.5 (CE-IVD)

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Background: To consistently evaluate product performance during development and as part of the post-market surveillance (PMS) process, altona Diagnostics participates regularly in External Quality Assessments (EQAs).

Aim: altona Diagnostics participated in the QCMD* HDV EQA scheme with the AltoStar® HDV RT-PCR Kit 1.5, initially twice during the development phase, then once for the PMS to assess and monitor the assay's performance over time.

Material and Methods: All samples of the QCMD HDV EQA schemes 2023 to 2025 contained the highly heterogenic genotype 1, most common in Europe. Samples were processed using the AltoStar® HDV RT-PCR Kit 1.5 / AltoStar® Automation System AM16 (Hamilton) workflow.

Results: All samples of the QCMD HDV EQA schemes were correctly tested positive or negative for HDV RNA using the AltoStar® HDV RT-PCR Kit 1.5. Quantitative analysis and comparison of the data to "mean consensus commercial" lead to an overall $\Delta\log_{10} = 0.07$ difference between results (standard deviation: 0.28). A slope of $y=0.90$ and intercept $b=0.37$ were calculated by Passing-Bablok regression.

Discussion: Results of the AltoStar® HDV RT-PCR Kit 1.5 match excellent to the expected results (qualitative) and to the mean quantitative results of all commercial assays. The dataset confirmed no systematic difference and no proportional difference to mean consensus data.

Conclusion: The AltoStar® HDV RT-PCR Kit 1.5 shows consistently accurate quantification of the 1st WHO International Standard for Hepatitis D Virus RNA (PEI code 7657/12) and for clinical HDV

samples of the QCMD EQA scheme with an average of $\Delta\log_{10} = 0.07$ difference between results.

*Quality Control for Molecular Diagnostics



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Ambient-Stable Air-Dryable qPCR Enzyme for STI Diagnosis: Analytical Performance and Operational Advantages

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Background: Molecular diagnosis of sexually transmitted infections (STIs) is central to modern public health strategies due to their rising global prevalence and associated gynecological, obstetric, and epidemiological complications. qPCR platforms constitute the analytical gold standard for detecting pathogens such as *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, *Gardnerella vaginalis*, *Trichomonas vaginalis*, HSV 1/2, *Ureaplasma parvum*, and *Ureaplasma urealyticum*, owing to their high sensitivity, specificity, and robustness. However, the structural dependence on cold chain logistics remains a major barrier to broad diagnostic deployment in decentralized settings and resource limited regions. This study provides a comparative evaluation of an air dryable qPCR enzyme, allowing mastermix stabilization at ambient temperature, against a reference enzyme used in conventional qPCR kits.

Materials and Methods: Multiplex qPCR reactions were performed in parallel using both enzymes under strictly harmonized experimental conditions, including primer/probe design, reagent concentrations, and thermal parameters. All reactions were run using a single, unified qPCR cycling program to ensure complete methodological consistency across conditions. The evaluation panel comprised clinically characterized specimens and controlled dilution series spanning the critical interval of the limit of detection (LoD). Comparative performance was assessed using three parameters: Cq value distribution, LoD estimation, and inter assay reproducibility. A stability study examined the analytical consistency of the air dried formulation during extended storage at ambient temperature, without refrigeration or dry ice.

Results: The air dryable enzyme demonstrated analytical performance strictly comparable to that of the reference enzyme across all evaluated pathogens. Cq values between the two formulations showed minimal and consistent variation, supporting equivalent analytical behavior, and LoDs were similar for all targets assessed. The air dried formulation maintained stable analytical characteristics throughout ambient temperature storage, with no detectable drift in Cq values or loss of sensitivity. Eliminating the requirement for cold chain conditions removes major logistical constraints typically associated with qPCR reagent transport and storage, providing broad applicability in resource limited environments and decentralized care settings.

Conclusions: The air dryable qPCR enzyme exhibits analytical performance equivalent to that of a reference enzyme while offering a major operational advantage: robust stability at ambient temperature enabling distribution without cold chain dependency. This capability represents a critical lever for expanding molecular STI testing in settings where logistical constraints limit diagnostic accessibility. By combining analytical rigor with simplified logistics, this technology has the potential to significantly enhance global access to STI diagnostics, strengthen early detection strategies, and support public health initiatives aimed at reducing transmission.



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SARS-CoV-2 Excretion in Patients as a Model of Viruses with Respiratory and Digestive Shedding

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Aim: The emergence of SARS-CoV-2 and multiple successive variants favored the viral dispersion to human populations, adaptative tropism to patients' tissues and continuous viral circulation. Viral spreading in stools is poorly known for COVID-19, justifying its exploration in respiratory and digestive compartments by viral loads, variability and infectivity in patients from a University hospital.

Methods: From the end of 2024, nasopharyngeal swabs (NPS) and stools from patients infected by SARS-CoV-2 were collected, in Intensive Care Unit (ICU) and Infectious Diseases Department: NPS at Day-0 (D0), Day of discharge; stools at Day-0 (D0), D2, D4, D-exit, D15 post-exit, to compare digestive and respiratory viral excretion according to time. SARS-CoV-2 PCR by Panther Fusion (Hologic) was used with an internal control (IC1 Biomerieux). Whole Genome SARS-CoV-2 Sequencing (WGS, ARTIC protocol) analyzed the variants, while viral infectivity was studied on VERO-E6 cells.

Results: One cluster of four SARS-CoV-2 infected patients (hospital medical consultation, Infectious Diseases) was explored by four NPS and two stools. For six other hospitalized patients, 12 NPS and 21 stools were collected. At the hospital consultation, in NPS the D0 Ct scale ranged from 21.3 to 33.4 Ct. In the hospital, for NPS the D0 Ct scale was at 16.1 to 29.6 Ct [16.1 Ct at 6.59E+07 cg/mL UTM], while for stools D0 Ct next to 27 Ct to not detected [27 Ct, 9.49E+06 cg/g]. From the day

of diagnosis of SARS-CoV-2 infection by PCR, viral excretion dynamics were patient-dependent, for viral loads and decay kinetics. But overall, the viral load (VL) decline in respiratory and digestive compartments was rather homogeneous for 5/6 patients, more often rapidly decreasing before the patients' discharge.

By WGS, 13/18 samples (12 NPS, 6 stools) showed (i) 231 variants for the cluster, one infective NPS positive strain; (ii) in the hospital, successive strains were 24C, 24E, 24F, XEC, 24E and 25C variants, infectivity observed only in NPS for 3/6 patients but not in stools. On low digestive viral loads, partial S genes were analyzed by NGS for 5/6 stools with NPS/stool pairs for three patients: (i) for one ICU patient, in Spike a T696N mutation was present in NPS but absent in the four successive stools with adequate coverage. For this patient in Spike RBD domain, S(ser) was observed at residue 371 in stools D4, versus F(Phe) in NPS D0 and stools D3. (ii) For the three patients, in RBD at residue 403, R(arg) was identified in stools at different dates, whereas K(lys) was detected in their corresponding NPS D0, suggesting again differences between respiratory and digestive compartments.

Conclusions: This ongoing study aims to characterize SARS-CoV-2 dynamics in respiratory samples and stools. SARS-CoV-2 viral load (VL) and sequence diversity appeared to be time-/patient-dependent while a mathematical modeling of SARS-CoV-2 spreading in stools is a perspective for most patients. Moreover, as viral diversity can influence vaccine and neutralizing antibodies efficacy, Spike amino acids in respiratory versus digestive compartments should be characterized.



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Unified Amplicon-Based Whole-Genome Sequencing of Influenza, RSV, and SARS-CoV-2 from Routine Diagnostics: Performance and Variant Reporting

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Background: Influenza, RSV, and SARS-CoV-2 co-circulate and evolve under immune and therapeutic pressures, complicating decision-making for both vaccine formulation and antiviral use. Fragmented, pathogen-specific sequencing approaches limit cross-virus comparability. Genomic surveillance is essential but often fragmented due to pathogen-specific workflows, limiting cross-virus comparability. A unified sequencing strategy could streamline surveillance and enhance early detection of antigenic drift and resistance markers.

Materials and Methods: We applied a standardized, multiplexed, amplicon-based next-generation sequencing (NGS) workflow to 34 diagnostic specimens (Ct <35) positive for influenza A/B, RSV-A/B, or SARS-CoV-2. Whole-genome amplification used DeepChek[®] assays (ABL Diagnostics), followed by Illumina MiSeq 2×250 bp sequencing. Although the wet-lab workflow is standardized across pathogens, consensus generation and annotation utilized two different analysis environments: Geneious Prime for influenza and MicrobioChek[®] for RSV and SARS-CoV-2, with Nextclade used for RSV clade assignment. Quality metrics included genome breadth and depth of coverage. Phylogenetic reconstruction used maximum-likelihood inference with 1,000 bootstrap replicates.

Results: Near-complete genomes (mean coverage ≥98%) were recovered for all samples. Influenza A(H1N1)pdm09 sequences clustered in clade 6B.1A; A(H3N2) clustered in subclade 3C.2a1b.2a.2; and influenza B belonged to the

Victoria lineage V1A.3a.2. RSV sequences were assigned to Nextclade clades A.D.5.1, A.D.1.10, A.D.2.1, and A.D.3 (RSV-A) and to B.D.4.1.3 and B.D.E.1 (RSV-B), consistent with the ON1 (RSV-A) and BA (RSV-B) genotypes prevalent in recent seasons. Clinically relevant mutations included influenza A/H1N1 HA substitutions (A186T, Q189E, K142R) and NA V453M; influenza B HA Δ162–164 deletion and NA D197N/H273Y; RSV-A F-protein T122A; and SARS-CoV-2 spike L455F/L455S and F456L with ORF8 truncation (G8*), consistent with recent Omicron sublineages. Sequencing quality metrics (mapped reads, Q30, uniformity) met thresholds for reliable full-genome reporting across all three viruses.

Conclusions: A unified amplicon-NGS approach yields harmonized genomic data across respiratory viruses, enabling timely detection of antigenic drift and resistance markers while supporting integrated, cross-pathogen surveillance. By coupling standardized mutation profiling with phylogenetic reconstruction, the approach reduces reliance on pathogen-specific strategies and offers a scalable solution for strengthening early-warning systems and supporting informed vaccine and antiviral policies.



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Pathomorphology of Respiratory Organs in Deceased Patients with COVID-19

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Background. COVID-19 can still have an unfavorable outcome in patients with a complicated premorbid background. A thanatological analysis allows us to assess the significance of the factors leading to severe disease progression and death despite ongoing therapy.

Aim of Study: To present the pathomorphology of lung in patients with severe COVID-19, resulting in death.

Materials and Methods: A retrospective analysis of medical records and autopsy reports was conducted for 41 patients hospitalized and deceased in an infectious clinical hospital with a diagnosis of COVID-19 between 2021 and 2024 (among them, 18 (43.9%) patients died during the pandemic period (2021-2022) and 23 (56.1%) in the post-pandemic period (2023-2024). Statistica package, v. 10 was used. The data are presented as median and interquartile range.

Results: The age of the patients was - 74.0 (46.0; 89.0) years; 19/46% were men; 22/54% were women ($p > 0.05$). In 100% of cases, all patients had a variety of combined somatic pathologies (arterial hypertension, coronary heart disease, heart rhythm disturbances, diabetes mellitus, obesity, kidney pathology, etc.), represented by 3-5 nosologies. All patients had severe lung damage (pneumonia), with complications such as grade 2-3 acute respiratory failure (ARF), acute respiratory distress syndrome (ARDS), and sepsis. The length of hospitalization from the onset of the disease was 4 days (1.0; 30.0). Hospitalization in the intensive care unit on the first day of admission was required for 25 patients (61%), with 10 (24%) of them being transferred to non-invasive/invasive ventilation on the same day. The duration of treatment in the clinic before death was 10 days (1.0; 33.0). According to the autopsy results, the

most pronounced pathomorphological changes were found in the lungs. The main morphological manifestation was diffuse alveolar damage. Viral and bacterial bilateral pneumonia was present in 29 cases (71%); total/subtotal pneumonia – in 11 cases (27%); interstitial pneumonia – in 2 cases (5%); pneumonia in the organizing stage – in 15 cases (37%); hyaline membranes were found in 31 cases (76%); microabscesses in the lungs – in 2 cases (5%). Thrombosis/thromboembolism of small branches of the pulmonary artery – in 10 cases (24%), thromboembolism of large branches of the pulmonary artery – in 2 cases (5%). Bacteriological examination of autopsy lung tissue revealed *Klebsiella pneumoniae* in 19 cases (46%), *Candida* in 12 cases (29%). In addition to lung changes, autopsies revealed a variety of lesions in other organs and systems, the pathogenesis of which is multifactorial. Complications leading to death included: ARDS in 32 cases (78%), acute pulmonary and heart failure in 38 cases (92%), vascular thrombosis and/or pulmonary embolism in 4 cases (10%). No differences were found in the frequency and nature of pathological changes in the lungs between patients who died during the pandemic and post-pandemic periods ($p > 0.05$).

Conclusion: In severe cases of COVID-19, the most pronounced changes were found in the lungs, which became the leading causes of death in the study group, underscores the importance of preventive measures against the development of COVID-19 in patients with multiple comorbidities in the post-pandemic period.



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Evaluation of HIV-1 Proviral Whole-Genome Sequencing for Detection and Interpretation of Archived Drug Resistance Mutations

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Background: In virologically suppressed people with HIV (PWH), Genotype Resistance Testing (GRT) from HIV-1 RNA is unfeasible. In these cases, sequencing of proviral DNA can provide information on archived resistance-associated mutations (RAMs). However, the proviral reservoir contains a mixture of intact and defective genomes, which may complicate interpretation of resistance data. We evaluated the performance of proviral Whole Genome Sequencing (WGS) for detection of pol RAMs and its suitability for GRT.

Methods: Proviral HIV-1 DNA was extracted from 200uL of frozen whole blood and quantified using the HIV-1 DNA assay (Diatheva) on the Elitech Ingenious automated platform. Whole-genome amplification was performed using the AD4Seq HIV Whole Genome assay (Arrow Diagnostics) and sequencing was carried out on the Illumina iSeq100 platform. FASTQ files were processed through an in-house bioinformatics pipeline to evaluate genome coverage, while RAMs within the pol region were interpreted using the Stanford HIV Drug Resistance Database and confronted with those detected from the reference pol-based assay with a 20% variant frequency threshold. Proviral defectiveness was assessed by identifying APOBEC-associated hypermutation patterns, premature stop codons and unusual mutations.

Results: HIV-1 DNA levels ranged from 34 to 830 copies per 10⁶ cells (median 141, IQR 71–165). Genome amplification occurred in 21 samples out of 23 tested (91.3%). Median genome coverage at 100× was 69.5% (IQR 51.4–78.9), with pol median

coverage of 100% (IQR 39 - 100). No correlation was observed between HIV-DNA levels and genome coverage (Spearman $\rho=0.09$, $p=0.71$). Markers of proviral defectiveness were also detected, including APOBEC-related mutations in 28.6% of samples, premature stop codons in 23.8%, and unusual mutations in 19.0%. At least one pol RAM was identified in 61.9% of samples. Comparison with the reference pol-based assay showed variable concordance across drug classes. Several discrepancies were attributable to genomic regions not covered with at least 100 reads by either assay. After excluding these regions, concordance between WGS and the reference assay was 72% for protease inhibitors (PI), 65% for nucleoside reverse transcriptase inhibitors (NRTI), 63% for non-nucleoside reverse transcriptase inhibitors (NNRTI), and 71% for integrase strand transfer inhibitors (INSTI). Among discordant variants, 40% occurred in APOBEC-associated mutational contexts, including D30N and M46I in protease, D67N and M184I in reverse transcriptase, E138K in reverse transcriptase/integrase, and G163R in integrase.

Conclusions: Proviral WGS demonstrated acceptable agreement with standard pol-based resistance testing and enabled the detection of archived RAMs in a substantial proportion of PWH in which GRT from RNA was not feasible. However, the frequent detection of APOBEC-associated signatures indicates that some RAMs identified in proviral DNA may originate from defective proviruses, highlighting the need for careful interpretation of resistance results derived from the viral reservoir. Interpretation of discordant mutations remains challenging, as proviral DNA sequencing is influenced by stochastic sampling of low-copy archived variants. These discrepancies likely reflect the intrinsic biological and technical variability of proviral DNA genotyping, including limited template availability, PCR sampling effects, uneven genomic coverage, and the contribution of defective or hypermutated proviruses.



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HIV Drug Resistance in Angola among Individuals Receiving Art with Viral Load Non-suppression: Comparison of Insti-Naïve and Insti-Experienced Patients

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Background: The WHO recommends dolutegravir (DTG), an integrase strand transfer inhibitor (INSTI), for first, second, and third-line ART. DTG is well tolerated, co-formulated, widely available as a generic, and has a high genetic barrier to resistance. Although DTG resistance remains uncommon in INSTI-naïve patients (INP), increasing use may lead to increasing levels, particularly among treatment experienced individuals.

Methods: We analyzed 201 plasma and dried blood spot specimens (convenience sampling) from adults (age ≥ 15 years) and children (0–14 years) treatment experienced with viral load $\geq 1,000$ copies/mL, including 92 INP and 109 INSTI-experienced patients (IEP), recruited nationwide. HIV-1 genotyping was performed using Sanger sequencing of the HIV-1 pol gene (protease/reverse transcriptase: HXB2 positions 2253–3353; integrase: 4239–5096). HIV subtypes were assigned with COMET and REGA v3.0. Drug resistance was predicted using Stanford HIVdb v10.1 and statistical analyses were conducted using SPSS and Python.

Results: Out of the 201 participants enrolled, including 42 children (20.9%) and 159 adults (79.1%). The median age was 34.0 years (IQR 18–

42.5), and 76.6% were female. Successful genotyping was obtained from 102 specimens, corresponding to 48/92 (52.2%) INP and 54/109 (49.5%) IEP. The median HIV-1 RNA was 5.50 log₁₀ copies/mL. Subtype C was the most prevalent (21.6%), followed by A1 (15.7%), F1 (13.7%), CRF02_AG (10.8%), G (8.8%), and other subtypes and recombinants (29.4%). In general, resistance to NNRTIs was most common (68.2%; 95% CI 56.2–78.2), followed by NRTIs (48.5%; 95% CI 36.8–60.3). Resistance to PIs and INSTIs was rare, observed in 1.5% (95% CI 0.3–8.2) and 3.5% (95% CI 1.2–9.8) respectively. The prevalence of resistance associated mutations differed between groups, with higher frequencies observed in INP compared to IEP for NNRTIs (76.3% vs 57.1%; $p=0.09$) and NRTIs (65.7% vs 25.0%; $p=0.001$), while INSTI resistance was low in both groups (5.3% vs 3.6%; $p=0.74$). PI resistance was observed only in IEP (3.6%). Overall, the most prevalent NNRTI mutations were K103N (31.8%), G190A (18.8%), and E138A (10.6%), while NRTI mutations were M184V (37.8%), K70R (7.6%), and T215Y (6.1%). INSTI mutations were rare (R263K, G140R, Q148H; 1.16% each), and PI mutations included I54V and V82A (1.5% each). Among INP, NNRTI-associated mutations were predominantly K103N (47.4%), G190A (21.1%), and E138A (13.2%), compared with 32.1%, 14.2%, and 10.7% in IEP, respectively. NRTI-associated mutations were likewise more prevalent in INP, particularly M184V (57.9%), K70R (18.4%), and T215Y (13.2%), whereas lower frequencies were observed in IEP (10.7%, 3.6%, and 3.6%, respectively). Major INSTI resistance mutations G140RG (2.4%) and Q148QH (2.4%) were identified in INP, while R263RK (2.2%) was detected in IEP. PI-associated resistance mutations were observed exclusively in the IEP.

Conclusion: Although resistance to INSTIs among INP remains rare, their expanded use in highly treatment experienced populations may increase the risk of resistance emergence. The detection of NRTI and PI resistance in IEP, components of first and second-line regimens, underscores the need for continuous and standardized HIV drug resistance surveillance in Angola to detect emerging mutations, guide therapeutic decisions, and preserve the long-term effectiveness of dolutegravir-based regimens.



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Real-World Hepatitis C Virus (HCV) Resistance Testing in Portugal: Genotype Distribution and Resistance-Associated Substitutions from 2022 to 2026

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Background: There is a lack of real-world data on hepatitis C virus (HCV) resistance testing, particularly from reference laboratories that serve diverse patient populations who are often treatment-experienced.

Materials and Methods: We conducted a retrospective review of HCV resistance tests performed in a molecular biology laboratory in Portugal between August 2022 and February 2026. Viral RNA extracted from plasma samples was analysed using next-generation sequencing (NGS) with the Sentosa SQ HCV Genotyping Assay (Vela Diagnostics), targeting the NS3, NS5A and NS5B genomic regions associated with resistance to direct-acting antivirals (DAAs). The following information was collected from the report or the geno2pheno ([hcv]) website (<https://hcv.geno2pheno.org>) for each test: genotype/subtype; reported substitutions in the NS3, NS5A, and NS5B regions; and interpreted DAA susceptibility. Detected substitutions were classified as resistance-associated substitutions (RAS) according to either the geno2pheno ([HCV]) interpretation algorithm or the Vela Genomics HCV Drug Resistance Algorithm.

Results: Sixty-five tests were available for analysis. Genotype 1 predominated (47.7%; 1a = 38.5%, 1b = 6.2%, and mixed 1a/4d = 1.5%), followed by genotype 4 (35.4%; 4d = 23.1%, 4a = 9.2%, and 4c/4f/4k = 1.5%), genotype 3 (15.4%; 3a = 14.3% and 3b = 1.5%), and genotype 2a (1.5%). 19/65 samples (29.2%) showed reduced susceptibility to one or more DAAs. The most clinically relevant

patterns were found in the NS5A region, particularly in genotype 1a (including M28T/M28V, Q30R/Q30L, L31M and Y93H/Y93N combinations) and genotype 3 (including A30K and Y93H combinations). Reduced susceptibility associated with NS3 was less frequent and mainly involved Q80K, V36M, Q168K/R and Y56F. Reduced susceptibility associated with NS5B was uncommon, being largely linked to L159F/C316N and the S282T variant. Repeated identifiers and report comments also indicated reinfection/genotype replacement and previous DAA exposure in several cases.

Conclusions: The majority of the studied population remained fully susceptible. Nevertheless, clinically relevant resistance-associated substitutions were observed in only a minority of cases involving prior treatment exposure or suspected reinfection. Routine resistance testing is most useful for planning retreatment and identifying unexpected genotype shifts, particularly in genotype 1a/3 infections.



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Comparative Evaluation of MGI DNBSEQ-E25 and Illumina MiSeq for HIV-1 Genomic Surveillance Using QCMD Panels

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Background: Next-generation sequencing (NGS) platforms are increasingly central to HIV-1 drug resistance monitoring and minority variant detection. While Illumina MiSeq remains the reference standard in clinical genomics, alternative platforms such as MGI DNBSEQ-E25 emerge as cost-competitive options. However, their comparative performance for HIV-1 genomic surveillance under standardized conditions has not been systematically evaluated. We aimed to compare sequencing performance, coverage depth, and resistance mutation detection between MGI DNBSEQ-E25 and Illumina MiSeq using standardized HIV-1 QCMD panels processed through a unified DeepChek[®] workflow.

Methods: HIV-1 QCMD panel samples were amplified and sequencing libraries constructed using DeepChek[®] HIV kits (ABL Diagnostics), followed by parallel sequencing on MGI DNBSEQ-E25 and Illumina MiSeq. Raw FastQ files were analysed with MicrobioChek[®] software (ABL) for quality control, reference mapping, and mutation calling across protease, reverse transcriptase, and integrase regions. Performance metrics included mapping rate, mean sequencing depth, and concordance of resistance-associated mutation profiles.

Results: Both platforms achieved high mapping rates (>80%), confirming robust read quality across all samples. MiSeq showed marginally higher alignment accuracy (mean mapping rate: 82.6% vs. 82.1%), while MGI DNBSEQ-E25 delivered significantly greater sequencing depth (~113,000 vs. ~95,000 reads per sample), enhancing sensitivity for minority variant detection. Resistance mutation profiles were highly concordant for major markers (M46I, I54V, V82A,

M184V, G190A). MGI DNBSEQ-E25 additionally detected low-frequency substitutions below the 10% allele frequency threshold in pooled samples, not consistently identified on MiSeq.

Conclusions: MGI DNBSEQ-E25 and Illumina MiSeq deliver comparable performance for routine HIV-1 genomic surveillance when combined with the DeepChek[®] workflow. MGI DNBSEQ-E25 offers a specific advantage for minority variant detection owing to its higher sequencing depth, while MiSeq retains a marginal edge in mapping accuracy. These findings provide a practical basis for informed platform selection in clinical and public health HIV surveillance settings.



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Partial Protection: Condom Use Patterns Across Vaginal and Anal Sex among American Women

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Background: Cisgender women remain at elevated risk for HIV acquisition through sexual activity, including both vaginal and anal sexual intercourse. Despite the substantially higher per-act risk associated with anal sex, education and prevention messaging related to heterosexual anal sex is inconsistent. Understanding women's condom use behaviors across both vaginal and anal sexual activities may inform more accurate HIV and sexually transmitted infection (STI) risk assessment and targeted prevention strategies.

Methods: We analyzed baseline data from 1,728 women enrolled in a longitudinal cohort study examining predictors of HIV-related sexual risk across the United States and its territories. Participants reported lifetime and past six-month engagement in vaginal and anal sex. Those reporting sex in the past six months reported condom use frequency for each sexual activity on a 5-point Likert scale (1=never, 5=every time). Analyses focused on a subsample of women reporting both vaginal and anal sex in the past six months. We examined the association between condom use across behaviors using Spearman's correlation and characterized concordant and discordant use patterns. Participants also provided brief open-ended responses regarding reasons for condom non-use, which were grouped into thematic categories.

Results: Among enrolled participants, 491 (28.4%) women reported both vaginal and anal sex in the past six months and were included in the analytic sample. Participant ages ranged from 16–62 years (mean 35.6 years). Condom use was generally infrequent: 87.8% of participants (n=431) reported "never" or "some of the time" for at least one behavior. Condom use across vaginal and anal sex

was moderately correlated (Spearman's $\rho=0.45$, $p<.001$), suggesting partial behavioral consistency. Over half of participants (n=272, 55.4%) reported identical condom use frequency for vaginal and anal sex. However, concordance mostly reflected shared non-use: 221 women (45.0% of the analytic sample) reported never using condoms for either mode of sexual activity. Discordant patterns were also common (n=220, 44.8%). Among those with discordant use, a greater proportion reported more frequent condom use during vaginal sex than anal sex (66.8% vs. 33.2%). Patterns of regular use were inconsistent across modes of sexual activity, with women who used condoms "every time" during anal sex reporting substantially different condom use frequency during vaginal sex. Condom non-use was more common among participants aged ≥ 40 and those with public insurance; differences by race and Hispanic ethnicity were minimal. Open-ended responses regarding general condom non-use reflected multiple domains, including individual factors (e.g., latex allergy, substance use, personal preference), partner and relationship factors (e.g., monogamy, partner refusal, erectile dysfunction, heat of the moment), risk perception (e.g., reliance on STI testing or contraceptive use), and access-related barriers (e.g., cost, lack of availability).

Conclusion: Among women engaging in both vaginal and anal sex, condom-use patterns demonstrate partial behavioral consistency that frequently reflects persistent non-use rather than uniform protection. Variation in condom use, shaped by multiple individual, relational, and structural factors, may be obscured when condom use is assessed as a single behavior rather than a behavior associated with separate sexual activities.



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Next-Generation HIV Prevention: Leveraging Digital Tools and Community-Based Interventions to Revolutionize Testing, Treatment, and Prevention in Nairobi

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Background: The HIV epidemic continues to present a major challenge, especially in high-risk communities where stigma, fear of testing, and inaccessibility hinder individuals from getting tested and starting treatment. Despite advancements in antiretroviral therapy (ART) and pre-exposure prophylaxis (PrEP), millions of individuals remain undiagnosed and untreated. In Nairobi, we face a rapidly growing HIV burden, particularly among key populations such as men who have sex with men (MSM), sex workers, and youth, who experience disproportionately high rates of HIV transmission. This study presents a cutting-edge model that combines digital health tools with community-driven interventions to address these barriers, increase HIV testing, promote prevention strategies, and ensure rapid linkage to care. This data-driven model is designed to overcome traditional barriers and make HIV prevention more accessible, effective, and scalable.

Materials and Methods: In partnership with local community organizations, we deployed an mHealth platform that provides personalized HIV testing reminders, real-time prevention tools, and location-based testing information. Alongside this, we implemented community-led mobile testing units offering free, confidential HIV tests and counseling in high-risk communities. A total of 2,000 high-risk individuals, including MSM, youth, and sex workers, were enrolled in the study. Data were meticulously collected on HIV testing uptake, PrEP adherence, condom use, and new HIV diagnoses. The goal was to assess the effectiveness of combining digital tools with community engagement in increasing testing uptake and reducing HIV transmission rates.

Results: HIV Testing Uptake: HIV testing uptake increased by 70%, from 40% to 100% in high-risk communities ($p < 0.001$), marking a groundbreaking shift in the accessibility and engagement of HIV testing services.

PrEP Adherence: PrEP uptake surged by 50%, from 30% to 80% ($p < 0.001$), demonstrating how real-time personalized reminders and digital tools can significantly enhance prevention adherence.

Condom Use: Consistent condom use in the target group increased by 40% ($p = 0.004$), reflecting a major behavior shift and increasing risk-reduction practices.

Linkage to Care: 85% of those testing positive were successfully linked to care within 30 days ($p < 0.001$), ensuring that early diagnosis quickly translates into treatment.

Reduction in New HIV Diagnoses: The intervention resulted in a 25% reduction in new HIV diagnoses in the intervention group compared to a control group ($p = 0.01$), demonstrating the real-world impact of increased testing and immediate care linkage.

Conclusions: This innovative, scalable model demonstrates the transformative potential of combining digital health technologies with community-driven interventions in HIV prevention. By leveraging personalized digital tools and engaging local communities, we have dramatically increased HIV testing uptake, PrEP adherence, and linkage to care, while simultaneously reducing new HIV diagnoses. This model is not only cost-effective and replicable, but it also addresses the key challenges of stigma and inaccessibility that continue to hinder HIV care. It offers a powerful solution that can be implemented globally, providing better outcomes and accelerating early HIV diagnosis and treatment access. The future of HIV prevention is smarter, digital, and community-powered—this model is a proven pathway to revolutionizing the fight against HIV.



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Occupational Precarity and Engagement in HIV Prevention among Trans People in São Paulo, Brazil

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Background: The disproportionate exposure to HIV among trans people reflects structural and social determinants beyond individual-level factors. In Brazil, HIV prevalence in the general adult population is approximately 0.7%, whereas estimates among transgender women and travestis range from 12% to 36.7%, highlighting a markedly disproportionate burden. Exclusion from the formal labor market, income instability, and fragmented social protection create a structural environment limiting sustained engagement in prevention strategies, including pre-exposure prophylaxis (PrEP) offered within Brazil's Unified Health System (SUS). The São Paulo Metropolitan Region (SPMR), the largest urban agglomeration in Latin America, provides a strategic setting to investigate how labor conditions shape upstream vulnerabilities in HIV prevention.

Objective: The aim characterises occupational precarity, discontinuities social protection and markers of economic vulnerability among trans people in the São Paulo Metropolitan Region. The structural implications for engagement in HIV prevention strategies, including PrEP within the SUS, are discussed.

Materials and Methods: This cross-sectional study included transgender individuals (≥18 years old) residing in the São Paulo Metropolitan Region (n = 136). Participants completed a structured questionnaire collecting information on employment status, income, job formality, social security contributions, workplace inclusion experiences, and history of exchanging sex or erotic/pornographic services for material resources. Descriptive analyses characterized occupational precarity and structural vulnerability

as upstream determinants within the HIV prevention continuum.

Results: Among the 136 participants, 62.5% were currently employed; however, labor market participation was characterized by occupational mismatch and limited social mobility, with only 32.6% working in their trained field. Among employed participants, 50.6% of the workers had formal employment, while half worked in informal or precarious arrangements. Income distribution revealed substantial economic vulnerability: Income distribution indicated substantial economic vulnerability: 19.1% earned less than half the Brazilian minimum wage per month (≈US\$140), 24.3% up to one minimum wage, and 31% between one and two minimum wages; only 3.7% reported earnings above five minimum wages. Regarding social protection, 36.8% currently contribute to social security systems, while 34.6% reported having contributed in the past but discontinuing contributions, reflecting unstable employment trajectories. Career progression opportunities were perceived as limited or nonexistent by 55% of participants. Although 52.5% reported satisfaction with their immediate work environment, fewer than half (48.3%) positively evaluated organizational support for trans inclusion, indicating persistent institutional barriers to retention and career advancement. Occupational precarity also translated into survival strategies: 67% reported having exchanged sex or erotic services for material resources, reflecting the intersection of labor market exclusion and socioeconomic vulnerability.

Conclusions: These findings highlight occupational precarity as a critical structural factor in HIV prevention among transgender individuals. The sustained effectiveness of pre-exposure prophylaxis (PrEP) depends on supportive structural environments, including economic stability, social protection, and inclusive workplaces. Therefore, effective HIV prevention governance should adopt an intersectoral approach that integrates health systems, labor policies, and anti-discrimination mechanisms. The context of the studied region indicates that equitable prevention requires health systems that are structurally competent and aligned with inclusive labor and social protection strategies. These strategies must be capable of integrating biomedical approaches into sustainable HIV prevention among transgender populations.



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Identifying Patterns of HIV Risk Behavior among a National Cohort of Cisgender Women in the United States

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Background: Cisgender women account for approximately 19% of new HIV diagnoses in the US, yet women remain underrepresented in HIV prevention research. Further, women substantially underutilize services such as pre-exposure prophylaxis (PrEP). Women comprise only about 9% of PrEP users nationally. Latent class analysis (LCA) provides a method to identify underlying patterns of HIV risk behaviors within heterogeneous populations, which could inform more effective interventions for HIV prevention and PrEP uptake. This study used LCA to identify distinct profiles of HIV risk behaviors among cisgender women with increased need for HIV prevention.

Materials and Methods: Baseline data collected between 2023 and 2026 from participants in the ongoing national AWARE cohort study of women with increased need for HIV prevention in the US were analyzed. LCA was conducted to identify subgroups of women based on self-reported STI history and sexual risk behaviors (i.e., partners with unknown/elevated HIV risk; inconsistent condom use; transactional sex, anal sex, sex under the influence of alcohol or drugs; multiple sexual partners, concurrent sexual partners; and frequent sexual activity).

Results: A total of 1,807 participants were included (mean age 34.6±8.68 years; 523 (29.6%) were White; 507 (28.7%) were Black; and 449 (25.4%) were Hispanic). Three distinct classes were identified, each associated with probabilities of sexual risk behaviors and/or STI histories: Class 1 was characterized by infrequent sexual activity (57.2%); Class 2 showed frequent sexual activity (63.8%) and recent anal sex (54.1%); and Class 3

demonstrated the highest probability of multiple partners (72.8%), concurrent partners (60.7%), anal sex (58.7%), and history of STIs (58.5%). Across all three classes, participants reported higher probabilities of inconsistent condom use (≥88%), sex under the influence of alcohol or drugs (≥79%), and partners with unknown/elevated HIV risk (≥93%). Class 1 comprised 35.81% of participants, while Class 2 comprised 33.77% and Class 3 had 30.41%.

Conclusions: Unique classes of women were identified based on their individual HIV risk behaviors, despite all participants having a high probability of reporting inconsistent condom use, sexual partners with unknown/elevated HIV risk, and sex under the influence of alcohol or drugs. Distinct behavioral risk profiles exist among cisgender women with increased HIV prevention needs, highlighting substantial heterogeneity in risk patterns. Ongoing analyses will examine associations between these classes and longitudinal STI/HIV outcomes as well as structural determinants of risk and underscore the importance of tailoring HIV prevention strategies, including PrEP delivery and behavioral interventions.



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Estimation of Liver Fibrosis Risk Using Non-Invasive Biomarker Tests in People Living with HIV and Pre-Diabetes in Tanzania

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) and liver fibrosis are increasingly common comorbidities among people living with HIV (PLHIV), particularly in ageing populations with rising metabolic dysfunction. Data on fibrosis risk in sub-Saharan Africa remain scarce. We aimed to estimate liver fibrosis risk and the 10-year risk of cirrhosis using non-invasive tests (NITs) among PLHIV with pre-diabetes in Tanzania.

Materials and Methods: We conducted a cross-sectional analysis of baseline data from 1,691 PLHIV with pre-diabetes enrolled in the META Trial, a phase III randomised controlled trial of metformin, in Tanzania. Liver fibrosis risk was assessed using the Fibrosis-4 (FIB-4) Index, Metabolic Dysfunction-Associated Fibrosis-5 (MAF-5) score, NAFLD Fibrosis Score (NFS), and the LiverRisk score; cirrhosis risk was estimated using the Cirrhosis Outcome Risk Estimator (CORE). Clinical and demographic variables were summarised, and risk categories were defined using validated and age-adjusted cut-offs.

Results: Participants had a mean age of 49.4 years (SD 9.4), 75.0% were female, and 99.2% were Black. Using age-adjusted FIB-4 thresholds, 31.8%

were classified as indeterminate risk and 3.0% as high risk, while NFS classified 50.9% as indeterminate and 8.3% as high risk. In contrast, MAF-5 categorised 59.9% of participants as high risk, whereas fewer than 1% were classified as medium or high risk by LiverRisk. Using CORE, 5.8% of participants were estimated to have a $\geq 1\%$ 10-year risk of developing cirrhosis or a cirrhosis-related outcome. Females were more often classified as low risk than males by FIB-4 (70% vs. 50%, $p < 0.001$), while males had higher risk classifications by MAF-5, LiverRisk, and CORE; no sex differences were observed for NFS.

Conclusion: Non-invasive fibrosis tests yielded divergent estimates of liver fibrosis risk among PLHIV with pre-diabetes in Tanzania. While FIB-4 and NFS identified most as low risk, MAF-5 suggested nearly half were high risk. These discrepancies underscore the need for locally validated NITs and integration of liver health assessments into HIV care in Africa, particularly as MASLD/MASH and metabolic comorbidities are common in this population.



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Community-Based HIV Prevention and Self-Testing among Transgender People: Preliminary Findings from a Population-Based Mapping Study in São Paulo, Brazil

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Background: Transgender populations experience a disproportionate burden of HIV worldwide. In Brazil, HIV prevalence among adults aged 15–49 years is approximately 0.7%, whereas studies among transgender women and travestis report prevalence from 12% to 36%, reflecting major epidemiological inequalities (Veras et al.). These disparities are driven by structural determinants including stigma, discrimination, social exclusion, and barriers to healthcare access. Brazil has implemented universal HIV prevention policies through its public health system, including free HIV testing, pre-exposure prophylaxis (PrEP), and post-exposure prophylaxis (PEP). However, structural stigma and inadequately adapted services limit the uptake of these strategies among transgender populations (Grinsztejn et al.). Community-based and peer-led interventions are promising strategies to expand prevention engagement among underserved populations. This study presents preliminary findings from the Trans Population Mapping Project in the Metropolitan Region of São Paulo, an initiative mapping living conditions, health needs, and service access among transgender people across the State of São Paulo.

Methods: The Trans Population Mapping Project is a mixed-methods population-based study generating epidemiological and social evidence about transgender populations across the State of São Paulo. The project combines community-

based recruitment, peer-led data collection, and structured interviews to reach populations underrepresented in health surveillance systems. In the Metropolitan Region of São Paulo, trained peer interviewers from the transgender community conduct structured interviews covering sociodemographic characteristics, healthcare access, HIV prevention knowledge, and use of biomedical prevention technologies. Participants receive an HIV self-test kit after each interview to expand testing access and strengthen prevention engagement. This analysis includes preliminary data from 135 participants interviewed between 2025 and 2026.

Results: Among the 135 participants interviewed, 86% reported their last HIV test result as negative. Awareness of prevention strategies was high: 93.3% had heard of PrEP and 89.6% of PEP. However, differences emerged between knowledge and use of prevention technologies. While 78% reported having used PEP at least once, 60% had never used PrEP. Among those who had used PrEP (40%), continuous use was reported in 74% of cases. Access to condoms occurred mainly through health services (57%). Most participants (83%) reported using the public health system for gender-affirming healthcare. Nevertheless, barriers persist: 75% reported fear of being mistreated by healthcare professionals, indicating ongoing stigma and discrimination in healthcare settings.

Conclusions: Despite high awareness of biomedical HIV prevention strategies, PrEP uptake remains limited among transgender people in the Metropolitan Region of São Paulo, suggesting gaps between prevention policies and implementation. Institutional stigma, insufficiently adapted services, and structural barriers may reduce engagement with prevention strategies. The peer-led approach combined with HIV self-testing represents a promising strategy to expand testing access and strengthen prevention outreach among hard-to-reach populations. As the Trans Population Mapping Project expands across the State of São Paulo, the findings may inform improvements in HIV prevention policies and community-based strategies.



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Study of the Association between Behavioural and Programmatic Indicators and HIV Incidence in Angola: An Ecological Study by Province

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Introduction: HIV infection remains a major global public health challenge, with profound impacts on the social, economic, and health dimensions of populations, particularly in sub-Saharan Africa. Angola has an estimated national prevalence of 1.6%, marked by substantial regional inequalities that reinforce the urgency of a territorialised analysis.

Objective: To analyse the behavioural, social, and programmatic access factors associated with HIV incidence and the number of new infections across different regions of Angola.

Methods: An ecological study with a quantitative approach was conducted using secondary data aggregated at the provincial level, extracted from the National Institute for the Fight Against AIDS (INLS) database and the Multiple Indicator and Health Survey (IIMS) 2015–2016. The study population included individuals aged 15 to 49 years receiving clinical follow-up across the 18 provinces of Angola. The main outcomes were HIV incidence per 1,000 population and the number of new infections. Explanatory variables included indicators of sexual behaviour, access to healthcare services, and social determinants. Statistical analysis was performed using Spearman's correlation and simple linear regression. Authorisation for the use of data was granted by INLS.

Results: In 2023, the highest HIV incidence rates per 1,000 population were observed in the

provinces of Lunda Sul (0.91/1,000), Lunda Norte (0.86/1,000), and Moxico (0.80/1,000), while Uíge and Zaire recorded the lowest rates (0.16/1,000). Luanda reported the highest absolute number of new cases (4,546), followed by Cunene (1,464) and Benguela (1,143). Antiretroviral therapy (ART) coverage ranged from 44% to 60%, and testing coverage showed considerable disparities by sex and province. Knowledge of HIV prevention was low, ranging from 11% to 51% across Angolan provinces. A strong negative correlation was observed between male condom use and new infections ($\rho = -0.753$; $p = 0.003$), as well as between HIV testing among women and new infections ($\rho = -0.637$; $p = 0.019$), while ART coverage showed a positive correlation with new infections ($\rho = 0.585$; $p = 0.036$). In linear regression analysis, HIV testing among women was negatively associated with new infections ($\beta = -0.605$; $p = 0.028$), whereas ART coverage showed a positive association ($\beta = 0.628$; $p = 0.022$).

Conclusion: The HIV epidemic in Angola is heterogeneous and influenced by multiple social, behavioural, and programmatic determinants. The findings reveal marked territorial inequalities and persistent barriers in access to prevention and treatment services. These results highlight the urgent need for integrated combination prevention strategies, strengthened epidemiological surveillance, and the reduction of social vulnerabilities as key pillars for achieving global HIV epidemic control targets.



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Developing a Remote e-Peer Navigation Model to Support PrEP Access among Young Men Who Have Sex with Men: The MyPEEPS Plus Intervention

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Background: Young men who have sex with men (YMSM), particularly those from racially and ethnically minoritized communities, continue to experience disproportionately high rates of HIV infection. Although pre-exposure prophylaxis (PrEP) is highly effective, uptake and persistence remain suboptimal among YMSM due to limited access to youth-friendly services, medical mistrust, stigma, and geographic barriers. Scalable, remote prevention strategies are urgently needed to expand equitable access to PrEP.

Methods: We developed MyPEEPS Plus, a combination HIV prevention intervention integrating a mobile health (mHealth) application with a remote electronic peer (e-peer) PrEP navigation program. The intervention was designed for delivery within a national randomized controlled trial among 500 YMSM in the United States. Intervention development followed a multi-method, community-engaged process including: (1) expert review of HIV prevention and adolescent health content; (2) in-depth qualitative interviews with 20 PrEP navigators from diverse clinical settings; and (3) iterative refinement through five Youth Advisory Board sessions (n=23). Feedback informed curriculum updates, communication strategies, and navigation workflows.

Results: The finalized e-peer navigation model delivers one-on-one, remote PrEP counseling sessions via videoconference or telephone, led by certified peer navigators trained in motivational interviewing and youth-affirming communication. Core components include standardized HIV risk screening, readiness assessment, tailored PrEP education, shared decision-making, and direct linkage to in-person or telehealth PrEP providers. The intervention emphasizes participant

autonomy, confidentiality, and flexibility, allowing navigation to occur entirely outside traditional clinic settings. Integration with tele-PrEP platforms enables immediate linkage to care regardless of geographic location.

Conclusions: MyPEEPS Plus represents an innovative, scalable approach to HIV prevention that combines digital education with personalized peer support to address persistent structural and psychosocial barriers to PrEP uptake among YMSM. This remote e-peer navigation model has potential relevance for HIV prevention programs seeking to expand PrEP access among youth and other key populations, particularly in settings with limited provider availability or high stigma. Ongoing evaluation will assess the intervention's impact on PrEP initiation, engagement, and retention.



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Forming Genuine Bonds: Retention Strategies for Cisgender Women in HIV Research

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Background: Cisgender women (CGW) face significant HIV risks in the United States, yet retaining CGW with increased HIV prevention needs in prevention research is challenging. The same factors that increase HIV vulnerability also create barriers to research participation. This qualitative study explored multilevel barriers and facilitators to retaining CGW in HIV prevention research using the social ecological model (SEM).

Materials and Methods: Semi-structured interviews were conducted with 114 participants across three groups: CGW at higher risk for HIV seroconversion (n=34), CGW living with HIV (n=40), and HIV prevention/treatment stakeholders (n=40). Participants were recruited nationally through convenience sampling via social media and community organizations. All interviews were conducted via virtual meeting platforms. Directed content analysis was used to identify retention factors across individual, environmental, intervention, and structural levels.

Results: Barriers included substance use affecting contact maintenance, concerns about study time commitments, frustrations with unclear study procedures, poor staff rapport, safety concerns around intimate partner violence, historical research mistrust, and systemic vulnerabilities like housing instability. Facilitators included creating affirming environments that fostered purpose, flexible scheduling with incremental compensation, clear study expectations and health education/support, authentic staff relationships that demonstrate genuine care, discrete communication methods, transparent study procedures and approaches that acknowledge historical context, virtual visit options, and availability of multiple contact methods.

Conclusions: Findings corroborated a key paradox: those at highest HIV risk may be least likely to complete longitudinal studies due to structural barriers. Successful retention requires comprehensive, flexible approaches that address barriers at all SEM levels. These findings provide strategies for researchers and HIV service providers to improve retention among vulnerable CGW, strengthening HIV prevention research representativeness and effectiveness.



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Navigating Criminalization: Community - Led HIV Prevention and Implementation Strategies in Ghana

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Criminalization of same sex relations in Ghana continues to create major barriers to effective HIV prevention, service uptake, and treatment continuity for key populations. Fear of arrest, extortion, and public exposure discourages individuals especially MSM, transgender people, and other LGBTQ+ groups from seeking essential HIV services. This expanded abstract presents community driven HIV prevention strategies that have proven successful despite these structural barriers, integrating community approaches, door-to-door outreach, trans-affirming care, and the training of community members as case managers.

Between 2022 and 2024, community led organizations in the Ashanti and Greater Accra regions engaged over 1,200 key population members, providing HIV testing to 780 individuals, enrolling 312 people on PrEP, linking 275 individuals to ART, and delivering STI screening and treatment to 420 community members. Additionally, 52 emergency cases involving violence, extortion, eviction, or blackmail were responded to through rapid crisis intervention and psychosocial support.

A community centered approach formed the foundation of all interventions. Peer educators, paralegals, and trained volunteers conducted community mapping, discreet outreach, and personalized follow ups. Community members were not treated as passive beneficiaries but as active partners in prevention. Trust building within hidden networks was essential for overcoming fear caused by the criminalization of homosexuality.

Door-to-door HIV prevention services emerged as one of the most effective strategies. Trained peer teams visited individuals discreetly in their homes, delivering HIV self-test kits, condoms, lubricants,

PrEP follow-up, mental health support, and referrals. This model reduced transportation barriers, enhanced privacy, and allowed individuals who feared health facilities to receive services safely. Out of the 780 individuals tested, approximately 45% received their test through door-to-door or home-based services, demonstrating the effectiveness of mobile approaches in criminalized settings.

To strengthen service continuity, community members were trained as case managers, including MSM, transgender women, and peer educators from local networks. A total of 26 case managers received training covering HIV prevention, PrEP navigation, ART adherence support, motivational counseling, documentation, and safety/security protocols. These case managers provided personalized care plans, escorted referrals, treatment reminders, and follow up calls. Their involvement contributed to an 82% PrEP continuation rate (measured at 3 months) and improved ART retention among newly diagnosed individuals.

Recognizing the heightened vulnerability of transgender people, the intervention embedded Trans Care strategies, including gender affirming counseling, linkage to trans-friendly providers, mental health screening, and assistance with stigma related emergencies. Trans community members received tailored support, with 67 transgender individuals directly benefiting from HIV prevention and referral services. Trans led peer networks helped improve uptake among individuals who previously avoided services due to discrimination.

Despite the legal and societal hostility surrounding LGBTQ+ issues in Ghana, these community led, door-to-door, and case management strategies demonstrated strong potential for scaling and sustainability. The findings affirm that criminalization undermines national HIV prevention goals, but that resilient, peer driven approaches can maintain and even expand service access. Strengthening these community structures and advocating for rights-based protections is essential for achieving and building an HIV response that leaves no one behind.



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High-Coverage Telehealth Education for HIV-Related NCD Comorbidities in Siha District Clinic Setting

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Background: Among people living with HIV (PLWH), non-communicable disease (NCD) comorbidities—including depression and hypertension—remain under-detected and inadequately addressed, particularly in settings where stigma limits participation in group-based education. At Siha District Hospital HIV clinic, serving 418 PLWH, persistent barriers to group assembly necessitated an alternative education delivery model.

Methods: A telephone-based NCD education program was implemented targeting 250 PLWH (60% of the clinic population), designed and coordinated by the District Research and Mental Health Office. Five trained healthcare providers conducted three sequential outreach cycles, delivering individualized education during evenings and weekends to accommodate employment-related constraints. Educational content focused on HIV–NCD comorbidities (mental health and hypertension), symptom recognition, and antiretroviral therapy (ART) adherence strategies. A dedicated clinic phone line supported post-session follow-up. Care and Treatment Clinic (CTC) clinicians monitored clinic attendance patterns before and after implementation.

Results: The intervention reached 210 of 250 targeted clients (84%) across three cycles. On average, 40 clients per cycle (16%) were unreachable, primarily due to work-related scheduling conflicts. Depression and hypertension were the most frequently identified comorbidities, including cases not previously documented during routine clinic visits. CTC clinicians reported improved clinic attendance following implementation, suggesting enhanced engagement in HIV care. Clients actively utilized the follow-up phone line, indicating high acceptability of the telehealth approach.

Conclusion: High-coverage telephone-based NCD education was feasible and effective in reaching PLWH despite stigma and structural barriers that impeded group-based delivery. Achieving 84% reach demonstrates that HIV clinics should not delay NCD education while awaiting group formation. Evening and weekend telehealth outreach represents a pragmatic, stigma-resilient strategy for early comorbidity detection and improved engagement in HIV care. HIV programs should consider integrating individualized telephone outreach alongside group based approaches to accelerate NCD integration and support sustained ART adherence.



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SLC26A3-Mediated Mitochondrial Chloride Overload Drives T Cell Apoptosis in Immunological Non-Responders

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Background: Despite sustained viral suppression with combination antiretroviral therapy (cART), 10-40% of HIV-infected patients remain immunological non-responders (INRs) characterized by persistently low CD4+ T-cell counts and increased risk of opportunistic infections. The underlying mechanisms remain elusive, and no targeted therapeutic strategies exist. While metabolic dysfunction has been implicated in INR pathogenesis, specific molecular mechanisms linking mitochondrial impairment to T-cell apoptosis remain elusive, precluding targeted therapeutic strategies. Here, we identify the solute carrier SLC26A3 as a potential pathogenic target, connecting ion transport dysregulation to mitochondrial-mediated cell apoptosis in INRs.

Methods: We performed single-cell RNA sequencing (scRNA-seq) on peripheral blood mononuclear cells (PBMCs) from INRs (CD4<350/ μ L, n=5), immunological responders (IR; CD4 \geq 500/ μ L, n=4), and healthy controls (HC; n=5). To mechanistically dissect the role of SLC26A3, we established an SLC26A3-overexpressing HIV latent cell model (J-Lat 10.6) treated with efavirenz to simulate clinical cART conditions. Comprehensive functional analyses included Western blotting, flow cytometry, Seahorse metabolic flux analysis, and transmission electron microscopy to assess apoptosis execution and mitochondrial bioenergetics. Pharmacological interventions utilized the anion channel inhibitor DIDS and the specific SLC26A3 inhibitor tenidap.

Results: Transcriptomic analysis revealed significant SLC26A3 upregulation in INR PBMCs coinciding with enhanced apoptotic signatures. Importantly, SLC26A3 expression showed strong

inverse correlation with circulating CD4+ T-cell counts. Mechanistically, SLC26A3 overexpression activated the intrinsic mitochondrial apoptosis pathway through excessive chloride ion influx, resulting in electron transport chain disruption and ATP depletion. Notably, both DIDS and tenidap rescued mitochondrial respiration and reduced apoptosis.

Conclusions: This study establishes SLC26A3-driven mitochondrial chloride dysregulation as a novel pathogenic mechanism underlying INR. By demonstrating that SLC26A3-mediated chloride overload disrupts mitochondrial oxidative phosphorylation and triggers T-cell apoptosis, we provide the first mechanistic link between ion transport dysregulation and immune reconstitution failure. The clinical anti-inflammatory drug tenidap exhibits significant efficacy in reversing mitochondrial dysfunction and reducing apoptosis. These findings open new avenues for metabolic-immune intervention strategies in HIV functional cure research.



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Analytical and Clinical Validation of a 4-Targets Multiplex Digital PCR HIV-2 IPDA

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Background: Multiplex digital PCR is a technique that allows absolute quantification of multiple DNA targets without standards. HIV viral reservoir is composed of integrated proviruses that may be intact or defective, i.e., with deletions and/or hypermutations. We wanted to further study HIV-2 viral reservoir by developing a digital PCR method targeting the Psi(Ψ), Gag, Pol, and Env regions of the genome, in order to better identify intact proviruses, similar to the Q4ddPCR for HIV-1 (Scheck et al, 2026). By targeting several genomic regions, this limits the impact of genetic variability and the overestimation of the defective reservoir due to primer and/or probe hybridization failure. The aim of this study was to set up a specific Q4dPCR for HIV-2 and assess viral reservoir among people with HIV-2 (PWH2).

Materials and Methods: To assess analytical performances of our assay, we used DNA extracts from U937-HIV-2 MS cells (CVCL_X608), a cell line containing at least one copy of integrated virus per cell. In order to adjust the quantification with the fragmentation rate and to control the quality of the DNA extract, digital PCR (QiaCuity, Qiagen) targeting two regions in RPP30 was performed, and tests to compare different extractions methods were carried out (QIA Symphony, EZ1, and DNA Blood Mini Kit). We determined the limit of blank (LoB) and the limit of detection (LoD), which corresponds to the minimum concentration likely to be detected in at least 95% of tests, from duplicates of three distinct dilution ranges (250 to 2.5 copies/reaction). This method was used to investigate viral reservoir of PBMC collected from 20 ARV-naïve PWH2 (2 Group A, 11 Group B and 7

amplification failure) included in the national ANRS COHIV2 cohort.

Results: The estimated LoB is 0 copies/uL for all targets, so the digital PCR positivity threshold is 1 positive partition/reaction. The LoD obtained was 10 copies/reaction for each single quantification by Ψ , Env, Gag, and Pol, as well as positive partitions combining Ψ -Gag-Pol, Gag-Pol-Env, Gag-Env, Gag-Pol, Psi-Gag and Ψ -Env. The LoD is estimated at 12 copies/reaction for positivity at 4 targets, Ψ -Pol-Env and Ψ -Gag-Env. Linearity was satisfactory ($R^2 \geq 0.99$) with an acceptable correlation between the number of copies measured and the number of copies expected (slope: 0.79-0.88). The fragmentation rate is lower after extraction by QIA Symphony (33% versus 45% and 70%), with a distribution of values similar to those reported in the literature (Bruner et al, 2019). Among PWH2, HIV-2 DNA was detected in 9/20 samples (45%). Detection of intact proviruses was observed in 1/9 (11%) samples. Defective proviruses were detected in all nine samples, the major form being 3' defective proviruses.

Conclusion: The use of HIV-2 4-targets multiplex dPCR makes it possible to quantify intact and defective proviruses. This assay provides a more accurate and representative measure of biological reality than standard two-target tests such as IPDA, while limiting the effect of point mutations linked to viral genetic diversity. Viral reservoir was limited in size and intactness in our PWH2 population, in concordance with the attenuated phenotype of HIV-2 infection.



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Breaking the Cycle of HIV Inflammation: Investigating Novel Anti-Inflammatory Therapies as a Pathway to HIV Remission in Nairobi

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Background: Chronic HIV inflammation continues to be a significant barrier in the management of HIV-related comorbidities and the persistence of viral reservoirs, despite the success of antiretroviral therapy (ART). This inflammation contributes to immune dysfunction, accelerating the development of cardiovascular disease and neurological decline in people living with HIV (PLWH). At CIHEB-Kenya, we recognized the urgent need to explore novel anti-inflammatory therapies that could reduce this chronic inflammation, promote immune recovery, and potentially pave the way for HIV remission. This study investigates the impact of these therapies in reducing HIV-related inflammation and improving health outcomes in patients on long-term ART.

Materials and Methods: In a randomized controlled trial, 500 HIV-positive individuals, all on ART for over 2 years, were enrolled from five healthcare facilities in Nairobi: Mbagathi Hospital, Kangemi Hospital, Mathare North Hospital, Mama Lucy Kibaki Hospital, and Dandora2 Hospital. Participants were randomly assigned to receive either a novel anti-inflammatory drug or a placebo. Primary outcomes included changes in C-reactive protein (CRP) levels, immune function (measured by CD4 count and T-cell function), and viral suppression. Secondary outcomes assessed improvements in cardiovascular health, neurocognitive function, and gut microbiome diversity.

Results: Inflammation Reduction: The treatment group demonstrated a 50% reduction in CRP levels, from a baseline of 10.2 mg/L to 5.1 mg/L ($p < 0.001$), indicating a significant decrease in systemic inflammation.

Immune Recovery: Participants in the treatment group saw a 25% improvement in CD4 counts, increasing from a baseline of 350 cells/mm³ to 437.5 cells/mm³ ($p = 0.002$). Additionally, T-cell function improved by 30% ($p = 0.003$).

Viral Suppression: 80% of participants treated with anti-inflammatory therapy achieved sustained viral suppression for 6 months, with viral loads remaining below 50 copies/mL ($p = 0.004$).

Comorbidities: Significant improvements in cardiovascular health ($p < 0.01$) and neurocognitive function ($p = 0.005$) were observed.

Gut Microbiome: Participants treated with the anti-inflammatory drug showed a 10% increase in gut microbiome diversity ($p = 0.006$), suggesting the broader benefits of inflammation reduction.

Conclusions: This study provides compelling evidence that novel anti-inflammatory therapies can reduce inflammation, boost immune recovery, and suppress HIV replication in PLWH. By targeting the underlying inflammation associated with HIV, we are opening new pathways toward functional HIV remission. The study also demonstrates the significant benefits these therapies can have on cardiovascular health, neurocognitive function, and the gut microbiome. This innovative approach represents a paradigm shift in HIV care, offering the potential to not only improve long-term health but also pave the way toward a functional cure for HIV. The scalability of this treatment model could transform HIV management globally, providing more effective solutions for PLWH in resource-limited settings.



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Emergency Department Screening Reveals Declining HCV Viraemia but Stable HBV Prevalence in Western Europe: A Sentinel Surveillance Opportunity

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Background: The World Health Organization aims to eliminate viral hepatitis as a public health threat by 2030. Achieving this goal requires scalable, integrated strategies for hepatitis B virus (HBV) and hepatitis C virus (HCV), particularly in underserved populations. Emergency departments (EDs) provide a unique opportunity for opt-out blood-borne virus (BBV) screening, enabling early diagnosis and real-world monitoring of elimination efforts. We evaluated temporal trends in HCV and HBV prevalence from ED-based screening initiatives in Western Europe.

Material and Methods: We included ED-based screening studies for HCV and HBV conducted in Western Europe between 2009 and 2024. Included studies reported HCV antibody positivity with confirmatory HCV-RNA testing and/or HBsAg positivity. Studies with ≥ 30 HCV Ab+ or HBsAg+ individuals per year were eligible. For articles containing data for more than 6 months per year over multiple years, we contacted corresponding authors of the published study to provide data broken down for annual years. Weighted prevalence estimates were used to evaluate temporal trends in HCV viraemia and HBsAg positivity across countries and time periods.

Results: Seventeen HCV screening studies from five countries included 1,500,659 individuals; 26,081 were anti-HCV positive and 4,347 were HCV-RNA positive. The weighted proportion of viraemic infection among antibody-positive individuals declined from 56% in the pre-DAA period (2009–2015) to 10% in broad DAA access period (2022–2024), a trend consistently observed in Spain, England, and Ireland. Ten HBV studies including 1,425,954 individuals showed a stable HBsAg prevalence of 0.70% over time. Only 20% of studies reported HBV-DNA levels, limiting assessment of disease control.

Conclusions: ED-based BBV screening captures meaningful population-level changes in viral hepatitis. The marked reduction in viraemic HCV reflects the impact of widespread DAA access, whereas stable HBsAg prevalence in the absence of consistent care status reporting, underscores ongoing challenges due to the lack of curative therapy. Routinely collected ED screening data could serve as a low-cost sentinel surveillance system to monitor hepatitis elimination and guide public health policy in Europe.



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Viral Kinetics, Pharmacokinetics, and Exposure-Response Analysis of Monotherapy of Multiple Ascending Dose Asopegvir, a New-Generation Integrase Strand Transfer Inhibitor, Followed by Combination with Emtricitabine and Tenofovir Alafenamide in Treatment-Naïve People Living with HIV-1: A Two-Period, Randomized, Placebo-Controlled Phase 1/2 Study

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Background: Asopegvir (ASU) is a new-generation HIV-1 integrase strand transfer inhibitor (INSTI) with potent antiviral activity, a distinct in-vitro resistance profile compared with existing INSTIs, and favorable preclinical safety and pharmacokinetic characteristics. This study evaluated viral kinetics, pharmacokinetics (PK), exposure-response relationships, and short-term safety of ASU administered as monotherapy and subsequently in combination with emtricitabine (FTC) and tenofovir alafenamide (TAF) in treatment-naïve people living with HIV-1 (PLWH).

Methods: This two-period, randomized, placebo-controlled phase 1/2 study enrolled antiretroviral-naïve PLWH allocated to three dose cohorts (5 mg, 40 mg, or 80 mg). In Period 1, participants within each cohort were randomized (5:1) to receive ASU or placebo once daily for 10 days. In Period 2, all participants received open-label ASU at their original dose plus FTC/TAF (200 mg/25 mg) for 18 days. The primary endpoint was HIV-1 RNA decline from baseline at D11. ANCOVA and linear mixed-effect model were performed.

Results: 36 participants were enrolled. Significant HIV-1 RNA reductions from baseline to D11 were observed in all ASU dose groups compared with placebo ($P < 0.001$), with adjusted mean decreases of 1.49-2.36 \log_{10} copies/mL and a clear dose-response relationship during monotherapy (P for trend < 0.001). Following combination therapy, adjusted mean HIV-1 RNA decline at D29 was 2.81-2.83 \log_{10} copies/mL, with no significant dose-response relationship ($P = 0.929$). Maximum viral load declines ranged from 1.56-2.41 \log_{10} copies/mL during monotherapy and 2.82-2.85 \log_{10} copies/mL during combination therapy. Viral load decline slopes were 0.15-0.25 \log_{10} copies/mL/day to Day 11 and 0.10-0.11 \log_{10} copies/mL/day to D29. At D29, virological suppression was achieved in 9/10, 9/10, and 8/10 participants in the 5 mg, 40 mg, and 80 mg groups, respectively. ASU exposure increased slightly less than dose-proportionally, with steady state reached within 3-4 days and minimal accumulation. ASU was well tolerated, with an adverse event profile similar to placebo and no treatment discontinuations.

Conclusions: ASU demonstrated potent antiviral activity, favorable PK and exposure-response characteristics, and good short-term tolerability in treatment-naïve PLWH. Based on integrated efficacy, PK/PD, and safety assessments, ASU 40 mg once daily is recommended for pivotal clinical trials.



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HIV Testing among Patients with Sexually Transmitted Infections: Evidence of a Testing Gap in Primary Care Emergency Units

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Background: Sexually transmitted infections (STIs) remain a major driver of HIV transmission, and European HIV elimination strategies recommend routine HIV testing in patients diagnosed with STIs. STIs are well-recognized HIV indicator conditions. While HIV testing is widely implemented in specialized and hospital settings, its uptake in Primary Care Emergency Units (PCEUs) remains poorly characterized. These units frequently attend young adults and other populations at increased risk of HIV infection and may represent an underutilized opportunity for early diagnosis. We evaluated HIV testing practices among patients diagnosed with STIs in PCEUs and compared them with those observed in primary care centers and hospital emergency departments.

Materials and Methods: We conducted a retrospective observational study (2024–2025) including all microbiologically confirmed STI episodes identified from samples submitted from Primary Care Emergency Units (PCEUs). Samples included urine and genital, pharyngeal, anal, or ulcer swabs. Testing targeted common STIs (*Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Trichomonas vaginalis*, *Treponema pallidum*) and herpes simplex virus. For each patient with confirmed STI, electronic records were reviewed to determine whether HIV serology was performed during the same episode, later, or never during the study period. HIV testing practices were compared with those in primary care centers and hospital emergency departments.

Results: A total of 426 STI diagnostic episodes were identified in PCEUs, of which 140 corresponded to microbiologically confirmed STIs. Seven patients had previously known HIV

infection. Among the remaining 133 patients, HIV testing was performed during the same episode in only 14 cases (10.5%), while 78 patients (58.6%) underwent testing later, with a median delay of 29.5 days (IQR 10.75–52.5). Thirty-nine patients (29.3%) never underwent HIV testing during the study period; 35 of them had subsequent healthcare contact without testing being performed. One patient initially diagnosed with an STI without HIV testing was subsequently newly diagnosed with HIV infection following delayed testing 10 days after the initial episode.

In primary care centers, 2,447 STI-positive episodes were recorded, including 68 patients with previously known HIV infection. Among the remaining 2,379 patients, HIV testing was performed during the same episode in 1,866 cases (78.4%), delayed in 324 (13.6%), and never performed in 189 (7.9%). Three new HIV diagnoses were identified, all detected through same-episode testing.

In hospital emergency departments, 596 STI-positive episodes were recorded, including 13 patients with known HIV infection. Among the remaining 583 patients, HIV testing was performed during the same episode in 449 cases (77.0%), delayed in 91 (15.6%), and never performed in 43 (7.4%). Two new HIV diagnoses were identified, both detected through same-episode testing.

Conclusions: Primary Care Emergency Units showed markedly lower rates of immediate HIV testing in patients diagnosed with STIs compared with primary care centers and hospital emergency departments. HIV testing in this setting was frequently delayed or omitted, representing a critical gap in HIV testing implementation and limiting timely diagnosis. Strengthening HIV testing protocols in PCEUs, including systematic or opt-out testing at the time of STI diagnosis, could improve early detection and support HIV elimination strategies.



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Clinical, Biochemical and Fibrosis Profiles of HCV Infections Detected through Opportunistic Screening in an Emergency Department

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Background: Early detection of hepatitis C virus (HCV) infection remains challenging, particularly among patients without identifiable risk factors. Emergency Departments (EDs) represent a strategic setting for opportunistic screening. This study analysed differences in hepatic biomarkers and fibrosis indices between HCV RNA-positive and RNA-negative patients identified through ED-based screening.

Materials and Methods: A retrospective observational study was conducted in the ED of Torrecedenas University Hospital (Almería, Spain) from August 2021 to October 2024. Residual serum samples from routine blood tests were used to perform anti-HCV testing, followed by HCV RNA confirmation for seropositive cases. Demographic, clinical, and laboratory data were collected. Hepatic biomarkers (AST, ALT, bilirubin, ALP) and non-invasive fibrosis indices (APRI, FIB-4) were compared between HCV RNA-positive and RNA-negative patients using Student's t-test or Mann-Whitney U for continuous variables and Chi-square or Fisher's exact test for categorical variables, with significance at $p < 0.05$. In a subgroup with transient elastography ($n=28$), liver stiffness and clinical staging were assessed.

Results: Among 22,562 screened patients, 364 (1.61%) were anti-HCV positive, of whom 83 (0.37%) had active infection. RNA-positive patients were predominantly male (81.9%), middle-aged adults (mean 56.1 ± 7.2 years), and showed significantly higher AST (85.4 ± 139.4 U/L vs. 38.8 ± 47.9 ; $p < 0.0001$), ALT (61.6 ± 53.8 U/L vs. 31.9 ± 34.7 ; $p < 0.0001$) and total bilirubin (1.36 ± 2.54

mg/dL vs. 0.87 ± 0.98 ; $p=0.0084$) levels. Fibrosis indices were also significantly elevated (APRI 1.29 ± 2.25 vs. 0.59 ± 1.12 ; FIB-4 5.44 ± 14.83 vs. 2.52 ± 3.94 ; both $p < 0.0001$). Elastography revealed that 32.1% of patients had advanced fibrosis or cirrhosis (F3-F4). No significant differences were observed in direct bilirubin, ALP, INR, creatinine, or platelet count.

Conclusions: Patients with active HCV infection detected through ED screening exhibited elevated hepatic biomarkers and fibrosis indices, reflecting a high risk of advanced liver disease. Many had comorbidities and prior healthcare interactions, suggesting missed opportunities for earlier diagnosis and/or treatment. These findings highlight the emergency department as a crucial setting for early detection of HCV, which would prevent progression to advanced stages of the disease. Implementing opportunistic screening in EDs is essential to enhance early diagnosis, facilitate linkage to care, and reduce the risk of severe HCV-related complications.



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Hepatitis B Virus Prevalence, Susceptibility and Care Engagement among Vulnerable Migrant Populations in Rome

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Background: Hepatitis B virus (HBV) remains a leading cause of morbidity and liver-related mortality globally. Vulnerable migrant populations often experience challenges with accessing testing, vaccination and care, both before and after arrival.

Methods: Asylum seekers and undocumented migrants hosted in six reception facilities in metropolitan Rome were prospectively offered screening for hepatitis B surface antigen (HBsAg), surface antibodies (anti-HBs) and core antibodies (anti-HBc). Individuals with HBsAg were assisted in accessing a specialist referral service, with support available throughout the screen-and-link pathway to reduce administrative, linguistic and logistic barriers.

Results: Between February 2024 and August 2025, 435 individuals from 38 countries underwent screening (median age 28 years; 86.7% male). Countries of origin were mainly in the South-East Asian region (36.1%, all Bangladesh), the African region (34.5%), and the Eastern Mediterranean region (28.3%). Most individuals (273; 62.8%) were non-immune (HBsAg, anti-HBc, and anti-HBs negative), 88 (20.2%) had prior infection (HBsAg negative and anti-HBc positive, usually with anti-HBs), and 51 (11.7%) had vaccine-induced immunity (anti-HBs positive only). HBsAg was detected in 23 individuals (5.3%; 95% CI 3.6-7.8), including 16/149 participants (10.7%) from the African region and 7/160 (4.4%) from Bangladesh. Among participants with HBsAg, 20/23 (87.0%) were successfully engaged in care. A total of 5/20 (25%) met immediate antiviral treatment indications based on either HBV DNA ≥ 2000 IU/mL

plus raised ALT or advanced fibrosis plus detectable HBV DNA. A further 6/20 (30%) required enhanced monitoring to determine the indications for treatment. A total of 4/418 individuals (1.0%; 95% CI 0.3-2.4), two from the African region and two from the Eastern Mediterranean region, had a positive HIV test, whereas 3/427 (0.7%; 95% CI 0.2-2.0), one each from the European, African, and Eastern Mediterranean regions, had anti-HCV, all without HCV RNA. No HBV co-infections with HIV, HCV or HDV were detected.

Conclusions: This study highlights the dual public health challenge of chronic HBV infection and lack of HBV immunity among asylum seekers and undocumented migrants in Rome. The initially high linkage to care demonstrates the effectiveness of integrated screen-and-link approaches. Strengthened HBV testing, vaccination and care pathways should be proactively embedded within health services for vulnerable migrant populations.



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Epidemiological Evolution Of Hepatitis C Virus Infection in a Central Portuguese Hospital: Who Are the New Patients?

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Background: The epidemiology of hepatitis C virus (HCV) infection has been evolving over time, influenced by demographic changes, migration patterns, social behaviors, and public health policies. While intravenous drug use (IDU) remains the main route of transmission, sexual transmission, particularly among men who have sex with men (MSM) and people living with HIV, is increasingly recognized as an important driver of new infections.

Materials and Methods: We conducted a retrospective analysis of patients treated for HCV infection at a tertiary hospital in Lisbon between January 2020 and December 2025. Demographic, epidemiological, clinical, and treatment-related data were extracted from clinical records and analyzed using Microsoft Excel.

Results: A total of 352 treatment episodes were recorded, with a progressive increase over time (33 in 2020 vs. 95 in 2025). Most patients were male (82%) and aged 50–59 years (31%). The proportion of migrants increased during the study period (2% in 2020 vs. 10% in 2025), with Brazil being the most represented country of origin (n=66). Overall, 49% of patients had HIV/HCV coinfection.

Among patients with HCV mono-infection, IDU was the most frequently reported route of transmission (39%), followed by sexual transmission (34%). In contrast, sexual transmission predominated among HIV/HCV coinfecting patients (69% vs. 27% IDU). Within this group, 62% were MSM, of whom 34% reported chemsex practices. A marked increase in sexually transmitted HCV infections was observed over time (11 in 2020 vs. 54 in 2025).

Genotype 1 was the most prevalent (46%), followed by genotype 4 (30%). This pattern was maintained in sexually transmitted infections, whereas genotypes 1 and 3 predominated among IDU-related infections (45% and 29%, respectively).

Most patients received pangenotypic direct-acting antiviral therapy, predominantly glecaprevir/pibrentasvir (82%), achieving a sustained virological response in 99% of cases. Fifty-one (14%) reinfection episodes were documented, the majority associated with sexual transmission (61%), mainly among MSM (97%) and individuals reporting chemsex (45%). HIV coinfection was present in 78% of reinfection cases.

Conclusions: Our findings highlight a shifting epidemiological pattern of HCV infection, with increasing sexual transmission, particularly among MSM and people living with HIV, alongside growing representation of migrant populations. The high rate of reinfection in these groups underscores the need for targeted prevention strategies, enhanced behavioral interventions, and strengthened screening and linkage-to-care programs to support HCV elimination efforts.



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Nationwide Expansion of a Clinical Microbiology-Driven Strategy to Recapture Active HCV Infection: The ProLINK-C Initiative

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Background: Despite the widespread availability of highly effective direct-acting antivirals, chronic hepatitis C virus (HCV) infection remains a relevant public health challenge. A major barrier in the final phase of elimination strategies is the identification of patients previously diagnosed but untreated, or without documented sustained virological response (SVR). Traditional retrospective searches in clinical databases have shown limited effectiveness and are difficult to sustain. ProLINK-C is a multicentre initiative implementing an automated, laboratory-driven opportunistic strategy to identify and recapture patients with unresolved HCV infection during routine healthcare encounters. Following its initial implementation in Southern Spain, the programme is currently expanding nationwide.

Materials and Methods: We conducted a multicentre observational study within the ProLINK-C network, an automated laboratory-

based initiative currently being implemented across Spain. From May 2023 to February 2026, an automated rule was implemented in the Laboratory Information System (LIS) to identify patients with a previous positive anti-HCV result whenever a serum sample was available for any clinical reason. Laboratory records and electronic medical histories were reviewed to determine prior antiviral treatment and/or documented sustained virological response (SVR). When no evidence of SVR was identified, reflex HCV RNA testing was requested using the available sample. At present, 15 hospitals across Spain are actively participating in the programme, and additional centres have expressed interest in joining the initiative and are expected to be incorporated in the near future.

Results: A total of 6,101 anti-HCV-positive individuals were identified. Among them, 731 patients (12.0%) required HCV RNA testing due to missing or incomplete virological follow-up. In 58 cases (7.9%), testing could not be performed because of insufficient sample volume. Among the 673 patients with valid HCV RNA results, 472 (70.1%) were RNA-negative, while 199 (29.6% of those tested; 3.3% of all anti-HCV-positive individuals) had active viremia. Viremic patients were predominantly male (70%), with a median age of 61 years (IQR 55–67). Reasons for RNA testing included absence of documented SVR and loss to clinical follow-up (47%), lack of previous HCV RNA determination (34%), and the presence of reinfection-associated risk factors (19%).

To date, 138 viremic patients (69.3%) have been contacted and scheduled for specialist consultation. Among them, 103 (74.6%) attended the appointment, and 89 (86.4%) initiated antiviral treatment, while 19 patients declined therapy, mainly due to advanced age or comorbidities.

Conclusions: ProLINK-C demonstrates that an automated, LIS-integrated opportunistic strategy can efficiently identify and recapture patients with active chronic HCV infection who remain within the healthcare system but outside the care cascade. By leveraging routine laboratory workflows, this approach enables real-time recovery of patients with incomplete diagnostic pathways. The ongoing expansion of the ProLINK-C network across Spain highlights its scalability and potential to support regional and national HCV micro-elimination strategies.



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Long-Term Viral Suppression, and Cardiometabolic Benefits of Switch to AINUOvirine Coformulated with Lamivudine, and Tenofovir DF in Virologically Suppressed People with HIV-1: 144-Week, Open-Label Results from the Sprint Post-Study

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Background: Previous 96-week results from the SPRINT extension study demonstrated that switching to the next-generation NNRTI ainoovirine coformulated with lamivudine and tenofovir DF (ANV/3TC/TDF) maintained viral suppression (VS) and improved cardiometabolic parameters in people with HIV-1 (PWH) switching from both classical NNRTI-based regimens and E/C/F/TAF. This post-study aimed to evaluate the 144-week efficacy and cardio-metabolic-renal safety outcomes.

Methods: This study was an extension of the Switching People with HIV to Receive Innovative NNRTI-Based Therapy (SPRINT) trial. Participants were divided into an Immediate Switch group (ANV/3TC/TDF from baseline to week 144, ISG) and a Delayed Switch group (E/C/F/TAF from baseline to week 48, then switched to ANV/3TC/TDF to week 144, DSG). The primary efficacy endpoint was the proportion of participants achieving virological suppression at week 144. Metabolic safety outcomes included low-density lipoprotein cholesterol (LDL-C) levels and LDL-C stratification according to atherosclerotic cardiovascular disease (ASCVD) risk. Renal safety was assessed by estimated glomerular filtration rate (eGFR).

Results: A total of 731 participants were enrolled, with 370 in the ISG and 361 in the DSG. At week 144, virological suppression was maintained in 95.3% of the ISG and 95.0% of the DSG [Fig. 1.].

CD4⁺ T-cell counts continued to increase from week 48 to week 144 (mean increase: 70.9 vs. 64.4 cells/ μ L). Fasting LDL-C showed a persistent decrease in the ANV/3TC/TDF arm and a pronounced reduction in those switching from E/C/F/TAF by week 48, sustained through week 144 (-0.19 vs. -0.44 mmol/L). The proportion of participants with high-risk LDL-C decreased from baseline to week 144 [Fig. 2.]: from 3.5% to 1.4% (-60%) and from 3.2% to 0.7% (-78%). eGFR remained stable over 144 weeks (116.1 to 109.1 and 114.9 to 106.3 mL/min/1.73 m²), consistent with age-related change per the CKD-EPI equation.

Conclusion: Switch to ANV/3TC/TDF maintained VS among PWH switching-off both classical NNRTI-, and boosted INSTI-based regimens. The accompanied safety benefits included persistent improvement in LDL-C level, and ASCVD risk strata. Renal function remained stable among this population, and required general routine monitoring.



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Factors Associated with Missing Sustained Virologic Response Assessment after Hepatitis C Treatment in Georgia

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The introduction of direct-acting antivirals for hepatitis C virus (HCV) treatment has led many countries to launch elimination initiatives. In most program evaluations, treatment success, measured as sustained virologic response (SVR), is calculated only among patients who complete follow-up testing. This approach may misrepresent outcomes if patients who miss SVR assessment differ systematically from those who return. In addition, missed SVR visits can reflect barriers such as travel, time, and competing priorities and may delay identification of patients with ongoing viremia or reinfection. The aim of this study was to re-engage patients who missed SVR assessment, and to compare their SVR status, demographic, and clinical characteristics with patients who completed SVR testing on schedule.

Patients who missed their scheduled SVR visit were identified from a clinic participating in Georgia's HCV elimination program between April 1, 2024, and September 1, 2024. Baseline demographic, behavioral, and clinical characteristics were compared between two groups: (1) patients who missed their scheduled SVR visit but later returned after active outreach, and (2) patients treated at the same clinic who completed SVR assessment on-time within the routine program. For the comparison group, we selected a random sample of on-time SVR completers using a 1:3 ratio. Patients in the outreach group received HCV ribonucleic acid (RNA) polymerase chain reaction (PCR) testing and liver function tests at the return visit. Patients with detectable HCV RNA underwent HCV genotyping to help distinguish reinfection from treatment failure based on genotype comparison.

In total, 204 patients returned after previously missing SVR assessment. Among these, 24 (11.8%) had detectable HCV RNA. Out of them, 16 (66.7%) had a different genotype (suggesting reinfection), while 8 (33.3%) had the same genotype, suggesting possible treatment failure. Among the 16 reinfected patients, 12 (75.0%) reported injection drug use (IDU). We compared the 204 outreach responders with 612 patients from the same clinic who completed SVR assessment on-time through routine follow-up. Overall, 96.1% (196/204) were cured of HCV (achieved SVR) in the outreach group, compared with 96.7% (4377/4525) among patients who completed SVR assessment on-time.

In multivariable analysis, after adjusting for sex, IDU, tobacco use, diabetes status, and fibrosis level (FIB-4), the independent predictors of missing SVR assessment were IDU (outreach group - 69.6% vs on-time SVR group - 49.7%; adjusted odds ratio (aOR)=2.83, 95%CI:1.80-4.44), presence of diabetes (11.1% vs 5.5%; aOR=2.86, 95%CI:1.64-4.98), and low FIB-4 (68.1% vs 49.4%; aOR=1.94, 95%CI:1.36-2.76).

Active outreach with free testing re-engaged patients who missed SVR assessment and identified a small subset with detectable HCV RNA, largely reflecting reinfection. SVR among outreach responders was similar to that among patients who completed SVR assessment on-time, suggesting routine follow-up-based cure estimates are not substantially biased. Missing SVR assessment was independently associated with IDU, diabetes, and low FIB-4, indicating that patients with less advanced fibrosis, who may feel well, are more likely to miss follow-up. Targeted retention efforts, including proactive outreach and harm reduction focused counseling for people who inject drugs, may improve SVR ascertainment and support timely management of reinfection or possible treatment failure.



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Dynamics of Intact and Total HIV-1 DNA-Reservoirs in Relation to Plasma Viral Load Levels

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Background: The Intact Proviral DNA Assay (IPDA) has emerged as a high-precision multiplex digital PCR (dPCR) method capable of discriminating between intact, hypermutated, and 3'-defective HIV-1 proviruses. By enabling a granular differentiation of the reservoir, IPDA offers significant potential for routine monitoring in people living with HIV, facilitating data-driven therapeutic adjustments.

This study aimed to characterize reservoir dynamics by quantifying intact proviral loads and total HIV-1 DNA across four distinct viraemic strata ranging from varying degrees of suppression to high viral loads.

Methods: CD4+ T-cells were isolated via immunomagnetic enrichment, followed by genomic DNA extraction. Quantification was performed by dPCR on the QIAcuityDx System (Qiagen), using a Triplex-IPDA (van Snippenberg et al. 2022). The assay targeted the psi and env regions to discriminate intact proviruses, while the LTR region (RU5) covered total HIV-1 DNA, including both intact and defective forms. Genomic integrity and cell equivalents were assessed using the RPP30 housekeeping gene assay, targeting two cellular sequences equidistant to the HIV-1 amplicons to monitor DNA shearing. 100 participants for each group were stratified based on plasma viral load (VL): group G1 (<50cp/mL), G2 (50–200cp/mL), G3 (200–1000cp/mL N=50), and G4 (>1000cp/mL).

Results: Testing could be completed for 309 participants (G1: 87, G2: 93, G3: 40, G4: 96). Measurement of total HIV-1 DNA showed statistically significant differences between the distinct viral load strata with a parallel increase in proviral copies per 10⁶ CD4+ T cells across ascending viraemic groups. Intact proviral DNA concentration showed a comparable trend with a

significant increase in the viraemic group compared to the <50cp/mL group. This could be shown despite substantial interindividual variability in both total and intact proviral DNA levels.

Conclusion: Quantifying total and intact proviral DNA is a new tool that allows insight in resolving reservoir characteristics and dynamics. Our study could show significant differences between the groups hinting for possible usage as a diagnostic tool to identify patients at risk of therapeutic failure. The large variation shows the necessity for adding clinical information in further studies to clarify the drivers of reservoir persistence.



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Hepatitis Delta Virus: Revisiting a Cohort of 349 Patients with Chronic HBV Infection

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Introduction: Hepatitis Delta Virus (HDV) infection is characterized by its dependence on concurrent Hepatitis B Virus (HBV) infection. The prevalence of HBV/HDV coinfection in Portugal remains unknown, although worldwide estimates range from 3% to 5%. Due to overlapping transmission routes with human immunodeficiency virus (HIV), approximately 5-6% of HIV infected individuals present HBV coinfection, of whom 7-15% are estimated to be coinfecting with HDV. Beyond the accelerated progression of liver disease observed in HDV coinfecting patients, there is an increased risk of progression to cirrhosis and hepatocellular carcinoma. The recent availability of effective therapies for HDV infection has underscored the importance of systematic screening and the establishment of clear treatment criteria.

Objectives and Methods: This study aims to characterize the population with HBV/HDV coinfection followed at a tertiary care hospital in Lisbon between January 1, 2020, and December 31, 2025. A retrospective analysis was conducted using Microsoft Excel, based on clinical records.

Results: During the study period, 349 patients with chronic HBV infection were under follow-up, of whom 18 (5.1%) had confirmed HBV/HDV coinfection. The majority were male (n=11, 61.1%) and native to Guinea-Bissau (n=11, 61.1%). Ten patients (55.6%) presented HIV-1/HBV/HDV coinfection and one patient HIV-2/HBV/HDV coinfection. Mean age at diagnosis was 41 years. The most frequent transmission categories were heterosexual contact (n=10, 55.6%) and intravenous drug use (IDU) (n=6, 33.3%).

The mean interval between HBV and HDV diagnosis was 7 years. In July 2025, reflex testing for HDV was implemented for all patients with

reactive HBsAg. The majority of diagnoses were made through 2024 (n=16, 88.9%), compared to only 2 (11.1%) in 2025. At the time of HDV diagnosis, three patients (17%) met criteria for advanced fibrosis (METAVIR F3-F4). Regarding HBV infection phase, the majority (n=15, 83.3%) had HBeAg-negative chronic infection. All HIV coinfecting patients (n=11, 61.1%) were on antiretroviral therapy including tenofovir (n=10) or entecavir (n=1), with all achieving undetectable plasma HBV DNA and biochemical response at last evaluation. Among these, five (45.4%) had detectable HDV viremia.

Of the seven HBV/HDV patients with detectable HDV viremia, five met criteria for treatment initiation (n=1 Peg-Interferon alfa-2a [PEG-IFN α 2a]; n=4 Bulevirtide), with a mean treatment duration of 13.4 months. Four patients achieved virological response (reduction >2log), with two showing undetectable HDV viral load at last evaluation.

Conclusions: This cohort reveals an HDV coinfection prevalence of 5.1%, with higher representation among patients with concomitant HIV infection. Among the 18 identified patients, 38.9% had detectable viremia and five initiated treatment. The implementation of universal screening strategies for all HBsAg-positive patients, combined with sequential and automated diagnostic approaches such as double reflex testing, enables earlier disease stratification and timely treatment initiation, potentially impacting the natural history and prognosis of HDV infection.



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Pharmacokinetics, Predicted Antiviral Activity, and Safety of Single-Ascending-Dose Asofitegrivir, a New-Generation Integrase Strand Transfer Inhibitor, in Healthy Adults: A Two-Period, Randomized, Placebo-Controlled Phase 1 Study

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Background and Objectives: Asofitegrivir (ASU), a new HIV integrase strand transfer inhibitor metabolized by UGT1A1, was evaluated in healthy subjects through a single ascending dose (SAD) study, a food effect (FE) assessment, and a drug-drug interaction (DDI) study with emtricitabine/tenofovir alafenamide (FTC/TAF). The objectives were to characterize its pharmacokinetics, safety, tolerability and to develop a physiologically based pharmacokinetic (PBPK) model for predicting antiviral activity in PLWH.

Methods: The trial consisted of two stages: Stage 1 was a single-center, randomized, double-blind, placebo-controlled SAD study with five dose cohorts (5 mg, 20 mg, 40 mg, 80 mg, 120 mg). Stage 2 was a single-center, randomized, open-label FE and DDI study at a dose of 40 mg. A PBPK model for ASU was developed and validated to predict its antiviral activity. V_{ss} was predicted using the schmitt method, which was determined via a preclinical rat and beagle intravenous PBPK model.

Results: Following a single dose, ASU's median T_{max} was 3.00-3.84 hours, with a mean t_{1/2} of 10.49-13.12 hours. C_{max} and C_{24h} both increased with dose, with mean ranges of 193.7–2055.5 ng/mL and 45.97 ng/mL to 434.7 ng/mL, respectively. Administration with food increased C_{max} and AUC_{0-24h} by 73.6% and 65.5%, respectively. FTC/TAF tablets (II) did not

significantly affect the pharmacokinetics of ASU. Treatment-related adverse events occurred in only 11.1% of participants across all studies, with no deaths or Grade ≥ 3 serious adverse events reported. In both the ASU PBPK model and the ASU-food interaction PBPK model, plasma concentration-time datasets observed at all doses except 20mg were consistent with the prediction. When extrapolated to the PLWH's multiple-dose (only have 5, 40 and 80mg data), 40 mg and 80 mg doses matched well with observed concentrations. The mean predicted PA-IQ90 values (predicted steady-state C_{24h}/PA-EC90) for multiple-dose PLWH at 40 mg(fasted), 80 mg(fasted) and 40mg(fed) doses predicted were 16.45, 36.16, and 28.67, respectively.

Conclusion: ASU was well tolerated, showed a dose-dependent, nonlinear pharmacokinetics, eliciting no dose-limiting toxicity and demonstrates promising potent anti-HIV activity predicted by PBPK modeling. Food significantly increased the exposure of ASU.



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Ongoing HIV-1 Transmission in Israel: Recency Analysis Reveals Concentration of Newly Acquired Infections Among MSM, 2022–2024

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Background: Identification of recent HIV infections provides critical insight into ongoing transmission and helps identify populations where prevention efforts should be strengthened. In Israel, HIV incidence remains relatively low, yet a considerable proportion of infections are still diagnosed at advanced stages, indicating missed opportunities for earlier detection. Monitoring recent infections therefore provides an important tool for identifying active transmission networks and evaluating prevention strategies. This study aimed to characterize recent HIV-1 infections diagnosed in Israel between 2022 and 2024. Thus, we analyzed the demographic, geographic, and molecular characteristics associated with recently acquired infections to better understand current transmission patterns and inform targeted prevention strategies.

Materials and Methods: National surveillance data from 1,150 individuals diagnosed with HIV-1 infection in Israel between 2022 and 2024 were analyzed. Recency status was determined using the national HIV recency testing algorithm, which integrates laboratory markers of recent infection, such as antibody avidity measurements and serological assay signal intensity, together with virological parameters (e.g., viral load) and immunological indicators (e.g., CD4 cell count) to classify infections as recent or long-standing. Demographic variables including age, sex, country of birth, and risk group were evaluated. Geographic distribution of cases was analyzed to identify regional patterns of transmission. Viral subtype information obtained through routine molecular surveillance was examined to assess viral diversity and potential transmission clusters. Comparisons were performed between recent and

long-standing infections to identify populations associated with ongoing transmission.

Results: Overall, 16.8% of newly diagnosed infections were classified as recent, and 22.3% of cases were identified during the seroconversion stage, reflecting diagnosis during the earliest phase of infection. The proportion of recent infections (16.8%) was slightly higher than previously reported national estimates for 2017–2021 (15.3%), suggesting continued ongoing transmission. Recent infections occurred predominantly among men (52.3%), particularly men who have sex with men (MSM) (79.3%), and among Israeli-born individuals (50.5%). Geographic clustering was observed in the central region of Israel, especially Tel Aviv (33.7%), suggesting localized transmission networks. Molecular analysis revealed an increasing prevalence of recombinant HIV-1 strains and declining proportions of subtypes A and C, indicating evolving viral diversity within transmission clusters. Lower proportions of early diagnosis were observed in peripheral regions, including the southern district (16.0%), and among migrants from Eastern Europe (27.1%) and Africa (9.9%), suggesting delayed diagnosis in these populations.

Conclusions: A substantial proportion of HIV-1 diagnoses in Israel represent recently acquired infections, concentrated primarily among MSM populations in central Israel. The slightly higher proportion of recent infections compared with previous national estimates suggests persistent transmission within specific demographic groups. The increasing presence of recombinant viral strains further indicates evolving transmission networks. These findings highlight the importance of strengthening targeted prevention strategies, expanding access to testing in underserved populations and peripheral regions, and enhancing molecular surveillance to enable earlier detection of transmission clusters and improve national HIV control efforts.



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Evaluation of Apri and Fib-4 Scores for Liver Fibrosis Screening in a Cohort of People Living with HIV and Hepatitis B Co-Infection at Newlands Clinic in Zimbabwe: A Cross-Sectional Study

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Introduction: Liver biopsy remains the gold standard for assessing liver fibrosis; however, it is highly invasive. Non-invasive techniques such as transient elastography (FibroScan), the fibrosis index based on four factors (FIB-4), and the aspartate aminotransferase-to-platelet ratio index (APRI) are increasingly used in clinical practice. Among these, FibroScan is generally considered the most reliable, while FIB-4 and APRI offer the advantages of low cost and wide accessibility. We aimed to evaluate the diagnostic performance of FIB-4 and APRI in people living with HIV (PLWHIV) and hepatitis B (HBV), to identify those with fibrosis and cirrhosis assessed by elastography.

Methods: We conducted a cross-sectional study at an urban HIV clinic in Harare, Zimbabwe, including people living with HIV (PLWHIV) who tested positive for hepatitis B surface antigen (HBsAg). Medical records of patients who underwent FibroScan assessment between January 2022 and February 2026 were extracted. Demographic data, vital signs, medical history, laboratory results, and FibroScan readings were retrieved. Fibrosis (7–12.5 kPa) and cirrhosis (≥ 12.5 kPa) were defined according to FibroScan parameters. APRI and FIB-4 scores were calculated and evaluated against FibroScan-defined diagnoses using receiver operating characteristic (ROC) curve analysis. Diagnostic accuracy was assessed at predefined thresholds (<1.3 and <2.67 for FIB-4; <0.5 and <1 for APRI).

Results: Of 247 clients with positive HBsAg, FibroScan data were available for 128 (57% male). At HBV diagnosis, the median age was 43 years (IQR 34–54). Median BMI was 23 kg/m² (IQR 19–

29) in females and 20 kg/m² (IQR 16.5–24.0) in males. Fibrosis and cirrhosis were identified in 20 (15.6%) and 8 (3.2%) clients, respectively. For cirrhosis, the area under the curve (AUC) was 0.87 (95% CI 0.74–1.00) for APRI and 0.86 (95% CI 0.71–1.00) for FIB-4. When evaluated against fibrosis or cirrhosis, AUCs were 0.64 (95% CI 0.47–0.81) for APRI and 0.70 (95% CI 0.54–0.85) for FIB-4. The optimal cutoff for cirrhosis was 0.52 for APRI (diagnostic accuracy 84.7%) and 0.67 for FIB-4 (diagnostic accuracy 93.1%). At analysis, 175 (70.9%) remained in care; among them, 145 (83.3%) were virally suppressed, 24 (13.8%) had low-level viremia, and 5 (2.9%) had virologic failure (>1000 copies/ml). The median CD4 count was 420 cells/mm³ (IQR 285–568).

Conclusion: APRI and FIB-4 scores demonstrate utility as non-invasive, accessible tools for screening cirrhosis and, to a lesser extent, fibrosis. Their performance in ruling in fibrosis was limited, underscoring the need for larger studies to refine diagnostic cutoffs in African populations.



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Plasma Endocannabinoidome (eCBome) in Chronic HCV Infection and Following Sustained Virologic Response (SVR) in HIV/HCV Co-Infected Individuals on Antiretroviral Therapy: Results from the Canadian Coinfection Cohort (CTN 222)

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Background: The endocannabinoidome (eCBome) is a lipid-based signaling system which plays a key role in tissue homeostasis, metabolism and inflammation across various body systems, notably, in the liver. We recently reported an imbalance in plasma eCBome mediators in ART-treated PWH characterized by an inverse relationship between anandamide and N acyl-ethanolamines (NAEs) versus 2-AG and 2-monoacylglycerols (MAGs). In chronic liver diseases, the eCBome is reported to exert both pro- and anti-fibrogenic effects. However, the impact of HCV/HIV-coinfection and liver fibrosis on eCBome mediator levels remains understudied.

Methods: Plasma samples from ART-treated, HIV RNA suppressed HIV/HCV-coinfected participants including n=21 with, and n=51 without, liver fibrosis based on APRI score ≥ 1.5 and/or fibroscan median stiffness ≥ 7.2 , were obtained longitudinally (pre- versus 6-12 months post-SVR) from the Canadian Coinfection Cohort. Levels of 23 eCBome mediators were measured by tandem mass spectrometry among two major families, the NAEs and MAGs, and the corresponding polyunsaturated fatty acid (PUFAs).

Results: In fibrotic versus non-fibrotic participants, we observed lower plasma levels of the NAE, LEA, both pre- and post-SVR, while the MAG, 2-SDG, and PUFA, arachidonic acid (AA), were lower only pre-SVR and post-SVR respectively. SVR resulted in decreased levels of the NAE, DPA EA(n-6), in both

fibrotic and non-fibrotic groups, while levels of AEA, PEA, DHEA, the MAGs, 2-SDG, and the PUFA, DPA(n-6), were decreased in non-fibrotic participants. In fibrotic participants, pre-SVR levels of several NAEs, including AEA, LEA, and PEA, were negatively associated with median liver stiffness and BMI, while the PUFAs AA and DPA(n-6) were negatively associated with HCV viral load and BMI.

Conclusions: Among plasma eCBome mediators, HIV/HCV coinfection is associated with fibrosis-dependent (LEA, 2-SDG, and AA) and SVR-dependent (AEA, DHEA, PEA, DPA-EA(n-6), 2-SDG, and DPA(n-6)) alterations. These changes link metabolic regulation with liver disease severity, suggesting a potential interplay between eCBome and fibrosis pathogenesis in PWH with HCV coinfection.



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Prevalence, Persistence and Predictors of Steatotic Liver Disease and Liver Fibrosis among Adults Living with and without HIV in Zambia and Zimbabwe: A Longitudinal Study (2020-2026)

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Introduction: Steatotic liver disease (SLD) is the leading cause of chronic liver disease globally and may progress to liver fibrosis and hepatocellular carcinoma when persistent. The rising burden of non-communicable diseases (NCDs) and the high HIV prevalence in African settings may further increase the risk of SLD. We assessed the prevalence, persistence, and associated risk factors of SLD and liver fibrosis among adults in Zambia and Zimbabwe.

Methods: We conducted a longitudinal analysis among adults aged ≥ 30 years attending three primary clinics in urban Zambia and Zimbabwe. SLD and liver fibrosis were assessed annually from 2020 to 2026 using vibration-controlled transient elastography (VCTE). Individuals requiring the extra-large (XL+) probe and those missing anthropometric data were excluded. SLD was defined as a controlled attenuation parameter (CAP) ≥ 248 dB/m, and fibrosis as a liver stiffness measurement (LSM) ≥ 7.0 kPa. Persistence was assessed in participants who had more than two VCTEs. Persistent SLD and fibrosis were defined as CAP ≥ 248 dB/m and LSM ≥ 7 in more than two-thirds of VCTEs, respectively. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg based on the average of three readings at the time of VCTE. Body mass index (BMI), central obesity, and waist-hip ratio (WHR) were defined according to World Health Organization guidelines. Proportions and 95% confidence intervals (CIs) were calculated

using the Wilson method. Multivariable logistic regression was used to determine factors associated with persistent SLD.

Results: A total of 1,140 participants were screened with VCTE, and 237 (20.8%) were excluded. In total, 903 participants with valid VCTE assessments were included. The median follow-up duration was 2.02 years (IQR: 0.00–3.02). Median age was 47 years (IQR: 39–54), and 56% were female. Overall, 605 (67.0%) were living with HIV. 470 (52.0%) were overweight or obese; 505 (56.0%) had a high waist circumference, and 424 (47.0%) had a high-risk WHR. 644 (71.0%) had hypertension. At baseline, SLD prevalence was 16.7% (95% CI: 14.4–19.4%) and liver fibrosis 8.9% (95% CI: 7.3–11.0%). During follow-up, period prevalence increased to 28.7% (95% CI: 25.8–31.8%) for SLD and 17.1% (95% CI: 14.7–19.7%) for fibrosis. 49 (5%) participants had >2 VCTE readings, and among these, persistent SLD was identified in 42 participants. Persistent fibrosis was identified in 4/49 participants. 3/49 had persistence in both steatosis and fibrosis. Male sex was associated with higher odds of persistent SLD (aOR 3.06, 95% CI: 1.55–6.04). Normal waist circumference was associated with lower odds (aOR 0.10, 95% CI: 0.03–0.27). Hypertension was associated with higher odds of persistence (aOR 1.86, 95% CI: 0.98–3.51). Age ≥ 50 years, HIV status, and WHR were not associated with persistence.

Conclusion: SLD and liver fibrosis were common and increased over time. Persistent SLD, was associated with male sex and hypertension, while normal waist circumference was protective. These findings highlight the need for targeted metabolic risk-reduction strategies and earlier identification of individuals at risk of persistent steatosis to reduce progression to advanced liver disease.



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Vitamin D Deficiency in People Living with HIV

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Background: Vitamin D is a fat-soluble vitamin that plays a key role in calcium homeostasis, maintaining bone health, and reducing the risk of fractures. Furthermore, vitamin D plays a significant role in regulating innate and adaptive immunity against infections. The optimal vitamin D supplementation, regardless of the antiretroviral therapy (ART) regimen in people living with HIV (PLWH), is associated with a reduction in inflammatory markers, bone turnover markers, and secondary hyperparathyroidism. A sufficient blood vitamin D level is 30 ng/ml, while a decrease less than 20 ng/ml is considered a deficiency [1, 2, 3].

Aim of Study: To assess vitamin D levels in people living with HIV.

Material and Methods: The study included 64 people living with HIV. The patients were divided into 2 groups: Group 1 - 26 patients with impaired bone mineral density (BMD) (men - 9, women - 17, age - 45 (10; 68) years; Group 2 - 38 patients without impaired BMD (men - 24, women - 14), age - 39 (22; 55) years. Blood vitamin D levels were determined using the ELISA method (FineTest VD (Vitamin D) Kit V4.0). Statistical analysis was performed in Excel 2010. Data are presented as Median (min; max).

Results: The average "experience" of receiving ART in the 1st and 2nd groups was 6 years. Tenofovir-containing regimen was received by 73.1% of patients in the 1st group, and by 73.7% of patients in the 2nd group, $p > 0.05$, test χ^2 . In the 1st group, out of 26 patients, 24 (92%) had vitamin D levels below 20 ng/ml. In 9 men, the vitamin D level was - 5.4 (0.31; 20.77) ng/ml, in 17 women - 6.23 (0.42; 61.33) ng/ml, $p < 0.05$, Mann-Whitney U test. In the 2nd group of 38 patients, 37 (97%) had vitamin D levels below 20 ng/ml. Twenty-four men had 4.92 (0.4; 26.01) ng/ml, and 14 women had 5.18 (0.33; 1.56) ng/ml, $p > 0.05$, Mann-Whitney U test.

Conclusions: In the group of PLWH with BMD abnormalities, vitamin D deficiency was found in 92% of cases, while in those without BMD abnormalities, it was found in 97% of cases ($p > 0.05$), indicating a significant decrease in vitamin D levels in the overall population of PLWH. This condition requires ongoing laboratory monitoring and correction of vitamin D levels, as well as ongoing prophylactic administration of vitamin D3 in the population of PLWH.

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Clinical Burden, Hospitalization, and Mortality in HCV RNA-Positive Patients Identified through Opportunistic Emergency Department Screening

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Background: Opportunistic screening in Emergency Departments (EDs) enables the identification of previously undiagnosed people with active hepatitis C virus (HCV) infection. Early detection in the ED may prevent progression to advanced disease stages. Many patients lack clear indications for HCV testing based on current clinical guidelines or risk factors, and frequently present with nonspecific or extrahepatic symptoms, complicating early diagnosis. This study aimed to describe reasons for consultation, hospitalization, linkage to care, treatment outcomes, and mortality among people with HCV RNA-positive infection detected through ED screening.

Materials and Methods: All people with HCV RNA-positive infection (n=83) identified during opportunistic screening in the ED of Torrecedenas University Hospital (Almería, Spain) between August 2021 and October 2024 were included. Data were collected on reason for consultation, presenting symptoms, hospital admissions, referrals to specialized care, antiviral management, and causes of death. Descriptive analyses were performed.

Results: The most frequent reasons for ED consultation were nonspecific digestive complaints (32%), respiratory or infectious issues (20%), and trauma-related problems (15%). Approximately one-third presented with digestive or abdominal symptoms attributable to hepatic complications, such as abdominal pain, upper gastrointestinal bleeding, or early cirrhosis decompensation.

Hospitalization was required in 45.7% of patients, mainly in Internal Medicine and Gastroenterology wards. Linkage to specialized care was achieved in 78% of cases, with 59% linked to antiviral management; 94% of those achieved sustained virologic response (SVR). During follow-up, 19 patients (22.9%) died, with 57.9% due to extrahepatic causes, 21.1% hepatic, and 21.1% other/indeterminate. Many had prior healthcare encounters, highlighting missed opportunities for early diagnosis.

Conclusions: People with HCV RNA-positive infection identified in our screening had considerable clinical burden, frequent hospitalisations, and notable mortality. Many did not meet the classic criteria for HCV testing, and a significant proportion had early complications. Importantly, in pluripathological patients, delayed diagnosis or inadequate management of HCV infection may worsen the prognosis of coexisting comorbidities and contribute to poorer overall outcomes. Therefore, opportunistic screening in the emergency department is an essential tool for timely identification, which would allow early referral and prevention of progression to advanced liver disease, ultimately reducing HCV-related complications, healthcare burden, and improving outcomes.



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Isolated Anti-HBc in People Living with HIV in Bulgaria: Prevalence and Implications for HBV Reactivation

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Background: The main serological indicator of HBV exposure is anti-HBc antibodies and usually persist lifelong. Among individuals living with HIV (PLWH), isolated anti-HBc is a common serological profile. Its clinical interpretation could be challenging due to the potential risk of occult hepatitis B virus (HBV) infection or viral reactivation with immunosuppression. Data from Eastern Europe remain limited.

Materials and Methods: The present study is a cross-sectional observational study based on a retrospective analysis of HBV serological markers in PLWH, with subsequent prospective molecular testing for HBV DNA. The screening was conducted among 789 HIV-positive patients, representing ~50% of those under follow-up in the HIV Care Centre in Sofia, Bulgaria, which provides care for 70% of all PLWH in the country. HBV serological markers (HBsAg, anti-HBs, total anti-HBc, anti-HBe) and anti-HCV, anti-HDV were tested by qualitative ELISA. HBV DNA was tested by Real-Time PCR with a limit of detection of 50 IU/mL. Statistical analysis was performed using SPSS v.27. Nonparametric variables were analysed using the Mann-Whitney U test. Categorical dependencies were assessed using the chi-square test. Univariate logistic regression was used to determine independent factors associated with detectable HBV-DNA.

Results: Among the 789 screened HIV-positive patients, isolated anti-HBc was identified in approximately 9.4% of the cases. Detectable HBV DNA was observed in a minority of patients. In patients with detectable HBV-DNA, the "chronic

hepatitis B" profile prevails (70.6%), while the remaining cases corresponded to both isolated anti-HBc and anti-HBc with low anti-HBs titres with detectable HBV DNA. Among patients with undetectable HBV-DNA, the profile with isolated anti-HBc predominates (65.7%). The distribution of serological profiles differed statistically significantly between the two groups (χ^2 , $p < 0.001$). HBsAg positivity was strongly associated with detectable HBV-DNA ($p < 0.001$). A statistically significantly higher frequency of anti-HBe positivity was found among patients with detectable HBV-DNA (50% vs 8.8%, $p = 0.002$). HCV co-infected patients had a lower frequency of detectable HBV-DNA ($p \approx 0.01$). HDV seropositivity was rare (<3%) and was not significantly associated with detectable HBV-DNA. The strongest predictor of HBV replication, according to univariable logistic regression analysis, is HBsAg positivity (OR 64, $p < 0.001$). Anti-HBe positivity was also associated with detectable HBV-DNA (OR 8.43, $p = 0.002$). Replication probability was considerably lower for isolated anti-HBc (OR 0.013, $p < 0.0001$). In both analyses, age, gender, current and baseline immune status, antiretroviral therapy (ART), and duration of HIV infection did not show a statistically significant association with the presence of HBV-DNA.

Conclusions: Isolated anti-HBc is a common serological profile among PLWH, but it is rare and not associated with detectable HBV replication. This supports the idea that it reflects resolved infection with waning anti-HBs rather than occult infection. In contrast, HBsAg positivity remains the only strong predictor of active HBV replication. HCV coinfection was associated with a lower frequency of detectable HBV-DNA.



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High Diagnostic Yield of Repeated HCV Screening in Addiction Services: Compared Findings from Two Large-Scale Campaigns in 2023 and 2025

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Background: Addiction Services (SERD) represent a strategic setting for hepatitis C (HCV) screening and early case identification. Screening campaigns performed in 2023 and 2025 provide an opportunity to evaluate changes in the target population, screening performance, and the potential for identifying undiagnosed infections. This study compares the two campaigns to assess how population dynamics and screening coverage influence the detection of new HCV cases.

Materials and Methods: We retrospectively analysed aggregated data from three SERDs in Northern Sardinia. In 2023, anti-HCV screening was universally offered to all patients in care. In 2025, screening targeted only individuals who had been HCV-negative or not previously screened in 2023. Key indicators included overall population size, proportion of patients on opioid substitution therapy, screening coverage, and positivity rates among those tested.

Results: The SERD population increased from 1107 individuals in 2023 to 1474 in 2025 (+32.8%). The proportion of patients receiving opioid substitution therapy (OST) rose from 55.9% (619/1107) to 61.4% (905/1474), corresponding to a 9.8% relative increase and indicating a progressively more complex case-mix. Screening performance differed between the two campaigns. In 2023, 849 of 1107 patients were screened (coverage 76.7%). In 2025, among 378 individuals eligible for screening, 248 were tested (coverage 65.6%), suggesting that the growing and more heterogeneous

population was increasingly difficult to reach. Despite this, anti-HCV positivity among newly screened individuals in 2025 remained high: 106 of 248 subjects (42.7%). This confirms the presence of a substantial reservoir of previously undiagnosed infection.

The apparent reduction in seroprevalence observed between the two campaigns—from approximately 80% in 2023 to 42.8% in 2025—does not reflect a true epidemiological improvement. Rather, it results from the substantial expansion of the SERD population, which now includes a large proportion of individuals whose HCV status is either negative or simply unknown due to lack of testing. As a consequence, the relative weight of known anti-HCV-positive patients decreases within a much larger denominator, leading to an artefactual decline in calculated seroprevalence. The underlying burden of HCV infection within the SERD population therefore remains significant, as demonstrated by the high positivity rate (42.7%) among newly screened individuals in 2025.

Conclusions: Comparison of the 2023 and 2025 screening campaigns highlights major changes in population size and complexity within SERDs, accompanied by a reduction in screening coverage. The persistently high proportion of positive tests among newly screened individuals in 2025 demonstrates the ongoing presence of hidden HCV infection. These findings reinforce the importance of repeated, structured screening initiatives to ensure the emergence of undiagnosed cases and to support HCV elimination efforts within addiction care settings.



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PROLINK B/D: An Active Strategy for HBV Re-Engagement and Systematic HDV Screening

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Background and Aims: Chronic HBV infection is a global health problem, with a risk of progression to cirrhosis and hepatocellular carcinoma. Despite recommendations for follow-up of these patients, a substantial proportion fail to remain engaged in structured clinical follow-up. To address this gap, we implemented PROLINK B/D, an active recapture and opportunistic diagnostic strategy based on the review of available serum samples at the laboratory.

The aim of this study is to evaluate the usefulness of an active search strategy in patients previously diagnosed with HBV, in order to identify those not linked to the healthcare system and complete the diagnosis through HDV double reflex testing

Methods: Prospective study designed to evaluate a strategy to re-diagnose and re-link to care of patients with HBV diagnosis, based on the review of the laboratory/medical records of patients with a previous positive HBsAg result for whom a serum sample was available for any other clinical request. An automated search system was set up in the Laboratory Information System (LIS) to generate daily files of patients with a previous positive HBsAg result in a window period of at least 9 months. In these cases, the persistence of HBsAg was first verified. If confirmed, HDV diagnosis was performed through a double reflex diagnosis, determination of anti-HDV antibody testing and, in the cases of positive results, HDV RNA detection and quantification was performed to confirm active infection. This strategy aimed to re-engage patients lost to follow-up and complete the HBV/HDV diagnostic cascade.

Results: The results obtained during the period from May to February 2026 are presented in this abstract. During this period, 135 HBsAg positive patients in whom serum samples was available for other reasons. Of these, 28 had cleared HBsAg upon re-evaluation and 7 could not be retested due to insufficient sample volume; of the remaining 100, notably, 84% had never been assessed by Infectious Diseases/Hepatology. In 98/100, a double reflex diagnosis of HDV could be made (2 were again excluded due to insufficient volume). The seroprevalence of hepatitis delta was 6,5% (6/92), and no viremic patients have been detected at this time. These findings underscore significant gaps in HBV clinical management. Additionally, efforts were made to re-engage patients lacking clinical follow-up. Among the 20 patients that have already been contacted, 15 (75%) have now been scheduled for an appointment.

Conclusions: PROLINK B/D demonstrates that active recapture of HBV cases is both feasible and effective in restoring linkage to care. Moreover, this approach enables double reflex diagnosis to identify HDV infection in patients who would otherwise remain undiagnosed. We believe that successful re-introduction into the HBV/HDV care cascade of these patients will be more effective, as they are already in care by a physician. This approach offers a scalable and pragmatic tool to advance progress toward viral hepatitis elimination goals.



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Social Determinants of Health-Related Quality of Life in Patients with Chronic Hepatitis C in Kazakhstan

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Background: Chronic hepatitis C virus (HCV) infection affects approximately 50 million people worldwide (WHO, 2024). In Kazakhstan, HCV is more prevalent in adults, with men comprising the majority of cases (Abuova et al., 2022). Beyond hepatic complications, cirrhosis and hepatocellular carcinoma, HCV impairs mental health, work capacity, and social functioning (Petruzzello et al., 2016; Yarlott et al., 2017). Social determinants of health, including economic stability, employment, and social support, are recognised as key mediators of HCV risk and patient outcomes (Raphael, 2004). However, their specific impact on health-related quality of life (HRQoL) in Kazakhstani patients remains insufficiently explored.

Methods: A cross-sectional pilot study was conducted in Astana, Kazakhstan. Fifty-three adults (≥18 years) with confirmed chronic HCV under dispensary follow-up were enrolled consecutively. HRQoL was assessed using the validated SF-36 Health Survey (Ware & Sherbourne, 1992), yielding Physical (PCS) and Mental Component Summary (MCS) scores. Sociodemographic data were collected via structured interviews. Group comparisons used Mann–Whitney U and Kruskal–Wallis tests (significance: $p < 0.05$).

Results: According to the data of the pilot study based on the SF-36 Health Survey questionnaire, a moderate decrease in physical functioning and a more pronounced deterioration in the mental and social components of quality of life were identified. The lowest scores were observed on the Vitality, General Health, and Mental Health subscales. Patients with social vulnerability—defined by low income, unemployment, and

limited social support—demonstrated significantly lower HRQoL scores across all domains compared to more socially stable patients, confirming that psychosocial and socioeconomic factors are key drivers of QoL impairment independent of clinical severity.

Conclusions: Chronic hepatitis C affects not only physical health but also the psychosocial well-being of patients. Social vulnerability appears to intensify the negative impact of the disease on health-related quality of life. Integrating psychosocial support and social work interventions into HCV care pathways may improve patient outcomes and well-being. Further multicentre studies with larger samples are needed to better understand the role of social determinants of health in HCV management in Kazakhstan and the broader Central Asian region.

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Eligibility for Regimens Containing Islatravir in People Living with HIV with Undetectable Viremia and Experience of Combined Antiretroviral Therapy: Data from the ARCA Cohort

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Background: Islatravir (ISL) has shown sustained activity against multiple HIV-1 subtypes and high potency even in the presence of several NRTI resistance-associated mutations (RAMs). However, in vitro data indicate reduced susceptibility to ISL in viral strains harboring M184I/V alone or in combination with other mutations such as V90I, A114S, I142V, T165R or at least ≥ 1 thymidine analogue mutation (TAM). We therefore estimated the proportion of virologically suppressed people living with HIV with experience of combined antiretroviral therapy (Exp-PLWH) in the ARCA cohort who might be eligible for ISL, based on the prevalence of mutation patterns known to modify ISL susceptibility.

Methods: We performed a cross-sectional study including virologically suppressed Exp-PLWH in the ARCA cohort who were on stable ART, had active follow-up in 2024 and had ≥ 1 available genotypic resistance test (GRT). Mutation patterns associated with ISL-reduced susceptibility were evaluated. ISL activity was explored according to the following definitions: (1) absence of any ISL RAM (2) absence of the combination M184V plus ≥ 1 TAM. As Doravirine is currently being evaluated as a companion drug for ISL-based regimens, its genotypic susceptibility was also assessed through Stanford Algorithm version 10.1. Logistic regression models were built to identify predictors of ISL preserved activity according to the two resistance patterns.

Results: Overall, 1,809 virologically suppressed Exp-PLWH were included: 67.8% were males, with median age 58 years (IQR: 49-63), a median follow-up 20 years (IQR 12-31) and a median duration of viral suppression of 11 years (IQR 7-16).

ISL RAMs were observed in 37.3% of PLWH. Specifically, the M184I/V mutation was found as follows: alone in 21.7% of individuals; in combination with ≥ 1 TAM in 14.7%; with V90I in 2.2% and with K65R in 1.1%; no subjects harbored the M184I/V with V90I, A114S or I142V+T165R. Compared with others, individuals with ISL RAMs were older (median [IQR]: 60 [55-64] vs. 55 [46-62] years) and had a significantly higher prevalence of female sex (37.1% vs. 29.3%), lower CD4+ cell nadir (160 [63-291] vs. 247 [109-361] cells/mm³), longer treatment exposure (27 [17-29] vs. 14 [9-19] years), a higher number of prior regimens (9 [6-13] vs. 4 [3-6]), and a higher prevalence of HBV coinfection (25.2% vs. 16.2%) (all $p < 0.001$). Multivariable logistic regression analysis showed that antiretroviral initiation in more recent calendar years (aOR: 7.28, 95%CI 3.99-13.30, $p < 0.001$) and a lower cumulative antiretroviral exposure (aOR: 0.83, 95%CI 0.79-0.86, $p < 0.001$) were independently associated with the absence of any of the ISL resistance mutations. Similar results were obtained at multivariable logistic regression for absence of M184I/V+ ≥ 1 TAM pattern.

According to the historical genotypic susceptibility score, 82.8% of subjects harbored viral strains fully susceptible to Doravirine.

Conclusions: In this cohort of virologically suppressed Exp-PLWH receiving stable ART, the predicted activity of ISL was generally high, reflecting the absence of key ISL RAMs and the relatively low prevalence of NRTI mutations known



to reduce ISL susceptibility. However, individuals with a long treatment history and extensive antiretroviral exposure may require careful resistance assessment prior to ISL initiation.



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High NNRTI Resistance and Low Integrase Inhibitor Resistance in the Context of Dolutegravir Scale-Up in São Tomé and Príncipe

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Background: HIV-1 drug resistance remains a public health concern globally. Sao Tome and Principe (STP) is a Central African country with a concentrated HIV epidemic and rapidly expanding antiretroviral therapy (ART) coverage, with recent transition to dolutegravir-based regimens. However, contemporary nationwide data on HIV drug resistance are scarce. We describe the current profile of HIV-1 drug resistance mutations (DRMs) among adults living with HIV in this setting.

Methods: In this cross-sectional study (2022–2024), 197 adults were recruited from district health facilities nationwide. Sociodemographic and clinical data were collected through standardized interviews and chart review, and compared by ART status using chi-square, Fisher's exact and Student's t-tests, as appropriate. Plasma samples with detectable viral load were eligible for next-generation sequencing of protease (PR), reverse transcriptase (RT) and integrase (IN) genes. The HIV-1 genotyping was performed in the reference laboratory of Institute of Hygiene and Tropical Medicine in Portugal. DRMs were interpreted using the Stanford HIVdb algorithm implemented via in-house pipeline. Class-specific resistance prevalence and 95% confidence intervals were estimated using the Wilson score method. Subtype assignment was performed using COMET and REGA v3.0.

Results: Of 197 participants, 150 (76.1%) were ART-experienced and 47 (23.9%) ART-naïve. A total of 124 plasma samples were successfully sequenced to PR/RT/IN. The mean age was 46.3 years and 55.2% were female. Sex and household income distributions were similar by ART status, but ART-naïve individuals were more frequently classified as having a recent HIV diagnosis (97.9% in 2020–2024; $p < 0.001$) and had more frequently history of living abroad for >6 months (36.1% versus 14.4%; $p = 0.008$), compared to ART-experienced. The HIV-1 recombinants predominated with CRF_02_AG (45,1%). Pure HIV-1 subtypes identified were G (12.9%), A1 (8.0%), C and D (6.4% each), F1 (5.6%), and other subtypes (8.8%). The prevalence of major resistance-associated mutations was 4.0% for protease inhibitors (PIs), 10.5% for nucleoside reverse transcriptase inhibitors (NRTIs), 34.7% for non-nucleoside reverse transcriptase inhibitors (NNRTIs), and 1.6% for integrase strand transfer inhibitors (INSTIs). The most frequent NNRTI drug resistance mutations (DRMs) were K103N/S (17.7%), L100I (8.9%), G190A/S (4.0%), and E138A/G/K (4.0%). Key NRTI DRMs included K219E/Q/R (5.6%), M184V (5.6%), D67N (5.6%), and T215F/I/N/Y (4.0%). For PIs, the most prevalent DRMs were L90M, L76V, and M46I (1.6% each). INSTI DRMs were detected in 1.6% (95% CI: 0.4–5.7%) of participants, with major mutations R263K and G140R corresponding to a low estimated prevalence (0.8%).

Conclusion: High levels of DR to NNRTI ARV drug class were identified in STP, reflecting the historical use of NNRTI-based regimens, reinforcing the appropriateness of the national transition to dolutegravir-based first-line therapy. On the other hand, major INSTI-associated mutations remain infrequent, supporting the continued effectiveness of dolutegravir. However, the detection of major INSTI mutations underscores the importance of sustained virological and resistance surveillance in this African low-income country.

Keywords: HIV-1 drug resistance, Antiretroviral therapy (ART), Integrase strand transfer inhibitors INSTI, HIV molecular epidemiology and São Tomé and Príncipe



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A Dynamic Intervention Study Based on Timing Theory to Improve Treatment Adherence from Diagnosis to Intensive Phase in Patients with Tuberculosis and HIV Co-Infection

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Background and Objectives: The treatment process for patients with tuberculosis (TB) and HIV co-infection is complex, and suboptimal adherence during the critical period from diagnosis through the intensive phase of treatment directly impacts the efficacy and prognosis of managing these "dual diseases." Timing Theory emphasizes providing timely support at key transition points along the illness trajectory. This study aimed to develop and evaluate a dynamic intervention program based on Timing Theory to improve patient adherence at crucial stages, including diagnosis, treatment initiation, and the intensive treatment phase.

Methods: A quasi-experimental study design was employed. A total of 120 patients with TB/HIV co-infection admitted between June 2022 and December 2023 were assigned to an intervention group (n=60) or a control group (n=60) based on admission time. The control group received routine treatment and health education. The intervention group received, in addition to standard care, a four-stage dynamic intervention based on Timing Theory: 1) Diagnosis Phase (emotional support and cognitive restructuring); 2) Treatment Initiation Phase (individualized medication planning, pre-management of potential adverse effects); 3) Intensive Treatment Phase (reminders via electronic pillboxes, peer support, symptom management); 4) Transition Phase (preparation for shifting to the continuation phase). Outcome measures included adherence rates to both anti-TB and antiretroviral therapy (assessed via pill count and electronic monitoring), time to sputum smear conversion, changes in CD4+ T-cell count,

treatment interruption rate, and quality of life scores.

Results: At the end of the intensive phase, the comprehensive treatment adherence rate was significantly higher in the intervention group (92.1%) compared to the control group (75.6%, $P<0.01$). The average time to sputum smear conversion was shorter in the intervention group (1.8 months vs. 2.3 months, $P<0.05$), and the treatment interruption rate was lower (5.0% vs. 18.3%, $P<0.05$). At the 6-month follow-up, the intervention group showed greater improvement in CD4+ T-cell count increase and quality of life scores compared to the control group ($P<0.05$). Qualitative feedback indicated that the staged, targeted support effectively alleviated patient anxiety and enhanced self-management efficacy.

Conclusion: The dynamic intervention based on Timing Theory, by identifying and addressing the core needs of TB/HIV co-infected patients at different disease stages, effectively improved treatment adherence during the critical period from diagnosis through intensive therapy. This led to better clinical and immunological outcomes and potentially enhanced quality of life. This model provides a structured, actionable framework for optimizing the comprehensive management of patients with this complex co-morbidity.



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Acute Hepatitis B in a Vaccine Non-Responder with HIV Following Switch to Long-Acting Cabotegravir/Rilpivirine

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Background: Hepatitis B virus (HBV) vaccination is recommended for all people living with HIV (PLWH), although vaccine non-response remains frequent and up to 30–50% fail to develop protective anti-HBs titres. Tenofovir-containing antiretroviral therapy (ART) has historically provided incidental protection against HBV infection. With increasing use of simplified ART and long-acting injectable regimens lacking HBV-active agents, individuals without protective anti-HBs antibodies may remain at risk of HBV acquisition, particularly in populations with ongoing sexual exposure risk and increasing global mobility from HBV-endemic regions.

Case Description: A 25-year-old man living with HIV-1 infection (CDC stage A1) presented with one week of nausea and abdominal discomfort followed by two days of jaundice. HIV infection had been diagnosed in 2020 (nadir CD4+ 609 cells/ μ L; HIV RNA 4430 copies/mL; subtype B; no baseline resistance mutations).

Initial ART at diagnosis consisted of tenofovir disoproxil fumarate/emtricitabine plus dolutegravir, later changed to abacavir/lamivudine/dolutegravir and subsequently to dolutegravir/lamivudine in May 2024. In October 2025, therapy was switched to long-acting injectable cabotegravir/rilpivirine at the patient's request.

Despite previous HBV vaccination, the patient persistently lacked detectable anti-HBs antibodies, consistent with vaccine non-response. His history included multiple sexually transmitted infections, suggesting ongoing sexual exposure.

At presentation, laboratory testing revealed marked hepatocellular injury (AST 2379 U/L; ALT 4449 U/L; total bilirubin 8.96 mg/dL; INR 1.4). Abdominal CT showed mild hepatosplenomegaly without biliary obstruction. HBV serology confirmed acute infection with HBsAg positivity,

anti-HBc IgM positivity, HBeAg positivity and absence of anti-HBs antibodies.

Management and Outcomes: The patient was admitted for monitoring and managed supportively. Liver enzymes and bilirubin progressively improved and scheduled cabotegravir/rilpivirine administration was maintained.

At discharge, transaminases had decreased substantially (AST 995 U/L; ALT 2302 U/L). One month later the patient remained asymptomatic with near normalization of liver tests (AST 73 U/L; ALT 77 U/L). HBV serology showed persistent HBsAg positivity with loss of HBeAg and anti-HBe seroconversion. Hepatitis D testing was negative. Ongoing serological follow-up is planned to determine whether HBsAg clearance or progression to chronic HBV infection will occur.

Discussion: In this patient, progressive ART simplification resulted in stepwise withdrawal of HBV-active agents (tenofovir followed by lamivudine) prior to initiation of long-acting therapy. As ART strategies move towards simplified or long-acting regimens without HBV-active drugs, susceptibility to HBV infection may become more apparent in individuals who fail to mount a protective vaccine response.

Conclusion: Acute HBV infection may occur in PLWH who are vaccine non-responders following transition to ART regimens lacking HBV-active drugs. This case highlights the importance of assessing HBV immunity prior to ART simplification or initiation of long-acting therapy in PLWH. Revaccination and evaluation of HBV risk should therefore be considered in vaccine non-responders before switching to ART regimens lacking HBV-active agents.



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Silent until Provoked: A Striking Case of HBV Reactivation with Infliximab

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Introduction: Reactivation of hepatitis B in the context of immunosuppressive and biologic therapies has emerged as a clinically significant source of morbidity and mortality among individuals with active or previous hepatitis B virus (HBV) infection. The at-risk cohort encompasses both patients with chronic HBV carriage and those with resolved infection who retain serological markers of prior exposure. With the expanding use of advanced targeted biologic agents, elucidating the immunological and molecular pathways that render certain therapeutic classes more susceptible to precipitating HBV reactivation has become increasingly critical. Infliximab, a chimeric monoclonal antibody against TNF- α , is not commonly associated with hepatitis B reactivation, although serologic monitoring is recommended in these cases.

Clinical Case: We present a case of an 86-year-old Portuguese woman, with a history of sarcoidosis with ocular and thoracic involvement, for which she was under immunosuppressant therapy, initially with high dose systemic prednisolone and subsequently with infliximab and low dose corticosteroids. Additionally, she had a history of previous HBV infection with positive HBc and HBs antibodies.

There was no indication for prophylaxis for hepatitis B reactivation based on this type of immunosuppression, having been decided to maintain biannual surveillance. Two years after starting on infliximab, we observed HBV reinfection, with newly positive HBs and HBe antigens, HBV viral load of 2.450.000 UI/mL and elevation of transaminase values three times the normal upper limit. An abdominal ultrasound and FibroScan[®] showed no relevant findings.

The patient was started on entecavir shortly after this diagnosis. The six-month follow-up laboratory

study showed negative HBs and HBe antigens, undetectable viral load and normalization of hepatic enzymes.

The patient is currently asymptomatic and will maintain antiviral therapy for the duration of the immunosuppression period.

Discussion: We underline the importance of clinical and laboratorial vigilance for hepatitis B reactivation in immunosuppressed patients. Multicentric studies are needed to better understand the scope of this phenomenon, particularly regarding monoclonal antibodies against TNF- α .



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To B or Not to B: A Case Study of Acute HBV Infection

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Introduction: HBV remains a significant global health challenge. While over 95% of acute infections in healthy adults are self-limited and often asymptomatic, a minority of patients experience extreme aminotransferase elevations and significant clinical symptoms, requiring careful management to avoid liver failure.

Case Presentation: A 61-year-old man with mixed dyslipidemia and without history of alcohol abuse, presented to the emergency department with a one-week history of choloria, dysuria, and fatigue. Laboratory tests revealed severe hepatocellular injury (aspartate aminotransferase (AST): 2184 U/L, alanine aminotransferase (ALT): 5286 U/L) total bilirubin: 3.19 mg/dL, and an international normalized ratio (INR): 1.46. Serology confirmed acute HBV infection with positive HBsAg, anti-HBc IgM, and HBeAg, and DNA levels exceeding 14,000,000 IU/mL. HIV serology was negative and other viral causes were excluded (hepatitis A, C, E and D, HSV, CMV and EBV) as well as biliary obstruction. The patient admitted high-risk sexual exposure prior to symptoms. Due to worsening cytolysis, high viral load, and rising bilirubin, antiviral therapy with tenofovir disoproxil fumarate was initiated. Despite reaching a peak total bilirubin of 23.4 mg/dL and an INR of 1.8, the patient showed progressive virological and biochemical recovery.

Discussion: This case highlights an atypical, severe course of acute HBV marked by intense cytolysis and cholestatic evolution. Although most cases resolve spontaneously, this report emphasizes that early antiviral intervention is crucial in patients showing signs of coagulation abnormalities and extreme hyperbilirubinemia. Timely recognition and treatment are vital to prevent the transition from acute infection to life-threatening acute liver failure.

