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TREATMENT STRATEGIES & ANTIVIRAL DRUG RESISTANCE

ABSTRACT BOOK

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Oral Presentations

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Extensive Virologic Characterization in a Person Living With HIV, With Apparent HIV Remission for 2 Years After Allogeneic Stem Cell Transplantation With CCR5 Wild-Type Cells: A Case Study

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Background: Allogenic hematopoietic stem cell transplantation (allo-HSCT) with homozygous CCR5Δ32 donor cells is the only potentially HIV-1 curative intervention. However, allo-HSCT has been associated with a drastic reduction in the HIV reservoir independently of engraftment with CCR5Δ32 or CCR5 wild-type cells. In this study, we investigated ex-vivo the reservoir of an HIV-1 infected individual with no evidence of infection at 2 years after allo-HSCT with wild-type CCR5 genotype.

Case Report: The study participant was an adult male, HIV-1 infected since 1994 with 5 antiretroviral class multidrug resistance strain and under treatment with ART. On January 2020, at the age of 58, he received an allo-HSCT from an HLA-identical sibling donor for a Hodgkin lymphoma in second complete remission after 24 cycles of therapy with the anti- PD1 pembrolizumab. The individual received a reduced toxicity conditioning regimen, based on fludarabine and treosulfan at myeloablative dosage, and was discharge on day 47 after transplant without major complications. He never developed graft-versus-host disease, neither acute nor chronic. Despite positive HIV-1 serology, he maintained undetectable viremia and he was negative for HIV-1 DNA by routine diagnostic analysis. In March 2022 CD4+ T cells were isolated after leukapheresis and maintained in culture to determine: (i) the CCR5 genotype by Sanger sequencing, (ii) the amount of cell-associated HIV-1 DNA (CAD) and cell-associated HIV-1 RNA (CAR) by digital droplet PCR at baseline and after ex-vivo stimulation with ionomycin (ION, 1 μg/ml) plus phorbol-myristate-acetate (PMA, 50 ng/ml) using a protocol previously published. Briefly, 2-days post induction (dpi) 4 x 10^7 this individual derived CD4+ were co-cultivated with MOLT-4 CCR5, a cell line permissive for HIV-1 infection. CAR and CAD were quantified at baseline (T0) and 2-7-14-21 dpi (T2, T7, T14, T21). At T7, T14 and T21, the infectivity of the CD4+T/MOLT-4 CCR5 co-cultures was evaluated using a modified TZM-bl based assay (TZA) protocol. In addition, a positive control of 1.2 x 10^6 CD4+ T cells, deriving from an induvial living with HIV-1, and is virologically suppressed was analyzed in parallel.
DNA sequencing confirmed a wild type homozygous CCR5. CAR and CAD were negative at baseline and at all time points analysed. The CD4+T/MOLT-4 CCR5 co-cultures were not infectious in TZA at T7, T14 and T21. The HIV-1 positive control was quantifiable in CAD at T7, T14 and T21 with 931.2 ± 400.1, 68.9 ± 10.1, 128.6 ± 15.3 HIV-1 copies per 10^6 CD4+ T cells respectively and in TZA at T21 with 16.1 [5.4-47.8] IUPM/cells.

Conclusion: Two years after wild-type CCR5 allo-HSCT, this individual remained without measurable or inducible HIV-1 DNA and RNA in the blood compartment by multiple ultrasensitive assays on a total of 40 million CD4+ T cells. However, the presence of the virus in other tissues was unexplored. Ultimately, only analytical treatment interruption may reveal whether full remission has been achieved.

2

Sars-Cov-2 Omicron Variability: Focus in Immunocompromised Individuals

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Background: We aim to characterize SARS-CoV-2 omicron variability with a particular focus in individuals who are immunocompromised (IPs).

Material and Methods: This retrospective study included SARS-CoV-2 infected hospitalized and non-hospitalized IPs (HIP/NHIP) and non-IPs (HP/NHP). For each subject, nasopharyngeal swabs were collected from January to December 2022, and SARS-CoV-2 whole-genome sequencing was performed by Miseq-platform. Additional mutations (AMs) (intra-host prevalence >20%), not present in each sublineage consensus, were analyzed in spike, nucleocapisd and nonstructural proteins (RNA-dependent RNA polymerase, main-protease, papain-like-protease, helicase, Orf6 and Orf9b).

Results: So far, 211 SARS-CoV-2 omicron infected individuals (126 NHIP, 12 HIP, 57 NHP, and 16 HP) were characterized: 49.8% were female, with a median [IQR] age of 61 [50-72] years and 10.4% reporting no SARS-CoV-2 vaccination. A significant different rate of diagnosis of pneumonia was observed in the four groups, par-ticularly higher in HP (0%, 0%, 58.0% and 93.8% in NHIP, NHP, HIP and HP, respectively p<0.001). Interest-ingly, a statistical high frequency of no-vaccination was observed in hospitalized individuals (HP 37.5%, HIP 25%, vs 9.9% NHP and 5.5% NHIP, p=0.0002). Significantly, immunocompromised individuals were younger: with a median [IQR] age of 58 [48-69] years in HIP+NHIP vs 67 [53-77] years in NHIP+NHP, p= 0.0015.
Overall, 34 different Omicron sublineages were identified, and according to the temporal collection of samples, BA.1.1 was the most prevalent (21.3%), followed by BA.1 and BA.2 (17.5%), BA.2.9 (7.6%) and BA.5.1 (3.8%). Regarding AMs analysis, 128/211 (60.7%) individuals showed at least one AM, in at least one of the eight ana-lysed genes. A different prevalence of AMs among the 4 NHIP/HIP/NHP/HP groups was found only in spike (17.5%, 33.3%, 7%, 25% (p=0.041) including in the receptor binding domain (RBD, 3.9%, 16.7%, 0%, 12.5% p=0.015, respectively). An higher prevalence of AMs was observed in hospitalized (HIP+HP) vs non-hospitalized individuals (NHIP+NHP) in both spike (28.5% vs 14.2.%, p=0.054) and RBD (14.3% vs 2.7%, p=0.019). These results correlate with the Δ days from first COVID-19 symptoms to NS sampling, that were significant longer in individuals who are hospitalized (median [IQR] days: 9 [7-12] in HP vs 5 [3-8] in HIP vs 4[3-5] in NHIP vs: 4 [3-5] in NHP p=0.00001).
Characterizing the 2 regions associated with innate response, we observed in Orf6 the D61L mutation (typical of BA.2 and BA.4.) with an overall prevalence of 36.5%. Its prevalence (according to BA.2 and BA.4 infection) was higher in individuals who are immunocompromised (41.3% in HIP+NHIP vs 23.4% in HP+NHP, p=0.046) and less in individuals who are hospitalized (17.8% in HIP+HP vs 39.3% in NHIP+NHP, p=0.028). Moreover, within the individuals who are immunocompromised, a higher prevalence of AMs in Orf9b was found in individuals who are hospitalized (16.7% in HIP vs 2.4% NHIP, p=0.06).
AMs associated with resistance to mAbs (346K/446D/452R/445L/460KS) were found only in HIP and/or NHIP, while none of AMs associated with resistance to molnupiravir and nirmatrelvir were observed in the cohort.

Conclusion: We confirmed a higher variability in individuals who are immunocompromised, particularly when hospitalized. These results deserve further characterization, with a larger population, specifically for Orf6 and Orf9b, which are strictly related to the innate antiviral response.

3

Epidemiology and Molecular Analyses of Respiratory Syncytial Virus in the Season 2021-2022 in Northern Italy

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Background: Human respiratory syncytial virus (HRSV) is a common cause of acute respiratory infection, especially in children, causing asymptomatic or symptomatic infections ranging from mild to severe. In Europe, HRSV shows a seasonal distribution mainly from October to May, with seasonal peaks in January/February. HRSV has a single-stranded negative-sense RNA genome, encoding for 10 viral proteins; two of them (G and F) playing a significant role in viral pathogenicity and immune evasion. HRSV is classified into A and B subtypes, with wide genetic diversity, especially in G gene, resulting in divergence into many genotypes. HRSV-A infection progress to more severe clinical manifestations than HRSV-B, despite the association between HRSV genotypes and disease severity remains unclear. Aim of this study was to characterize the HRSV in the 2021-2022 season in a paediatric cohort.

Material and Methods: 104 samples were collected between November 2021-January 2022, from paediatric patients attending the "Vittore Buzzi"Children's Hospital in Milan. Samples were analysed using RT-PCR assay to discriminate type A and B. Subtype specific protocols were applied to obtain amplicons for NGS procedures. Maximum-Likelihood and Bayesian phylogenies were used to analysed Italian sequences in the European contest and dated Italian clusters.

Results: Males accounted for 58.7% and the median age was 87 days. 76.8% of subjects required hospitalization, with a median stay of 8 days. Stratifying subjects by age, a significant proportion of hospitalized subjects was observed in patients aged <3 months (p=.037). Subtypes A and B were equally distributed. Subtype A patients were significantly older than that of B subtype, (109 vs. 66 days, p=.007). Significant differences were found in length of hospitalization (8 for HRSV A vs. 6 days for HRSV B, p=.05), days of supplemental oxygen treatment (6 vs. 4 days, p=.004) and intravenous hydration duration (4 vs. 2 days, p=.018). Whole genome was obtained for 88 samples, 49 of A subtype, genotype GA2.3.5 and 39 of subtype B, genotype GB5.0.5a. HRSV-A sequences showed a higher heterogeneity compared with B by analysing number of substitutions. Phylogeny highlighted the presence of 20 clusters containing quite the totally of Italian sequences and 35 clusters involving 60% of Italian strains for HRSV-A and B, respectively. Clusters presented a tMRCA between 01/2012-09/2017 for A subtype, and 12/2013-02/2019 for B subtype. No differences were observed between sequences inside or outside clusters. Italian sequences showed a peculiar mutational patter compared to European sequences. Some mutations such as V279A in G gene, N117K in M2-1 portion, I7V and L422M in L gene for HRSV A and N5K in NS2 portion, V90A in N gene, T198A in G gene and A675V, UI677V, I1588, K1589, Y1590S, V1592L and T1596I for HRSV B were present only in Italian strains.

Conclusion: These data confirmed a more severe clinical course of HRSV-A, in particular in young children. Firstly, our study permitted the characterization of HRSV whole genomes of Italian strains highlighting the peculiar pattern of mutations that needs to be more investigated and monitored.

4

Expanding PrEP by 50% Of the Current Users on Long-Acting Cabotegravir vs Daily-Oral TDF/FTC PrEP Will Have a Similar Impact in Reaching the UNAIDS Goal to Reduce New Infections by 2030 Among MSM in the Netherlands. A Modeling Study

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Background: United Nations aims to end the AIDS epidemic in 2030 by reducing AIDS-related deaths and new HIV infections by at least 90% in 2030 as compared to 2010. In the Netherlands, 55% of annual new HIV diagnosis are concentrated among MSM, with 750 new diagnoses in 2010. Cabotegravir is a novel long-acting integrase inhibitor PrEP formulation allowing dosing once every two months compared to daily-oral TDF/FTC regimen currently rolled-out as PrEP globally. Currently, 8500 MSM use oral PrEP in The Netherlands, but it is unknown if this number is sufficient to reach the 2030 goals. For the Dutch context where the epidemic is rapidly declining, we investigated the impact of introducing cabotegravir PrEP among MSM to achieve the UNAIDS targets by bringing down to 75 new diagnoses by 2030, when compared to increasing the coverage of daily oral TDF/FTC users among MSM.

Material and Methods: A deterministic mathematical model was used including HIV transmission among men-who-have-sex-with-men, the acute stage, three chronic stages (stratified by CD4 cell count >500 cells/µl, 350-500 cells/µl, and 200-350 cells/µl), and the AIDS stage. Each stage has a different duration and infectivity. Virally suppressed individuals cannot transmit HIV to others. The model was calibrated to the Dutch HIV epidemic and includes the number of newly diagnosed MSM from 2017 to 2020, cumulative diagnosed in 2020, late diagnosis (CD4<350 cells/µl), advanced diagnosis (CD4<200), linkage to ART, viral suppression, individuals living with HIV in 2020, and the number of PrEP users. The epidemiological impact was evaluated as the number of infections forecasted until 2030.

Results: In our base-case scenario with current PrEP users, a median of 115 (interquartile range- IQR- 92-155) new infections and 109 (IQR 91-134) new diagnosis is predicted among MSM by 2030 in the Netherlands. Compared to this base-case scenario, increasing the coverage of PrEP users by 50% on daily-oral TDF/FTC will lead to 76 (IQR 52-107) new infections and 88 (IQR 67 to 114) new diagnosis. Whereas, as compared to baseline scenario, increasing the coverage of PrEP users by 50% on LA-PrEP will lead to 72 (IQR 44-103) new infections and 80 (IQR 59 to 112) new diagnosis in men.

Conclusion: Increasing the coverage of PrEP users among MSM at risk in the Netherlands by 50% on long-acting cabotegravir or daily-oral TDF/FTC will be sufficient towards declining new HIV infections by 90% in 2030 and achieving UNAIDS targets. Further budget allocative efficiency estimates are needed to rationalise expanding the PrEP program using long-acting cabotegravir instead of TDF/FTC.

5

Binational Dimensions of Human Immunodeficiency Virus-1 A6 Variant Clustering Pattern in Ukrainian Migrants Diagnosed in Poland

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Background: In the countries of the former Soviet Union, including Ukraine, the dominant variant of HIV-1 is sub-subtype A6. In Poland, we observed an increased frequency of A6 variant transmissions, likely from the East parts of the European continent. The migration of Ukrainian citizens to Poland has been observed continuously since 2010. The outbreak of the armed conflict in 2022 in East Ukraine caused an enormous influx of war refugees to Poland. Advances in high-throughput molecular epidemiology allow indicating molecular transmission clusters from the vast number of sequences. We set out to identify Ukrainian migrants and refugees diagnosed in Poland before and after February 2022 in a molecular transmission network to guide public health activities.

Material and Methods: We used HIV-TRAnsmission Cluster Engine (HIV-TRACE) to infer putative genetic transmission networks. A sensitivity analysis was performed to determine the optimal genetic threshold. The dataset comprised 2304 unique HIV protease and reverse transcriptase gene (pol) sequences sampled between 1997 and 2023. Polish cohort of 1 405 individuals included 304 isolates from migrants collected before February 2022 (UaM) and 147 sequences obtained from war refugees after this date (UaR). Ukrainian sequences (n=856) were retrieved from publicly available databases. The distance-based molecular network was based on the TN93 model. We performed logistic regression analysis to compare clustered and non-clustered individuals.

Results: The calculated genetic distance threshold among the A6 variant was ≤1.1%. 1074 of 2304 (46.6%) sequences were linked with at least one other, assembling 165 transmission clusters of 2–436 sequences. We identified three large transmission clusters (>15 sequences), whereas 119 (72.1%) comprised only two individuals. Clustering rates for the Ukrainian migrants before February 2022 and Ukraine refugees after the onset of the armed conflict were 31.6% (distributed across 53 clusters) and 10.9% (within 10 clusters), respectively. Nineteen UaM sequences belonged to 1 of 3 large clusters containing sequences from rapidly growing two Polish and one Ukrainian regional epidemics. Sequences in clusters of post-war Ukrainian refugees were most linked (60.0%) to other refugee individuals. Of the sequences from Ukrainian migrants before February 2022, links were primarily observed to Poland sequences (75.4%).

Conclusion: Migrants from Ukraine fueled the HIV-1 A6 variant epidemic in Poland. The increase in A6 prevalence is observed among war-displaced people. The clustering of Ukrainian refugee sequences reflects the potential for war-associated movement of people living with HIV from related populations or regions. There is an urgent need to targeting of public health prevention and treatment services for war-displaced migrants from Ukraine.

6

Use of Genotypic HIV DNA Resistance Testing: French DELPHI-Type Consensus

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Background: In certain clinical situations, HIV DNA sequencing is performed to search for antiretroviral (ARV) resistance mutations. As many disparities in practices have been observed and in the absence of recent national guidelines, a DELPHI-type consensus was initiated in France with the aim of homogenizing situations in which this technique could be used and guiding interpretation of results.

Material and Methods: After a literature review and sharing of clinical experiences, a Steering Committee (SC) composed of eight virologists and one infectious disease specialist formulated assertions. These were submitted to an independent and anonymous electronic vote of virologists and clinicians in France, selected for their expertise in the clinical follow-up of people living with HIV, between October and December 2022. Votes used a scale from 1 "strongly disagree" to 9 "strongly agree". Strong or good consensus was achieved when >75% of scores were ≥7 and/or the median score was ≥8. Assertions that did not meet these criteria were modified by the SC and then put to a second round of voting.

Results: The SC initially formulated 21 assertions grouped into 6 categories: clinical situations for the use of HIV DNA genotyping, techniques for performing the test, consideration of APOBEC mutations, reporting of results ARV recycling, and test availability. Twenty-one virologists and 47 clinicians participated in each of the two voting rounds. In the first round, 14/21 assertions received good or strong consensus and 7/21 no consensus. The SC reformulated 5 assertions with good consensus and the 7 with no consensus, of which two were merged. In the 2nd round, 2/20 assertions remained without consensus. Finally, 18/20 (90%) assertions reached a strong consensus (after 2 rounds of voting), including that: prior genotyping on HIV DNA is useful for clinical decision-making in considering a switch to a “long-acting” regimen or to reduce the number of ARV drugs in virologically suppressed patients for whom the RNA data are unavailable/not exploitable/insufficiently informative; it is not essential when switching to 4 or 5 days-on maintenance regimen without changing ARV drugs; resistance mutations attributable to APOBEC, if detected, must be mentioned in the final genotyping report. The virologist voters did not unanimously validate to report any detected minority population to discuss it in a multidisciplinary consultation meeting. Similarly, the clinician voters were divided on the possible risk on an impaired virological response when using an InSTI + XTC regimen in patients with an undetectable viral load ≥1 year in the presence of a documented M184V mutation within the past 5 years.

Conclusion: This DELPHI-type consensus will allow to strengthen and harmonize good practice in performing HIV DNA genotyping in France, particularly regarding the reduction in the number of ARV drugs and the use of “long-acting” regimens.

7

Factors Associated with M184V Mutation Clearance in the HIV Reservoir Over Time

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Background: With life expectancy of people living with HIV, optimization of antiretroviral therapy (ART) is a key challenge considering the resistance and toxicities past histories. Resistance associated mutations (RAMs) are archived in the HIV reservoir, at least for years and can re-emerge with an inappropriate ART use limiting treatment options. However, recent studies, using ultra deep sequencing (UDS), showed a decrease of the amount of quasispecies harbouring RAMs, suggesting that recycling some antiretrovirals could be considered. Some clinical trials and academic studies suggest also that this could be feasible in some cases. The aim of this study was to characterize, in treated people living with HIV, the kinetics of the M184V mutation decrease in proviral DNA and to determine associated factors with M184V mutation clearance over time.

Material and Methods: We studied 22 treated people living with HIV, virally supressed (HIV RNA <50 copies/ml) for at least 5 years, with, in all cases, the M184V resistance mutation detectable in DNA using Sanger or UDS sequencing at day 0 (D0). UDS was performed on HIV DNA from frozen blood cells at different time points to quantify the percentage of M184V positive quasispecies. The sequence reads were analysed with a minimum coverage set at 50 and an ambiguity filter (threshold) at 5% or 2%. A Kaplan-Meyer survival model and multivariate analysis were realized.

Results: We analysed the UDS results of 22 treated people living with HIV (18 male and 4 females, with median age of 56 years [49-65] and CD4 cell count of 560/mm3 [465-807]). They presented, in their therapeutic history, a median CD4 Nadir of 164/mm3 [77-259] and a median HIV viral load (VL) Zenith of 4.99 log10 copies/ml [4.20-5.56]. At the first time-point (D0) all the patients presented a M184V detected in the HIV reservoir. Using an ambiguity filter at 2% or 5% we obtained a median survival of 2.5 years (the M184V was not detected for 50% of patients at 2.5 years) for the two thresholds. Univariate analysis highlighted that a higher CD4 Nadir and a lower VL Zenith were correlated with a faster clearance of the mutation. Moreover, multivariate analysis with the 5% threshold showed that a higher VL zenith was negatively associated with the M184V clearance. Interestingly, the 3TC/FTC presence in the ART line therapy during the five years was not associated with the persistence of the M184V.

Conclusion: Our study provides new information concerning the clearance speed of archived M184V mutation over time in patients with fully suppressed viremia, opening the discussion about duration needed to consider a 3TC/FTC recycling and reinforces the association of the CD4 Nadir and VL Zenith values with the M184V mutation clearance. Importantly, receiving a treatment with or without 3TC/FTC had no impact on the M184V mutation clearance in the reservoir in people living with HIV with a fully suppressed viremia.

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Comparison of Different HIV-1 Resistance Interpretation Tools for Next Generation Sequencing in Italy

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Background: HIV genotypic drug resistance testing through next generation sequencing (NGS-GRT) is gradually replacing Sanger sequencing. This work aimed at evaluating the concordance in resistance detection among different interpretation systems for NGS data obtained from real-life settings.

Material and Methods: Routine NGS-GRT data from viral RNA generated through the HIV-1 Solution v2 kit (Arrow Diagnostics, Illumina MiSeq/iSeq100 platforms) and Sentosa® SQ HIV-1 Genotyping Assay (Ion torrent platform) was collected from 13 Italian laboratories. The interpretation of NGS results was performed by three online tools (Paseq, Hydra, HIVdb Stanford) and the SmartVir standalone software. FastQs were considered reliable for NGS-GRT when the coverage was >100 reads (100X) for at least three tools at each PR/RT/IN resistance associated position listed in the HIVdb algorithm ver.9.4. Reliability rate was stratified according to viremia levels at genotyping (≤1000; 1,001-10,000; 10,001-100,000; 100,001-1,000,000; >1,000,000 copies/mL) and subtype. Mutations detected were classified as follows: unreliable (frequency <5% by all tools), minority variants (mV: frequency 5%-20% by all tools), majority variants (MV: frequency >20% by all tools), unclassified5% (U5%: frequency <5% by at least one tool), unclassified20% (U20%: frequency 5%-20% by at least one tool). The coefficient of variation (CV) of mutation's frequency was calculated to estimate variability among interpretation tools.

Results: 738 NGS-GRT were evaluated. Subtypes detected were: B (58.9%), CRF02_AG (14.2%), F (6.1%), A (5.6%), C (5.3%), and others (9.9%). Viremia levels (copies/mL) were as follows: ≤1,000: 7.0%; 1,001-10,000: 14.5%; 10,001-100,000: 32.1%; 100,001-1,000,000: 33.7%; >1,000,000: 12.6%.
Reliable 100X coverage was obtained for 471 NGS-GRT (63.8%). The proportion of reliable samples was affected by viremia levels <10,000 copies/mL (≤1,000: 44.2%; 1,001-10,000: 55.1%; 10,001-100,000: 70.0%; 100,001-1,000,000: 65.1%; >1,000,000: 65.6%, P=0.011) and by non-B subtypes (total non-B: 52.5% [CRF02_AG: 52.4%; F: 57.8%; A: 58.5%; C: 59.0%; others: 42.5%]; B: 71.7%, P<0.001).
In reliable samples, 7452/9640 (77.3%) mutations detected were excluded as unreliable minority variants. Among the 2188 mutations evaluated (PR:703; RT:835; INT:650), 77.8% (MV: 59.9%; mV: 17.9%) were concordantly detected by 4, 3 or 2 tools (87.2%, 8.1% and 4.7%, respectively). Median (IQR) CV related to these mutations was low (1.3% [0.6%-4.6%]). INT mutations showed the highest CV, followed by RT and PR mutations (CV median [IQR]: INT: 3.1% [1.0%-8.1%]; RT: 1.4 [0.6%-4.2%]; PR: 0.8% [0.4%-2.0%], P<0.001 by Kruskal-Wallis test). CV was slightly higher for viremia levels ≤1,000 copies/mL (2.0% [0.8%-5.2%]) compared to viremia >1,000 copies/mL (1.3% [0.6%-4.6%], p=0.024). Overall, 492 (22.5%) mutations were considered as unclassifiable, 428 as U5% (19.6%) and 64 as U20% (2.9%). Median (IQR) CV was high both for U5% (34.0% [18.3%-52.2%]) and U20% (46.2% [18.3%-62.2%]). For these unclassifiable mutations, the median of maximum frequency detected among interpretation tools was 6.7% (5.7%-9.7%); the third quartile of this distribution (9.7%) suggests that results of NGS are more likely to be discordant for mutations with frequency below 10%.

Conclusion: This first survey on NGS resistance testing suggests that the reliability of NGS-GRT is negatively affected by viremia <10,000 copies/mL and non-B subtypes. At frequency >10%, mutations were detected with acceptable concordance among different interpretation tools.

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Evaluation of HIV-1 DNA Resistance Burden Through NGS in Highly Treatment-Experienced Multi-Resistant Individuals Under Virological Control Enrolled in the PRESTIGIO Registry

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Background: This study aimed to clarify whether NGS might be useful for resistance assessment in virologically suppressed highly treatment-experienced (HTE) individuals with multidrug resistance (MDR).

Material and Methods: Ninety-one HTE MDR individuals from the PRESTIGIO registry were analysed. HIV-1 DNA PR/RT/IN and V3 sequences were obtained through NGS on MiSeq platform. Major resistance mutations (MRM) and APOBEC editing estimation (APOBEC mutations [APO-M]; stop codons) were evaluated through HIVdb algorithm. NGS cut-offs at ≥1%, ≥5% and ≥20% were tested. Minority MRM with frequency ranging 1-5% (mV1%) and 5-20% (mV5%) and majority MRM (frequency >20%, mV20%) were compared to historical-GRT. Variants distribution was compared between individuals who experienced virological rebound after NGS-GRT and those who maintained virological control.

Results: At NGS-GRT, individuals had a median (IQR) cART exposure of 23 (21-25) years, had been virologically suppressed since 3 (2-5) years and had a total HIV-DNA of 2,377 (1,274-4,949) copies/106 CD4+ cells. X4 tropism was detected in 61.5% of individuals.

A total of 1,772 MRM were detected (median [IQR] mutational load: 424 [104-1,384] copies/106 CD4+ cells), of whom 361 (20.4%) exclusively by historical-GRT, 875 (49.4%) by both NGS- and historical-GRT, and 536 (30.2%) exclusively by NGS. The detection rate of historical MRM by NGS was 70.8%, 67.2% and 59.3% at 1%, 5% and 20% NGS cut-off, respectively. Specifically, median (IQR) individual detection rates were 81.3% (54.8-93.5), 77.8% (50.0-92.3) and 62.5% (30.4-89.4) with NGS set at 1%, 5% and 20%, respectively.

NGS set at 1% showed poor reliability, although associated with the highest detection rate of historical MRM. In fact, mV1% (N=337) were frequently detected in samples with stop codons (94.4%) or APO-M (97.4%) providing potential misleading resistance assessment.

Differently, among mV5% (N=370), a substantial proportion of cases was not affected by APOBEC editing and contributed in expanding detection of historical MRM (25.9%) or detecting new MRM (18.6%).

Regarding majority variants, mV20% (N=704) were marginally detected in samples with stop codons (2.9%) or APO-M (5.3%), and mostly contributed to detect (69.4%) historical MRM or detect new MRM (25.4%).

Regardless of the NGS threshold, detection rates were not associated with viro-immunological parameters.

After NGS-GRT, 21 individuals underwent virological rebound in a median time of 23 (10-33) months with a median (IQR) viremia at rebound of 365 (98-7,840) copies/mL. Among them, the median (IQR) number of mV5% detected exclusively by NGS-GRT was higher (2 [1-3]) compared to those who maintained virological control (1 [0-2], p=0.030 by Mann-Whitney test). No significant differences in the number of mV1% and MV20% were observed. The number of mV5% newly detected by NGS in failing individuals positively correlated with plasma HIV-RNA levels detected at virological rebound (Spearman test, Rho=0.474, P=0.030).

Conclusion: In HTE MDR virologically suppressed individuals, NGS-GRT on HIV-1 DNA allows detection of around 60-70% historical MRM and detects considerable new resistance. Our results confirm that setting NGS at 5% might be a good choice to obtain reliable sequence data. At this setting, an increased number of minority species correlates with loss of virological control and with viremia levels at virological rebound.

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Insufficient Drug Levels With Cabotegravir/Rilpivirin Based Long Acting Therapy

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Background: The relatively new therapy form of injectable drugs (cabotegravir and rilpivirine) is enjoying increasing popularity. It is especially aimed at patients for whom regular daily administration is difficult or burdensome. To improve adherence to therapy in these cases this new treatment form is seen as particularly advantageous. We have investigated the plasma drug levels of patients on long acting therapy.

Material and Methods: At a dosage of 1 x 600 mg cabotegravir every 2 months, a trough level (Ctau) of approx. 1600 ng/ml should be reached. For the injection of rilpivirine every 2 months at a dosage of 900 mg, the Ctau target is 65.6 ng/ml. We measured the plasma levels of 33 adjusted patients by LC/MS/MS.

Results: Of 33 measurements, 24 were diagnosed with a value below the target trough level for rilpivirine; in addition, no rilpivirine was detectable in the plasma of two patients (detection level 25 ng/ml). Six patients had cabotegravir levels below the target level; all of them also had reduced RPV levels. In four patients with low drug levels, HIV-1 viral load could be detected in parallel; for one patient, resistance to both drugs was detected: Integrase L74I, S119P, G140AG, Q148R and Reverse Transcriptase E138K in an HIV-1 subtype A6. In another patient with low RPV levels and suppressed viral load, proviral analysis revealed an emerging Y181C mutation alongside the wild type, apparently an evolving resistance to RPV. The integrase was still wild-type in an A1 subtype.

Conclusion: We strongly recommend checking drug levels when administering long-acting therapies to avoid insufficient levels and thus functional monotherapies. One patient with proven resistance to components of the therapy had an A6 subtype with the L74I mutation in the integrase and should not have been treated with cabotegravir according to the recommendations. The second had no L74I integrase mutation in a subtype A1.

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Prevalence of Underlying Viral Factors Associated With Treatment Failure in the Long-Acting Cabotegravir-Rilpivirine Regimen

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Background: The combination of cabotegravir and rilpivirine has recently been approved for use as long-acting antiretroviral therapy (LA-ART). This regimen is the first two-drug ART administered intramuscularly every two months for the maintenance of virological suppression in people living with HIV. Three factors associated with the likelihood of virologic failure have been identified: rilpivirine resistance mutations (RPV), HIV-1 subtype A6/A1, BMI ≥ 30 kg/m2. The combination of ≥ 2 of the following baseline factors has been associated with an increased risk of virologic failure (19%). The aim of this study is to analyze the presence of virologic factors associated with virologic failure to cabotegravir+rilpivirine-LA in individuals who have entered the CoRIS cohort in the years 2019 to 2021.

Material and Methods: A total of 1859 individuals were analyzed. As a "proxy" for archived mutations to rilpivirine, the presence of IAS-USA RPV associated mutations in plasma (L100I, K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, M230I/L), tested at diagnosis were evaluated. Rapid subtyping data provided by the Standford Algorithm (v9.1) was used for subtype estimation and for a more in-depth study, we performed a maximum likelihood based phylogenetic tree of the subtypes, using the Neighbor Joining algorithm and the Jukes and Cantor Nucleotide substitution model with the CLC Genomics Workbench tool.

Results: Of the 1859 individuals, 121 (6.5%) had a IAS-USA RPV associated mutations, with E138A (4.09%) being the most prevalent, followed by K101E/P (0. 75%), E138G (0.48%), H221Y (0.43%), E138K (0.32%), Y188L (0.27%), L100I and Y181C/I/V (0.11%) and E138Q and M230I/L (0.05%). Regarding subtype distribution, 25 individuals (1.3%) were infected by subtypes A6/A1 (16 subtypes A6-0.86%- and 9 subtypes A1-0.48%): 36% Spanish, 24% from Eastern Europe, 20% from Latin America, 12% unknown and 8% other nationalities. In the period analyzed, 143 individuals (7.7%) had one factor related to virological failure, while only three individuals (0.16%) had both factors analysed.

Conclusion: A very limited number of newly diagnosed individuals in the CORIS cohort from 2019 to 2021 have an increased risk of virologic failure to cabotegravir+rilpivirine-LA based on virologic factors. The presence of baseline IAS-USA RPV associated mutations is the most prevalent virologic failure associated factor in the cohort and the period analyzed.

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Kinetics of the Three HBsAg Isoforms Along With HDV-RNA Predict Virological Response in CHD Patients Treated With Bulevirtide for 48 Weeks

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Background: HDV exploits the HBV surface protein (HBsAg) for the release of its progeny and entry into hepatocytes. HBsAg consists of three different proteins: Large HBsAg (L-HBs), including preS-1, preS-2 and S regions; Middle HBsAg (M-HBs) including pre-2 and S regions and small HBsAg (S-HBs), containing only the S region. Among them, L-HBs is predominantly present in virions and is crucial for the binding to the NTCP receptor and thus for viral entry into the hepatocytes. Here, we investigate the still unknown kinetics of HBs forms in patients receiving the entry inhibitor bulevirtide (BLV).

Material and Methods: Consecutive patients with HDV-related compensated cirrhosis starting BLV monotherapy 2 mg/day were enrolled in this single-center retrospective/longitudinal study. All patients were under effective NUC treatment at entry. L-HBs, M-HBs and S-HBs (Beacle Inc.) were quantified by ad hoc ELISAs assays in baseline and week 48 samples. HDV-RNA was quantified by Robogene 2.0 (LoD: 6 IU/mL). Virological response (VR) was defined as HDV-RNA<6 IU/mL or >2 Log decline compared to baseline.

Results: Twenty patients with compensated cirrhosis were enrolled: at baseline, median (IQR) age was 50 (40-62) years, 65% males, liver stiffness measurement (LSM) 17.6 (13.1-28.4) kPa, median (IQR) ALT was 110 (83-147) U/L, serum HDV-RNA was 4.9 (4.4-5.7) log IU/mL and HBsAg levels 3.7 (3.4-3.9) log IU/mL. Pre-treatment median (IQR) levels of S-HBs, M-HBs and L-HBs were 3421 (1240-6209) ng/mL, 791 (260-1930) ng/mL and 7 (2-15) ng/mL, respectively. Following 48 weeks of BLV, serum HDV-RNA declined by 3.1 (1.8-3.6) log IU/mL and ALT normalized in 14 (70%) patients. VR was observed in 14 (70%) patients while HDV-RNA undetectability in 7 (35%) with 5 of them achieving ALT normalization. In particular, HDV-RNA<5 log IU/mL at baseline was predictive of HDV-RNA undetectability at week 48 (endpoint achieved in 60% with versus 10% without HDV-RNA<5 log IU/mL, p=0.05).
During BLV treatment, S-HBs, M-HBs and L-HBs decreased in 60%, 70% and 45% of patients with a median (IQR) decline of 1095 [839-2403] ng/ml, 145 [39-350] ng/mL, 10 [4-15] ng/mL), respectively. A level of L-HBs<9 ng/mL at baseline significantly correlated with the achievement of HDV-RNA undetectability (endpoint achieved in 54.5% with versus 11.1% without L-HBs<9 ng/ml, p=0.042). Superimposable result was observed in relation to the achievement of HDV-RNA undetectability plus ALT normalization (endpoint achieved in 50% with versus 0% without L-HBs<9 ng/mL, p=0.03). Even more, the combination of L-HBs<9 ng/mL+HDV-RNA<5 log IU/mL was the best predictor for achieving HDV-RNA undetectability plus ALT normalization (endpoint achieved in 66.7% with versus 7.1% without this combination, p=0.01).

Conclusion: Quantification of L-HBs along with serum HDV-RNA may reflect the burden of circulating infectious virions, possibly providing a new tool to identify patients more likely to respond to BLV monotherapy.

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Intrahepatic HDV Replication Is Sustained by an Abundant Production of HBsAg Derived From Integrated HBV-DNA, and Is Not Strictly Dependent From the Extent of HBV Reservoir

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Background: HDV exploits HBV surface glycoproteins (HBsAg) for viral morphogenesis and de novo entry into hepatocytes. The interplay between the two viruses is poorly understood and has been mainly evaluated in peripheral blood. Here, we investigate HBV and HDV replicative activity and their interplay by analysing a well-defined set of liver biopsies from patients with chronic HBV/HDV co-infection.

Material and Methods: Liver tissue was analysed from 25 patients (71% NUC-treated; 96% HBeAg negative, all infected with HDV genotype 1 and 84% with HBV genotype D). Intrahepatic levels of covalently closed circular DNA (cccDNA), pregenomic HBV-RNA (pgRNA) and HDV-RNA were quantified by highly-sensitive droplet digital PCR (ddPCR). ddPCR assays were also set up to quantify total HBs transcripts and to distinguish HBs transcripts deriving from cccDNA and from integrated HBV-DNA according to Grudda, 2022.

Results: Patients were characterized by high serum levels of HDV-RNA and HBsAg (median[IQR]: 6.3[3.8-7.7] logIU/mL and 14,460[5,207-21,118] IU/mL, respectively) and low HBV viremia (serum HBV-DNA detectable in only 48% of pts with a median[IQR] of 50[34-214] IU/ml). Median(IQR) ALT was 72(52-102) U/L and half of patients had a fibrosis score >F5.
Intrahepatic HDV-RNA was median(IQR) 787(1-2913) copies/1000cells and positively correlated with serum HDV-RNA (Rho=0.63, P=0.05). Regarding HBV intrahepatic reservoir, median(IQR) cccDNA was 3(0.1-24) copies/1000cells and pgRNA was 8(1-147) copies/1000cells. Despite a limited HBV reservoir, we observed an abundant production of total HBs transcripts (median[IQR] total HBs RNAs: 6,028[409-19,137] copies/1000 cells), positively correlated with serum HBsAg (Rho=0.54; P=0.04). Notably, by analyzing the source of HBs trancripts, we found that >90% of HBs transcripts derived from integrated HBV-DNA, with a limited contribution of cccDNA transcriptional activity, highlighting that HBsAg production is mainly derived from integrated HBV-DNA in HBV/HDV chronic co-infection. Finally, no difference in the levels of intrahepatic HDV-RNA was observed according to the size of HBV reservoir (median[IQR]: 787[1-5,495] and 880[1-3,338] copies/1000cells in patients with cccDNA<5 and >5 copies/1000cells, p=0.9). Differently, markers of HBV activity were significantly lower in patients with a more restricted HBV reservoir (median[IQR]: 1[1-10] vs 147[9-406] copies/1000cells for pgRNA and 0.3[0.2-1] vs 73[7-243] copies/1000cells for cccDNA-derived HBs transcripts in patients with cccDNA<5 vs >5 copies/1000cells, p<0.01 for both). Overall, these data suggest the existence of independent pathways underlying the replication of the two viruses.

Conclusion: Pathways sustaining HDV replication act independently from the extent of intrahepatic HBV reservoir and are fueled by an abundant production of HBs transcripts, mainly derived from integrated HBV-DNA. These issues are crucial for deciphering mechanisms underlying HDV persistence, that could jeopardise the success of anti-HDV therapeutic strategies.

Poster Presentations

European Meeting on HIV & Hepatitis 2023

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Resistance Analysis of Long-Acting Lenacapavir in Treatment-Naïve People with HIV at 80 Weeks

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Background: Lenacapavir (LEN) is a first-in-class HIV-1 capsid inhibitor approved for the treatment of HIV-1 infection in adults with multidrug resistance in combination with other antiretrovirals. CALIBRATE (NCT04143594) is an ongoing phase 2 open-label study evaluating subcutaneous (SC) and oral LEN-containing regimens in people with HIV-1 initiating antiretroviral therapy. At week 80, high rates of virologic suppression were maintained in people treated with SC LEN every 6 months in combination with tenofovir alafenamide (TAF) or bictegravir (BIC; B), or oral LEN daily (QD) in combination with emtricitabine (F)/TAF (87%, 76%, 87%, respectively), and treatment was generally well tolerated. Here, interim resistance analyses through week 80 are described.

Material and Methods: Participants were randomized (2:2:2:1) to 1 of 4 treatment groups (TG). TG1 and TG2 received SC LEN + oral QD F/TAF for 28 weeks, after which virologically suppressed participants continued a 2-drug regimen: SC LEN with QD TAF (TG1, n=52) or SC LEN with QD BIC (TG2, n=53). TG3 (n=52) received oral QD LEN + F/TAF, and TG4 (n=25) received oral QD B/F/TAF throughout. Genotypic and phenotypic analyses of HIV-1 capsid, protease, reverse transcriptase, and integrase were performed at confirmed virologic failure (VF: confirmed virologic rebound ≥50 copies/mL or <1 log10 decline from baseline at Week 10) visits.

Results: Seven of 182 participants met VF criteria for resistance analysis through week 80, including 4 participants with no emerging resistance in capsid, reverse transcriptase (RT), or integrase who resuppressed (HIV-1 RNA <50 copies/mL) while continuing treatment and were excluded from the resistance analysis population. The 3 remaining participants developed LEN-associated resistance mutations in capsid: 1 participant in TG1 developed Q67H+K70R in capsid (LEN fold change [FC]=57) at week 80 while on LEN + TAF, with no NRTI resistance; 1 participant in TG2 developed Q67H+K70R in capsid (LEN FC=20) and M184M/I in RT (conferring resistance to FTC) by week 10 while on LEN + F/TAF, with retrospective deep sequencing analyses indicating that M184 mutations emerged first; 1 participant in TG3 with documented non-adherence (based on pill count and plasma drug levels) developed Q67H in capsid (LEN FC=7) at week 54 and K70R in capsid (LEN FC=23) at week 80 while on LEN + F/TAF, with no emergent resistance to F/TAF components.

Conclusion: In this study, in which LEN was part of an asynchronous or multi-tablet regimen, emergent resistance mutations to LEN were infrequent through 80 weeks of treatment (2%, 3/157 participants). These findings support the ongoing evaluation of LEN in combination with synchronously-dosed, long-acting partner agents for the treatment of HIV-1 infection.

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HIV Drug Resistance Among Patients With Low Level Viremia: An Appeal for Revision of the Viral Suppression Threshold in Resource Limited Settings

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Background: Viral suppression (Viral load <1000 copies/ml) is considered as a threshold for therapeutic success and for ineligibility to HIV drug resistance (HIVDR) testing in resource-limited settings (RLS). However, drug resistance mutations (DRMs) have been seen at low-level viremia, with the likelihood to jeopardise long-term therapeutic response. In the frame of contextual gap in RLS, we herein sought to ascertain the sequencing success rate.

Material and Methods: A cross-sectional and analytical study was conducted among VSP at the Chantal BIYA International Reference Centre from January 2020 to August 2021. HIV-1 sequencing was performed in the reverse-transcriptase and protease regions of Pol gene. Sequences were analysed using the Stanford HIVDBv9.0 algorithm, and molecular phylogeny done using MEGA 11. Sequencing success rate and presence of DRMs were assessed according to viral load ranges; with p<0.05 considered statistically significant.

Results: Of the 132 participants enrolled (median age [IQR] 43[33-51], with a female to male ratio of 2.2. Median duration on antiretroviral therapy (ART) 19 [12-117] months, median viral load: 192[57-419] copies/ml, median CD4 count of 272 [145-528]). Overall sequencing success rate of 28.9% (38/132). Interestingly, sequencing was more successful with VL ≥ 200 copies/ml [47.2% (25/53)] versus VL< 200 copies/ml ([16.5% (13/79)]), p<0.001. Out of the 38 sequences successfully obtained, overall HIVDR rate was 92% (35/38) with 79.9% NRTI-resistance, 79.4% NNRTI-resistance and 15.3% PI/r-resistance. HIVDR was higher with VL ≥ 200 copies/ml (32.0%) versus VL ≤ 200 copies/ml (7.7%), OR 4.5; p=0.013. Seven viral clades were identified with a prevailing CRF02_AG (64%). M184V (74.3%) and K103N (45.7%) were the most frequent DRMs in reverse transcriptase region and M46I (14.2%) in the protease. With respect to viral susceptibility, 41.1 %(14/38) were on suboptimal ART in spite of VS.

Conclusion: In this RLS, sequencing appears to be efficient in VSP, specifically with PVL≥200 copies/ml. Of clinical relevance, nine of out ten VSP may harbour HIVDR with reduced viral susceptibility to antiretrovirals. Moreover, about 4 out of 10 VSP may be receiving a suboptimal regimen, henceforth underscoring the clinical benefit of HIVDR testing even in Sub Saharan Africa (SSA) context.

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Impact of PDR and ADR on Doravirine and Doravirine FDC in Portugal

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Background: Doravirine (DOR) has a very different resistance profile when compared with the former NNRTIs. Weight gain related to integrase inhibitors and TAF has been a matter of concern. Consequently, alternatives are needed when this subject is on the table. DOR regimens can be one choice to overcome this problem. Historically NNRTIs mutations are always the reason to not have this class present as an option.
Objective: To evaluate if DOR can be used in nowadays Portuguese population, as first-line option or subsequent choice after a virological failure. Pretreatment drug resistance (PDR) and acquire drug resistance were evaluated (ADR) in the recent last year.

Material and Methods: Between February 2022 and February 2023, 839 antiretroviral resistance tests (RT) were performed in the Molecular Biology Laboratory at the Centro Hospitalar Lisboa Ocidental in Portugal. 622 RT belonged to people who are treatment naïve infected with HIV and 217 to people under antiretroviral therapy with detectable viral load. RT were done through NGS (Vela Diagnostics NGS Assay) and quasispecies populations present in >5% were considered. Mutations that impact DOR and possible backbones were evaluated in both groups.

Results: In the 622 naive RT tests, most prevalent mutation on the NNRTIs was K103N (2,89%) that has no impact on DOR resistance. Mutations with impact on DOR V106AM, (0,16%), G190E (0,48%), Y188L (048%), Y318F (0,32%), L234I (0,16%) and F227L (0,16%) were found in very low percentages. Mutations that may impact the backbone were also present in very low percentages, M184V (0,64%) and K65R (0,32%). In the 217 treated individuals the most prevalent found mutation was M184V (18,89%) followed by K103N (12,90%). With impact

on DOR we found V106M (0,46%), Y188L (3,23%), G190E (0,92%) and Y318F (1,38%). K65R was found in 2,76% of the individuals.

Conclusion: Recent and present data show that resistance mutations with impact on DOR, both in people who are treatment naïve (PDR) and treated individuals (ADR) are present in really low numbers not compromising the utilization of DOR in first-line regimen or in switch one ever needed for simplification or to achieve viral suppression. Care should be taken when there are historically NNRTI accumulation of mutations.

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Pre-treatment HIV Drug Resistance and Genetic Diversity in Cameroon: Implications on Dolutegravir-Based Regimens

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Background: The efficacy of first-line antiretroviral therapy (ART) may be hampered by the presence of HIV drug-resistance (HIVDR) and viral clades. We sought to estimate HIV-1 pre-treatment drug resistance (PDR) patterns, its geographical dispersal, the effect of viral clades on PDR and their programmatic implications on dolutegravir-based regimens in Cameroon.

Material and Methods: A sentinel surveillance of PDR was conducted in eight regions of Cameroon from 2014 to 2019. Sequencing of HIV-1 protease and reverse transcriptase was performed, drug resistance mutations (DRMs) were interpreted using Stanford HIVdb.v.8.7, molecular phylogeny performed using MEGA 11 and statistical analyses using EPI-Info v7.2.2.6, with $p<0.05$ considered statistically significant.

Results: A total of 379 sequences were obtained from individual participants (62% female and average age 36 ± 10 years). Overall rate of PDR was as high as 15.0% [95% CI: 11.8-19.0] nationwide, with a significant disparity between the eight regions ($p = 0.03$). The ARV drug class with the highest-level of PDR was NNRTI, 12.4% [95% CI: 9.5-16.1], of which 7.9% [95% CI: 5.6-11.1] had DRMs to EFV/NVP. Two of the eight regions had EFV/NVP PDR above the critical threshold of 10%, namely the Far-north (15%) and East (10.9%). The rate of PDR was 3.2% [95% CI: 1.9-5.4] for NRTI as opposed to 1.3% [95% CI: 0.6; 3.1] for PI/r. The most prevalent mutation was K103N (5.5%). Overall, we identified a total of 18 viral strains, the most predominant being the CRF02_AG subtype (65.4%). Viral genetic diversity did not influence occurrence of PDR. Regarding ART predictive efficacy, TDF-3TC-DTG was superior (98.4%) compared to TDF-3TC-EFV (92%), $p <0.0001$.

Conclusion: The overall high rate of PDR in Cameroon underscores the poor efficacy of EFV/NVP-based first-line ART nationwide, with major implications in two regions of the country. This supports the need for a rapid transition to NNRTI-sparing regimens, with TDF-3TC-DTG having an optimal efficacy at programmatic level. Despite the wide HIV-1 diversity in Cameroon, the effect of circulating viral clades might not substantially affect the patterns of PDR nor the potential efficacy of first-line dolutegravir-based regimens in resource-limited countries with similar programmatic features like Cameroon.

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Transmitted Drug Resistance to Integrase Inhibitors in Recent HIV Diagnosis in Portugal

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Background: INSTIs are currently recommended for first-line treatment. However, although there has been an increased use of this drug class, a very low prevalence of TDR to INSTIs has been documented across Europe and the USA, until now. However, real time surveillance is mandatory.
Objective: To evaluate transmitted drug resistance (TDR) to integrase inhibitors during the last year in the Portuguese population, as well as TDR affecting the usual drugs used as backbones, namely emtricitabine (FTC), lamivudine (3TC), tenofovir DF (TDF) and tenofovir alafenamide (TAF).

Material and Methods: Between February 2022 and February 2023, 622 integrase resistance tests (RT) from people just diagnosed with HIV were performed at the Molecular Biology Laboratory at the Centro Hospitalar Lisboa Ocidental in Portugal. RT were done through NGS (Vela Diagnostics NGS Assay) and quasispecies populations present in >5% were considered.

Results: In the 622 naive RT tests, most prevalent major mutation to INTIs were N155H and R263K both representing 0,16%. Polymorphisms E157Q and T97A were found in 0.96% and 0,8% respectively. M184V was found in 0,64% and K65R in 0,32% of the population.

Conclusion: Recent and present data show that transmitted drug resistance mutations with impact on integrase inhibitors and on the usual drugs used as backbone are present in really low numbers, below 1%, in Portugal, not impacting the use of all available options with integrase inhibitors as first line choice. Although TDR to INTIs, as well as M184V and K65R mutations in TDR are still below 1% in Portugal, surveillance must continue to be carried out.

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HIV-1 Resistance Analysis of Participants With HIV-1 and Hepatitis B Coinfection Initiating Therapy with Bictegravir/Emtricitabine/Tenofovir Alafenamide or Dolutegravir + Emtricitabine/Tenofovir Disoproxil Fumarate

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Background: Study GS-US-380-4458 (ALLIANCE; NCT03547908) previously demonstrated the noninferior efficacy of bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) versus dolutegravir + emtricitabine/tenofovir disoproxil fumarate (DTG+F/TDF) in achieving viral suppression (HIV-1 RNA < 50 copies [c]/mL) in adults with HIV-1/HBV coinfection who were naïve to treatment for both viruses at Week 48. Here, the effect of baseline resistance on treatment response and complete details of the resistance analysis are described.

Material and Methods: Population sequencing of HIV-1 protease (PR) and reverse transcriptase (RT) was performed at screening. Historical genotypes for PR, RT and integrase (IN) were also collected, if available. Participants with virologic failure (defined as a rebound in HIV-1 RNA ≥ 50 c/mL that was subsequently confirmed and ≥ 200 c/mL at the next visit; or as HIV-1 RNA ≥ 200 c/mL at last visit or at Week 48) underwent genotypic and phenotypic resistance analyses for PR, RT and IN.

Results: Preexisting transmitted primary drug resistance substitutions were present in this population: 1.7% (4/241) with nucleotide reverse transcriptase inhibitor (NRTI)-resistance (R), including M184I in 1 participant in the B/F/TAF

group, the thymidine analog mutations M41L in 2 participants (1 in each treatment group) and K219N in 1 participant in the DTG+F/TDF group, 7.9% (19/241) with NNRTI-R, and 2.1% (5/241) with protease inhibitor (PI)-R. No integrase strand-transfer inhibitor (INSTI)-R was documented. High levels of virologic success were observed at Week 48 in both treatment groups, including for participants with preexisting resistance. The participant in the B/F/TAF group with M184I at baseline achieved HIV-1 RNA < 50 c/mL by Week 4 and remained suppressed through Week 48. No participant in the B/F/TAF group had HIV-1 treatment-emergent drug resistance. One participant in the DTG+F/TDF group with documented nonadherence by pill count experienced multiple episodes of virologic failure and developed the K70E mutation in RT at Week 24 (conferring low-level resistance to TDF) and M184V/I in RT at Week 36 (conferring resistance to emtricitabine [FTC]), and subsequently resuppressed HIV-1 RNA to < 50 c/mL.

Conclusion: Treatment with B/F/TAF or DTG+F/TDF achieved high rates of HIV-1 virologic suppression at Week 48 in adults with HIV-1/HBV coinfection who were previously treatment naïve. The presence of preexisting resistance mutations did not affect treatment outcomes. Development of primary drug resistance mutations to study drugs occurred in 1 participant with documented nonadherence in the DTG+F/TDF group.

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Laboratory Based Surveillance of HIV-1 Acquired Drug Resistance in Cameroon: Implications for Use of Tenofo-Vir-Lamivudine-Dolutegravir (TLD) as Second- or Third-Line Regimens

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Background: Increased HIV drug resistance (HIVDR) with antiretroviral therapy (ART) rollout may jeopardize therapeutic options, especially in this era of transition to fixed-dose tenofo-vir-lamivudine-dolutegravir (TLD). We studied acquired HIVDR (ADR) patterns and described potentially active drugs after first- and second-line failure in resource-limited settings (RLS) like Cameroon.

Material and Methods: We carried out a laboratory-based study among 759 patients (≥15 years) experiencing virological failure, was carried out at the Chantal Biya International Reference Centre (CIRCB), Yaoundé, Cameroon. Socio-demographic, therapeutic and immunovirological data from patient records were analysed according to HIV-1 genotypic profiles.

Results: The mean age ± SD was 42±14 years, with Majority of participants being female; 485/745 (63.9%; 95% CI: 60.4%-67.2%). Most patients (76%) were failing NNRTI-based first-line, with 24% failing PI/r based second-line regimens. The Median [IQR]

ART-duration was 63[50-308] months. Median CD4 and viremia were 153[IQR:50-308] cells/mm3 and 138,666[IQR:28,979-533,066] copies/mL respectively. Overall ADR was high (93.4% first-line; 92.9%-second-line). TDF, potentially active in 35.7% of participants after first-line and 45.1% after second-line suggested suboptimal TLD-efficacy in second-line (64.3%) and third-line (54.9%). All PI/r preserved high efficacy after first-line failure while only DRV/r preserved high-level efficacy (87.9%) after second-line failure. In this resource limited setting (RLS), ADR is high in ART-failing patients. PI/r remain potent back-bones for second-line ART, while only DRV/r remains very potent despite second-line failure. Though TLD use would be preferable, blind use for second- and third-line regimens may be sub-optimal (functional monotherapy with dolutegravir) with high risk of further failure, thus suggesting strategies for selective ART switch to TLD in failing patients in RLS.

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Prevalence, Clinical and Virological Characteristics and Short Term Prognosis of Hepatitis Delta Infection Among People Living With HIV and HBV in Nouakchott, Mauritania

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Background: Due to HDV common transmission routes, people living with HIV infection are at risk of acquiring HBV and HDV. However, datas remain scarce despite high prevalence of HBV and HDV in Mauritania. The present study aimed to determine the prevalence and characteristics of HDV-HBV co-infections among Mauritanian people living with HIV, in comparison with those living only with HBV co-infection; and to estimate and compare the severity and outcome of the liver disease.

Material and Methods: 292 consecutive people living with HIV showing a positive HBsAg rapid test confirmed by ELISA assay, visiting the CTA in Nouakchott were included between January 2018 and August 2019. Clinical and biological characteristics were recorded at inclusion and during the follow-up. Anti-HDV antibodies, HBV and HDV viral loads; and HBV and HDV genotypes were determined. Liver disease evaluation by blood tests (APR and FIB-4) and FibroScan® were also recorded.

Results: People included in the cohort were male at 54.8% with a mean age of 38 ± 8.5 years. At inclusion, among the 215 people under ART, tenofovir or 3TC were in the ART combination for 102 and 113 persons respectively. HBV strains clustered in genotype D (42.5%), E (38%), A (15.5%) and G (0.5%). The HBV viral load was detectable for 108 persons receiving 3TC and 25 receiving TDF, and mutations associated to HBV resistance were found in 20 viral strains. The HDV seroprevalence was 37%, and HDV RNA was positive in 40.7% of them. HDV genotype 1 was found in 93% and genotype 3 in 7%. At inclusion, no significant difference was found between people for all variables studied, including FIB-4 and APRI, regarding anti-HDV antibodies status or HBV DNA detectable or not, excepting a higher CD4T cell level and a lower HIV viral load in people with negative HBV DNA (p=0.001 and p=0.000). The mean duration was 24.55 ± 8.01 months in the 217 people having a follow-up. Between inclusion and the last evaluation, a highly significant worsening of APRI and FIB-4 scores was found whatever the HDV antibodies status was; interestingly, people with HDV infection had a more severe liver disease progression than those without HDV infection, using APRI and FIB-4 scores. The unique FibroScan® evaluation, performed at the end of the study, did not showed significantly difference regarding HDV Ab status, unless values were slightly higher in people living with a triply infection.

Conclusion: Through a substantial Mauritanian cohort of people living with HIV well characterized and benefiting of a two years follow-up, we found a high HDV seroprelavence and demonstrated the association with a worsening evolution of the liver disease. Our work claimed for developing validated tool and treatment access to manage HDV infection. We also underlined a need for ART with tenofovir instead of 3TC to avoid associated resistance mutation and well controlled HBV replication, and, as a consequence, a systematic

and regular screening of HBV and HDV infections and then an adapted management and treatment in people living with HIV.

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Seroprevalence of Transfusion Transmissible Infections Among SARS-CoV-2 Exposure Blood Donors in Luanda, Angola

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Background: Currently, the risk of transfusion-transmitted infections (TTIs) is lower, although the supply of safe blood products remains subject to contamination with human pathogens, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and Syphilis. Herein, we investigate the seroprevalence of TTIs (HIV, HBV, HCV, and Syphilis) among SARS-CoV-2 exposure blood donors in Luanda, Angola.

Material and Methods: This was a cross-sectional study conducted with 119 rejected blood donors in Luanda, the capital city of Angola, from March to July 2022. Parametric tests were used to compare means while Chi-square to check determinants related to TTIs among SARS-CoV-2 exposure donors and were deemed significant when $p<0.05$.

Results: Overall, 91.6% (109/119) of the studied population tested positive for SARS-CoV-2 antibodies, of which, 91.6% and 6.70% were IgG and IgM-positive, respectively. About 84.9% of the donors had a past SARS-CoV-2 infection and 6.70% had a recent SARS-CoV-2 infection. HIV (7.30%), HBV (69.7%), HCV (12.8%), and Syphilis (11.9%) infections were detected among these SARS-CoV-2 exposure blood donors. A statistically significant relationship was observed between HIV/SARS-CoV-2 coinfection ($p=0.018$). In addition, age was related to Syphilis/SARS-CoV-2 co-infection ($p=0.007$). Overall, the sociodemographic profile of donors co-infected with any TTIs/SARS-CoV-2 were adults over 20 years of age, male, a balance between residents in urbanized and non-urbanized areas, with a low level of education, employed and unmarried, although no statistical significance was observed ($p>0.05$).

Conclusion: Our results showed that emerging viral infections transmitted through transfusion are an area of increasing concern showing that the risk of occurrence of TTIs must be considerable in Angola.

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CMV/EBV Co-infection in Treatment-Naive HIV-1 Patients, Associated Factors, and Early Immuno-Virological Response

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Background: Viral coinfections are thought to impact the outcome during HIV infection. Besides classically screened agents such as HCV and HBV, the role of herpesviruses is increasingly investigated. For example, CMV replication, even subclinical, was associated with incomplete recovery of CD4+ T cells, higher CD8 T cells, lower CD4/CD8 ratio and increased systemic inflammation in treated HIV patients. HIV patients with positive CMV serology showed an increased risk of suboptimal immune recovery. The activation driven by herpesvirus replication was also shown to be associated with an expansion of HIV reservoir.
We investigated in HIV treatment-naive patients, the factors associated with CMV and EBV antibody (Ab) levels and replication, and sought for their impact on early on-treatment immuno-virological response.

Material and Methods: This monocentric study included patients living with HIV, who underwent HIV resistance testing and HIV DNA quantification before antiretroviral treatment (ART) initiation, with available samples for CMV/EBV investigation. Patients were retrospectively selected if they received an optimized ART without any treatment discontinuation, or regimen change during the first three months post initiation.
Total HIV-1 DNA quantification was performed using a real-time PCR assay (Biocentric). Levels of IgG Ab to CMV and EBV were determined with the LIAISON® assays (Diasorin). CMV and EBV DNA were detected and quantified using the AltoStar® PCR assays (Altona Diagnostics). Linear or logistic regression was performed when appropriate, to identify associated factors.

Results: A total of 93 patients were included. Briefly, patients were mainly young (median age of 32.5 years), male (76.3%), and infected with a subtype B (59.1%) virus. The median baseline plasma viral load was 4.68 Log copies/mL. Median CD4 count and CD4/CD8 ratio were 368 cells/mm3, and 0.46 respectively.
The median HIV-1 DNA level in whole blood was 3.14 Log copies/106cells. Anti-CMV IgG Ab were detected in 89.25% of patients while almost all subjects (98.92%) had already been exposed to EBV (anti-VCA and/or anti-EBNA Ab). CMV and EBV DNA were investigated in 70 patients, and were detected in 11.4% and 61.4% of subjects, respectively. Anti-CMV IgG levels were negatively associated with CD4/CD8 ratio (p = 0.03). Regarding EBV, anti-VCA IgG levels were only associated with age (p= 0.007), and EBV DNA detection was associated with higher HIV-1 DNA levels (p= 0.02).
We investigated factors associated with immuno-virological response at 3 months post ART initiation in 79 patients, of whom 46 (58.2%) reached an undetectable plasma VL. Only the use of INSTI was independently associated with undetectable plasma VL at 3 months. Regarding the CD4 count, a median increase of 136 cells/mm3 was observed at 3 months post ART initiation. Only the baseline CD4/CD8 ratio was positively associated with CD4 increase.

Conclusion: CMV IgG levels were negatively correlated with CD4/CD8 ratio, a marker of immune activation, while EBV replication was associated with increased HIV-1 DNA levels. No association was found between CMV/EBV markers and early immuno-virological response.

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Sociodemographic, Clinical, and Behavioral Factors Associated With STIs Among HIV-1 Positive Migrants in Portugal: Are There Differences Between Sex?

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Background: Sexually transmitted infections (STIs) continue to occur at high levels. According to the WHO, each year there are an estimated 374 million new infections with 1 of 4 curable STIs. STIs are associated with an increased risk of acquiring HIV infection. Migrants and specifically women are especially vulnerable groups.

Background: This study aims to characterize a population of HIV-positive migrants living in Portugal and determine factors associated with STIs.
Methodology: This is a retrospective cross-sectional study of 265 newly diagnosed HIV-1-positive migrants. Patients were part of the BEST HOPE study that was developed in 17 hospitals located in Portugal between September 2014 and December 2019, that included information collected through socio-demographic and behavioral questionnaires filled in by the patients, clinical questionnaires filled in by the clinicians and HIV-1 genomic sequences generated through resistance testing (Sanger sequencing). Univariate and multivariate statistical analysis to determine the association between sociodemographic characteristics, sexual behaviors, HIV testing and sexual infections were performed using SPSS v25.

Results: Most HIV-1 positive individuals included in the study were males (66.8%) and aged between 25-44 years old (57.4%). Men had a higher proportion of STIs when compared to women (38.3% vs 14.9%) as well as more homosexual contacts (52.8%). In the last year, most men had two or more casual sexual partners (85.7%). However, men showed a higher percentage of condom use with casual partners (40.9% vs 13.6% in women, p=0.001). Other risk behaviors for acquiring HIV, such as tattooing and performing invasive medical procedures, were more prevalent in men (38.0% and 46.2%, respectively), when compared to women (30.4% and 45.1% respectively) and 4.7% of men reported having already shared injectable materials, with no data for comparison in the case for women. Additionally, 23.9% of women reported having had a blood transfusion while only 10.3% of men reported having had this medical procedure. 27.7% of the individuals reported having been diagnosed with some type of STI in the last 12 months. The most prevalent STIs that were reported were syphilis and genital warts (41.0% and 17.9% respectively). Hepatitis B was reported in 43.3% of individuals who filled in that question, while hepatitis C infection was reported by 13.0% of individuals. According to the multivariate adjusted model, only age group was significantly associated with reports of previous STI infection: the 25-44 age group was 72.4% more likely to have been diagnosed with an STI in the past 12 months compared to those aged 45-63.

Conclusion: HIV-1 infected men were more likely to report previous STIs than women. However, women are less likely to have safe sexual practices. Gender inequalities and between some migrant communities may make women unable to negotiate safe sexual practices, resulting in increased susceptibility to infection. For this reason, the implementation of safer sex awareness campaigns for condom use and awareness of STI knowledge would need to be targeted to both women and their partners.

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Comparison of Four Predictive Scores for Cardiovascular Risk in Mexican People Living With HIV

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Background: The risk of a cardiovascular event in people living with HIV (PLWH) is increased due to constant immune activation, comorbidities and probably to the use of some antiretrovirals. Despite that, none of the cardiovascular risk (CVR) scores is directed specifically to PLWH or has been recently updated. Our objective was to evaluate the concordance of the following predictive CVR scores: 10 year for Framingham for cardiovascular disease (FRAM-CHD), Framingham for coronary disease (FRAM-HCHD), Atherosclerotic Cardiovascular Disease (ASCVD), and 5 year Data collection on adverse events of Anti-HIV Drugs (DAD) in Mexican PLWH, and the relation of these scores with clinical and demographic data.

Material and Methods: Descriptive cross-sectional analysis was made in 200 PLWH, Cohen's Kappa concordance test was used to analyze the agreement between the scores. Logistic regression models were performed to assess the association with other HIV-related clinical variables such as virological and immunological parameters, treatment type and lipids.

Results: A total of 200 participants with a mean age of 42 years, from an urban treatment center in the state of Mexico were included in 2017-2018, most on ARV treatment (83%) and 79.5% with undetectable viral load. We found a low frequency of moderate-high risk scores with higher values for FRAM-HCHD, and with a very low-concordance between all the scores. In the logistic regression models, an association was found between all or some CVR scores with baseline VL and CD4 cell counts, and elevated triglyceride levels (TG), but not with anthropometric measures such as body mass index and abdominal circumference. Treatment with integrase inhibitors (INSTIs) was associated with all predictive scores; notably with ASCVD (OR = 7.03, 95% CI 1.67 - 29.64).

Conclusion: CVR scores in PLWH have poor concordance, highlighting the importance of developing a specific score that includes comorbidities and ARV drugs. In spite of the young age of the studied population, we discovered significant correlations of moderate-high CVR with use of INSTIs, mostly first-generation, baseline viral load and CD4 cell counts, and triglycerides, factors not considered in most of the scores. Regardless of the real value of the scores, screening of CVR in PLWH is strongly recommended.

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Deaths Among Patients Living With HIV – A 10-Year Retrospective Analysis of Patients Hospitalised in the Infectious Diseases Department in the Lower Silesia Region of Poland

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People living with HIV (PLWH), especially those diagnosed too late or not treated with antiretroviral drugs for various reasons in the advanced stage of AIDS, develop additional opportunistic infections or AIDS-defining illnesses that may directly contribute to the death of these patients.

The study group (113 patients) consisted of patients with confirmed HIV infection and hospitalised in the Infectious Diseases Department of the Provincial Specialist Hospital in Wrocław, Poland, who died during hospitalisation in 2009-2018(the approximate number of PLWH hospitalisations in that period was 1,300).
Men predominated in the study group (82%). The average life expectancy was 40.4years and the median 39 years. The largest subgroup of patients was in the 30-39 age range. Infection through intravenous drug use (IVDU) was found to be the most common and likely route of HIV infection, with as many as 88(78%) patients being infected. The mean CD4 T-cell count from the last assay before death was 131 cells/mm3; median 59 cells/mm3. The group of late presenters comprised a total of 41 individuals (36%). A total of 61patients (54%) received antiretroviral therapy(ART), including 19patients(16.8%) in whom ART was started de novo during hospitalisation. The mean length from detection of HIV infection to death was 5.32years (range:1day–26 years). At the time of admission, 52% (59/113) of patients were in severe and extremely severe condition, 21%(24) in moderate to severe condition, 22%(25) in moderate condition and 4%(5) in fairly good condition, which most likely affected the average

length of hospitalisation that was 20days (median 15 days).
Consequences of HIV/AIDS infection as the initial cause of death were diagnosed according to ICD-10 diagnoses in 88 patients (78%). In the remaining patients, the non-HIV-related causes of death included cirrhosis with liver failure (17 patients), bacterial pneumonia(4), urosepsis (1), neoplastic disease (1), cerebral haemorrhage (1), intestinal obstruction (1).

In 31 cases (27.4%), an autopsy was performed. After the analysis of the autopsy results and the patient's final discharge diagnoses, discrepancies in diagnoses were found in eight cases (25.8% of post-mortem results). Examples include the diagnosis of cerebral toxoplasmosis that turned out to be a case of cerebral lymphoma in three post-mortem cases, and vice versa (1 patient); suspected pulmonary tuberculosis turned out to be pulmonary histoplasmosis in the post-mortem material; a brain tumour suspected during life was ruled out on post-mortem histopathology; pulmonary aspergillosis was not diagnosed (it was confirmed at autopsy), and disseminated tuberculosis was not diagnosed in two cases. 1.Therapeutic success in AIDS patients largely depends on early detection of HIV infection and implementation of ART. 2.In the majority of patients hospitalised in the Wrocław centre, the initial cause of death was related to the underlying disease, i.e. HIV infection and the consequences of the disease. 3.In the 21st century, despite the availability of many diagnostic methods there are still many cases of death in the course of HIV/AIDS infection without a final and clearly certain diagnosis. 4.Performing an autopsy makes it possible to definitively verify the cause of the patient's death and even to confirm a completely different comorbidity that was not diagnosed during life.

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Determinants Associated With Uptake of HIV Testing for Women Attending Sexual Health and Genitourinary (GUM) Clinics in Wales

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Women are often left behind when it comes to accessing HIV services which are essential for achieving the first 90% stipulated by the UNAIDS, of diagnosing people living with HIV. Due to structural barriers of HIV women are left undiagnosed as these societal drivers inhibit them from accessing essential HIV services. The research seeks to identify the determinants which have inhibited women from taking up HIV testing in sexual health clinics (SHCs) and genitourinary clinics (GUM).

Sexual Wales Surveillance (SWS) dataset was used to collect HIV testing data from 2015-2021. Univariate and multivariate logistic analysis was carried out to identify associations among the variables. The Mantel Haenszel test was used to get stratified odds ratios. Furthermore, the Welsh Index of Multiple Deprivation (WIMD) data was merged with the SWS dataset to identify deprivation across areas in Wales. Stata v.16.1 was used to carry out statistical analysis. Survey weights were applied as health boards were separated into clusters.
The prevalence of HIV testing in sexual health and GUM clinics in Wales for women in 2021 was 36.1% whilst men had a prevalence of 63.9% in the same year (n=9,868). Men attending sexual health and GUM clinics in Wales from 2015 to 2021 were aOR 3.05 (95% CI:3.01-3.09; p<0.001;) more likely to test for HIV than women. Moreover, women from Cwm Taf Morgannwg had an odds ratio of aoR 0.71(95% CI=0.70-0.74; p<0.001). Women testing from Aneurin Bevan Health board had an odds ratio of aoR 0.50(95% CI=0.49-0.51;p <0.001) whilst women from Swansea Bay health board had an odds risk aoR 0.83 (95% CI= 0.81-0.86;p<0.001).Women testing for HIV in the most deprived quintiles in Wales, that is 1 and 2 had an odds risk of aoR 0.87 (95% CI=0.86-0.89;p<0.001) and aoR 0.88(95% CI=0.86-0.90;p<0.001). Moreover, women who tested for HIV in in the deprivation quintiles 3 and 4 had an odds risk of aoR 0.97(95% CI=0.95-0.99; p<0.001) and aoR 0.97 (95% CI=0.13-0.17; p<0.001). There was strong evidence to show that gender was associated with HIV testing as men were 3 times more likely to test for HIV as compared to their female counterparts attending sexual health and GUM clinics in Wales. From the seven health boards in Wales there was good evidence to show that women from Aneurin Bevan, Cwm Taf Morgannwg health board and Swansea Bay were less likely to receive testing for HIV as compared to women from other health boards. Moreover, in terms of deprivation women there was strong evidence to show that women from the most deprived areas in Wales were less likely to test for HIV at sexual health and GUM clinics.

The decline in prevalence of HIV testing by women at SHCs and GUM clinics shows the need for targeted HIV testing and community-based HIV testing for women in Wales. Targeted testing and awareness campaigns might need to be rolled out in the health boards of Aneurin Bevan, Cwm Taf and Swansea Bay. Testing programs might need to be rolled out in the most deprived areas in Wales.

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Screening Strategies for Sexually Transmitted Infections in Transgender Women: Contributions From the +Screening=+Life Project

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Background: Transgender people are at high risk of HIV infection and other sexually transmitted infections, and prevention efforts specifically targeting this population are critical. The challenges include combining prevention strategies with the articulation of complementary medical, legal and psychosocial services to achieve a greater health impact. Home-based screening was a safe and effective method not only to test oneself, but also to promote linkage to other services.

Material and Methods: The transgender people, partners and clients who participated in the +Screening=+Life Project were all screened for HIV and other sexually transmitted infections. The sample relates to interventions conducted between March 2022 and February 2023. A specialist nurse and a psychologist were present at all home visits, providing pre- and post-test counselling, promoting moments of health education, conducting informal interviews and referring to other services, according to the participants' needs.

Results: 421 telephone contacts were made facilitating the dissemination of the project and the responses made available. As a result 202 home screenings were carried out, 18 of these people kept repeats (every 3 months). Of these people we know that 172 are trans women (called men at birth), all of Brazilian nationality, working in the sex industry, aged between 21 and 59. We reached 2 clients and 2 partners. 38 people with HIV were referred, 1 of them being a new case. Their ages range from 23 to 49 years. All of them were referred/followed up to the infectiology consultation at the two Hospital Centers of reference. In some cases, other infections are associated, namely HCV and Syphilis, all under treatment. We referred 49 people for PrEP, of which 18 will be new beneficiaries.
From the interviews carried out, the importance of home screening was reinforced because 1) it facilitates screening for different infections due to lack of knowledge of the places where they are carried out, 2) it reduces the fear of being identified as an undocumented person, 3) overcome logistical barriers associated with clinic/hospital-based testing, 3) encouraged participants to overcome their fear of testing, 4) provided privacy in the home, and 5) provided quality time with qualified health counselors and are 5) anonymous way to conduct the intervention.

Conclusion: The results suggest that the strategies used - making an initial telephone contact with subsequent scheduling of home screening proved to be very beneficial especially for connecting these people to health care and services. It encouraged participants to overcome their fear of testing through an environment that provided privacy and quality time with qualified health advisors. Facilitated referral to other services in health. These results also encourage the team to propose to policy-makers and other social partners to carry out actions and other programs that facilitate access to existing responses for trans immigrant people that they are often unaware of or afraid to join.

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The Immune Status Is a Predictor of Treatment Outcome Among Vertically Infected Adolescents Receiving Antiretroviral Therapy: The EDCTP READY- Study

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Background: WHO strongly recommends that all HIV patients entering or re-entering care should receive a CD4 test at treatment initiation and a close monitoring in case of treatment failure and/or advanced HIV disease. Since Immunological monitoring is less described in our context within the pediatric population, we sought to assess the rate of adolescent living with HIV (ALHIV) requiring immune monitoring and ascertain the association between immunological markers and virological failure (VF).

Material and Methods: A cross-sectional and analytical study was conducted among 283 ALHIV in the center region of Cameroon. Viral load (VL) and lymphocyte phenotyping were carried out on Abbott m2000rt and BD Facscalibur platforms respectively. Priority target for immune monitoring was defined as absolute CD4 <200 cells/mm3. Data were analyzed by SPSS; p <0.05 considered as significant.

Results: The mean age was 14±3 years, 152 (53.7%) were girls and 152 (53.7%) were young adolescents (10-14 years). Regarding VL, 39.7% (112/283) was on VF (VL ≥1000 copies/mL); with a median RNA VL of 4.66 [IQR: 3.89-5.33] log10 copies/mL. Considering the immune status, 17.7% (50/283) was severely immune-compromised (CD4 <200 cells/mm3). RNA VL ≥4.66 log10 copies/mL was significantly associated with a severe immune-suppression (p<0.0001). Compared to younger adolescents (10-14 years), older adolescents (15-19 years) were more severely immune-compromised (48.1% vs. 32.1% respectively (p<0.001)), and were more on VF (25.9% vs. 10.5% respectively, p= 0.004). Moreover, CD4/CD8 ratio was the immune parameter with the strongest negative correlation with VL (r=-0.62 [95% CI: -0.69-0.54], p<0.001) and was the only predictor of virological failure in regression analysis.

Conclusion: According to these findings, ALHIV aged 15-19 years are a priority target for the close immune monitoring. Also, the package of care strategy recommended by WHO for such cases should take into consideration the CD4/CD8 ratio, as it is the best immunological parameter predicting VF in this population.

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Characteristics of the Epidemic Process of HIV-Infection in Minsk, Belarus

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Background: According to UNAIDS data, there were 38.4 million people living with HIV in 2021. 85% of all people living with HIV know their status and about 5.9 million people don't know that they were living with HIV (2021). The number of new HIV infections in 2021 decreased by 54% compared to 1996 and amounted to 1.5 million people. In 2021, the rate of new HIV infections among children has also decreased by 52% from 320,000 to 160,000 (2010). The number of people who died from AIDS-related causes decreased by 68% from the peak in 2004 and by 52% compared to 2010, and amounted to 650,000 people (2021).

Material and Methods: The purpose of our study is to identify and evaluate the features of the epidemic process of HIV-infection at the present stage in Minsk, Belarus. The material of the study was the demographic indicators and epidemiological data of patients diagnosed with

HIV-infection, identified in 2000-2021 in Minsk. For the statistical processing of databases, standard packages of statistical programs Microsoft Excel 10, STATISTICA 10 were used.

Results: The incidence of HIV-infection in Minsk from 2000 to 2021 varied between 4.93-41.00 cases per 100,000 population. The dynamics of the HIV incidence was characterized by a significant upward trend with an average growth rate of 7.57% ($p<0.05$). During the analyzed time interval, the minimum and maximum incidence rates of HIV-infection differed by 8.32 times. The average long-term incidence rate was 15.22 per 100,000 population. Since 2013, the incidence of HIV-infection in Minsk has not fallen below 10.00 cases per 100,000 population of the analyzed territory. The maximum incidence rate of HIV-infection in Minsk was recorded in 2015 and amounted to 41.00 cases per 100,000 population, which is due to changes in the structure of HIV transmission routes (an increase in the proportion of people infected with HIV through injecting drug use).

Conclusion: In 2020, there was a decrease in the HIV incidence to 16.40 cases per 100,000 of the population, but later on, the incidence of HIV-infection increased and in 2022 amounted to 23.50 cases per 100,000 of the population. We believe that the decline in the HIV incidence in 2020 in Minsk was caused both by the reprofiling of laboratory facilities amid the spread of infection caused by the COVID-19, a decrease in the number of HIV tests, and a decrease in the activity and number of social contacts due to restrictions amid the development of the pandemic COVID-19 in the analyzed area.

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Synergistic Effects of Antivirals and Monoclonal Antibodies in Vitro Against SARS-CoV-2 Wild Type B.1 Strain and BQ.1.1 Omicron Sublineage

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Background: Combination regimens can enhance antiviral potency, limit emergent drug resistance and/or reduce drug dosage. Aim of this work was to determine, in a live virus cell-based assay, the potential synergistic effects of: (i) the combination of approved Directly Acting Antivirals (DAAs) against SARS-COV-2 wild type strain B.1 (WT) and the recently circulating omicron BQ.1.1 and (ii) the combination of the injectable DAA remdesivir (RDV) and licensed monoclonal antibodies (mAbs) against WT.

Material and Methods: The cytotoxicity of the active form of molnupiravir (EIDD-1931), RDV, nirmatrelvir (NRM), cilgavimab (CIL), tixagevimab (TIX), bebtelovimab (BEB) and sotrovimab (SOT) was determined by luminescence in VERO-E6 cell line treated with CP-100356 P-gp efflux inhibitor. Scalar dilutions of each DAAs were incubated in a 36 pairwise concentration matrix on VERO-E6 infected with WT and BQ.1.1 (MOI=0.01). After 72h, cytopathic effect was quantified by luminescence. The same experiments were performed with RDV in combination with each mAb. The synergistic score (SC) was calculated by SynergyFinder 3.0 using the Bliss/Loewe model and the the Multi-dimensional Synergy of Combinations (MuSyC) post-analysis option. SCs>10 was scored as synergistic, -10≥ SCs ≤10 as additive, <-10 as antagonist.

Results: Half-maximal μM inhibitory concentrations (IC_{50}) for EIDD-1931, RDV and NRM were 2.40±0.40/1.59±0.44, 0.06±0.03/0.03±0.01 and 0.10±0.03/0.10±0.01 μM against WT/BQ.1.1, respectively. CIL, TIX, BEB and SOT were active only against WT (IC_{50} of 0.20±0.13, 0.07±0.04, 0.03±0.01, 0.81±0.32 μg/ml, respectively). Considering global weighted SCs, additive effects were observed for all DAAs combinations against WT (-0.96±1.69 for EIDD-1931+RDV, -6.71±0.89 for EIDD-1931+NRM and 0.02±0.33 for RDV+NRM) and against BQ.1.1 (-0.33±4.10 for EIDD-1931+RDV, -0.47±0.37 for EIDD-1931+NRM and -2.12±3.8 for RDV+NRM). Similarly, additive effects were observed for all mAbs/RDV combinations against WT (-5.9±4.4 for SOT+RDV, -5.5±1.6 for RDV+BEB, 0.88±2.1 for RDV+CIL and -4.3±1.6 for TIX/RDV). SC>10 suggestive of synergism was observed for a few DAAs combinations at specific concentrations, including EIDD-1931 (0.05-0.19-0.75 μM) + RDV (0.4 μM), EIDD-1931 (0.075-1.5 μM) + NRM (0.05 μM) and RDV (0.02 μM) + NRM (0.01 μM) against both viral strains. Similarly, SC>10 was observed against WT for the following RDV+mAbs combinations: RDV (0.06 μM) + SOT (0.09-0.04-0.1 μg/ml); RDV (0.016-0.06 μM) + BEB (0.04 μg/ml); RDV (0.06 μM) + TIX (0.04-0.009 μg/ml). Synergistic potency shifts at

different concentrations were confirmed using MuSyC post-analysis. As a proof of concept, DAAs IC_{50} shifts were measured in infected cells treated with 3 fixed NRM concentrations (0.1, 0.05, 0.025 µM) and scalar EIDD-1931 (5 to 0.04 µM). The IC_{50} of EIDD-1931, with the addition of NRM 0.1, 0.05 and 0.025 µM, was reduced by >61.5-, 7.7- and 0.7-fold against WT and by >41- and by 10- and 2.6-fold against BQ.1.1.

Conclusion: Global weighted SCs indicated additive effects. However, each DAAs combination induced synergistic potency shifts against WT and BQ.1.1 when specific concentrations' combinations were evaluated. Despite the same effects were observed for RDV in combination with SOT, BEB or TIX against WT, it was not possible to evaluate this combination against the circulating BQ.1.1 strain due to its high resistance to all tested mAbs.

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Preliminary Results: of the Eucare School Studies and SARS-CoV-2 Trends in Italy and Germany and School Opening During the Omicron Variant

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Background: As part of the global reaction to stop the spread of SARS-CoV-2 during the first few months of the COVID-19 pandemic, primary and secondary schools were closed to on-site instruction in many countries. Contradictory results were reported on the role of school closure/reopening on the SARS-CoV-2 transmission rate.
The Lolli-Methode (LM) is a strategy for epidemiological surveillance and early intervention aiming at SARS-CoV-2 outbreaks' reduction in schools, relying on polymerase-chain-reaction analysis of saliva samples in two steps: on pooled samples and then on individual samples from positive pooled cases.

Material and Methods: We designed a cluster randomized trial, aimed to determine whether the use of the LM is useful to support schools opening and a prospective cohort study to investigate impact of preventive measures adopted [Raimondi, S. et al. BMC Infect Dis 23, 1, 2023]. We also investigated the potential impact of school reopening on SARS-CoV-2 transmission in Italy and Germany in autumn 2022. The investigation faced several methodological challenges, including the selection of the most appropriate statistics to investigate the link between the infection's spread and the different school reopening dates. We pooled data from regions/states that opened on the same day and compared the case reproduction number (Rc) curves by age groups in relation to school opening dates. We calculated the time from school opening to the day of increase or increase in velocity of Rc. We employed a staggered difference-in-differences analysis to assess whether the school reopening significantly affected the trend of infections while considering the different dates of reopening. We used different metrics (daily and weekly) by country, to verify stability of results.

Results: We enrolled 1453 subjects, 1127 in the randomized trial and 326 in the observational cohort study. Looking at trends in time of positive notifications we found greater positive rates in the Standard-of-Care (SoC) trial arm for teachers and lower in the Lolli arm in students. Overall rates of symptoms per week were significantly lower in the Lolli arm: 47% for the SoC arm versus 23% for the Lolli arm (p=0.009). No differences were observed between groups regarding adopted preventive measures, except for the use of ventilation and opening of windows which were found significantly less frequent in the Lolli arm (p=0.02). We found that the time parametrization for Rc regarding Omicron variant was the most appropriate. A significant decrease in Rc following school openings was found in the 6-19 years population (Overall average treatment effect for the treated subpopulation (O-ATT):-0.69 [95%CI:-0.99;-0.39] for Italy; O-ATT:-0.28 [95%CI:-0.32;-0.243] for Germany). No difference in the adult population was observed compared to the situation before the opening of schools (O-ATT:-0.02 [95%CI:-0.05; -0.02] for Italy; O-ATT: -0.06 [95%CI:-0.09;-0.03] for Germany). Multivariable models adjusting for confounders confirmed these results.

Conclusion: Preliminary results indicate that the LM may be useful to decrease Sars-cov2 infections, which are greater in teachers than in students. The increasing trend of the Sars-Cov-2 in autumn 2022 appeared to be driven mainly by the geographical location, seasonal changes and overall population behavior than by school openings.

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A Heparan Sulfate Proteoglycan Binding Tetrapeptide Successfully Inhibits SARS-CoV-2 Omicron Replication

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Background: The emergence of the SARS-CoV-2 omicron variant has led to increased transmissibility and immune escape, due to the high number of mutations in the Spike (S) region, particularly in the receptor binding domain (RBD) responsible for the interaction with the Angiotensin Converting Enzyme 2 (ACE2). At the same time, the mechanism of Omicron entry has evolved into endocytic dependent internalization, as opposed to membrane fusion dependent internalization, which was predominant with previous variants. In addition, a possible interaction of membrane Heparan Sulfate Proteoglycans (HSPG) with SARS-CoV-2 during virus internalization has been recently suggested. The aim of this work was to determine the role of HSPG in the infectivity of the Omicron variant, and the efficacy of an HSPG binding tetrapeptide (LB-1) in inhibiting Omicron entry in a live virus cell-based model.

Material and Methods: LB-1 was synthesized in a tetra-branched form on a multiple automated synthesizer using standard Fmoc chemistry. The infection model was set up with Omicron BA.1 and Delta SARS-CoV-2 strains in the adherent human intestinal Caco-2 cell line with 24h incubation followed by transfer of the virus supernatant to a highly permissive reporter cell line (VERO E6 monkey kidney cells). The half-maximal Tissue Culture Infectious Dose ($TCID_{50}$) was determined after 24h in VERO E6 measuring the viral nucleocapsid expression by ELISA assay. The experiments were performed in quadruplicate in Caco-2 cells, with and without treatment with heparinase to remove the HSPG chains. The half-maximal cytotoxic concentration (CC_{50}) of LB-1 was determined by luminescence. To determine the antiviral activity, serial dilutions of LB-1 starting from the not-toxic dose, were incubated with a fixed amount of BA.5, BA.1 and Delta strains (MOI=0.01) for 1h at 37°C on pre-seeded Caco-2 cells. After incubation, the virus-peptide mixture was removed, and fresh LB-1 was added. Viral supernatants were transferred in VERO E6 as previously described. The half-maximal inhibitory concentration of LB-1 (IC_{50}) was measured by SARS-CoV-2 nucleocapsid ELISA. Each experiment included a mock infection control, a virus control and two reference inhibitors with known IC_{50} (remdesivir and a previously tested human immune serum).

Results: LB-1 inhibited cell infection by BA.1 and BA.5 with 21,5 ± 2,8 and 19,5 ± 1,7 µM IC_{50}, respectively. By contrast, LB-1 had no effect against delta variant indicating a crucial role for HSPG in cell infection by Omicron but not Delta. Heparinase treated cells were not permissive to Omicron infection, confirming the role of HSPG, while a modest impact was measured on Delta (4.6-fold reduction).

Conclusion: The prototype HSPG binding tetrapeptide LB-1 selectively inhibited the replication of SARS-CoV-2 Omicron. The role of HSPG in Omicron infection and this proof-of-concept results support further development of HSPG targeting agents as a novel strategy to block SARS-CoV-2 infection.

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Post-Vaccine Anti SARS-CoV-2 Humoral Immunogenicity in Immunocompromised Children With Acute Leukemia

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Background: Deaths attributable to COVID-19 in children living with cancer are ten times higher than in general pediatric population. SARS-CoV-2 infection in this population also leads to a delay in starting chemotherapy, while vaccine responses are less efficient. The aim of this work is to assess the immunogenicity of BNT162b2 vaccine in a population of children with hematologic malignancies.

Material and Methods: The study cohort included 49 children with hematologic malignancies (mean age: 6,7 ± 1,0 years; 42 B lymphoid leukemia, 3 T lymphoid leukemia, 4 myeloid leukemia) and 12 healthy children from the siblings (mean age: 6,6 ± 1,1 years) as control group. Each children received 2 doses of mRNA vaccine (BNT162b2) separated from 21 to 28 days. Vaccine response was evaluated 6 weeks after the second injection, and non-responding children (defined by anti-Spike (S) IgG below 264 BAU/ml) received a third dose of vaccine. Humoral response was evaluated by high-input chemiluminescence assay (Alinity i, Abbott, Rungis, France) quantifying the anti-Spike (S) IgG, and by live virus neutralization assay performed in Vero TMPRSS2 cells. This functional assay quantified the neutralizing activity of sera against two SARS-CoV-2 isolates (Wuhan B.1 and Omicron BQ.1.1 lineages), assessed by cytopathic effect on microcope. Neutralizing antibody titers (NAbs) were defined as the reciprocal value of the dilution that showed a 90% protection of virus-induced cytopathic effect (NT90). Sera without neutralizing capacity were defined as negative and scored as 1 for statistical analysis.

Results: The median anti-S IgG titers in immunocompromised children after two doses was 250,4 BAU/mL (vs 2679,0 BAU/mL in the control group). 21 immunocompromised children (42,9%) received a third injection because of non-response. Median anti-S IgG titers reached 137,63 BAU/mL after the third dose in this sub-population, and 12 children (24,5%) were still non-responders after the vaccine boost. NAbs against B.1 variant and anti-Spike (S) IgG titers were significantly correlated (Pearson r=0,824, p=0,005). In immunocompromised children, the mean neutralizing activity against B.1 variant was 161,5 (CI95% : [96,7 ; 226,3]), which was significantly lower than in healthy children (412,5; CI95% : [230,3 ;594,3]) (p=0,001). Importantly, the mean neutralizing activity was low for the Omicron BQ.1.1 variant (29-fold reduction compare to B.1) and did not differ between immunocompromised and healthy children (7,3 [2,7-11,9] vs 18,5 [5,2-31,8]) (p=0,1)). Finally, in responding immunocompromised children, NAbs against B.1 variant were significantly correlated with lymphocyte count at vaccination (Pearson r=0,33, p=0,03), while there was no significant correlation between NAbs against Omicron BQ.1.1 variant and lymphocyte count (Pearson r=0,25, p=0,10).

Conclusion: These findings shows that vaccination with 2 BNT162b2 doses only induced a significant humoral response in half of the immunocompromised children, while lymphocyte count at vaccination could be predictive for humoral response. However, the recent BQ.1.1 variant displayed a significant NAbs escape, both in healthy and immunocompromised children. The evaluation of the vaccine response durability is currently in progress.

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Dynamic of the Inflammatory Profile in Hospitalized People With SARS-CoV-2 Infection With Post COVID-19 Symptoms

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Background: According to WHO "post COVID-19" is defined as the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation. The causes and the pathophysiological mechanisms remain controversial. A link between a persistent inflammatory environment and these long-term sequelae has been suggested. Herein, we assessed the dynamic of up- and downstream molecules of the NLRP3 inflammasome's pathway promoting inflammation.

Material and Methods: A prospective longitudinal study was performed by selecting individuals belonging to the Galicia Sur Health Research Institute COVID-19 Cohort. Three groups were defined: healthy blood donors without SARS-CoV-2 infection and donors with a confirmed SARS-CoV-2 diagnosis who had been hospitalized, the latter being further divided into post COVID (PC) and no post COVID (nPC) patients. The patients included in the study were those with samples at baseline (maximum 10 days after the confirmation of the SARS-CoV-2) and months 1 and 6 after the acute infection. PC patients were distinguished from nPC based on the presence or absence of symptomatology at month six, respectively. Individuals between groups and healthy donors were matched by sex/age. Epidemiological and clinical characteristics were collected (i.e symptoms on admission and month six, comorbidities or severity).
Milliplex assays were used to quantify the levels of plasma cytokines (TNF-α, IFN-γ, IL-18, IL-6, and IP-10) at baseline and during the follow-up (months 1 and 6) after the onset of the infection. Total peroxide level (TPX) measurement was also performed at the same time points. Differences were examined using Friedman and Kruskal-Wallis tests, respectively.

Results: A total of 54 individuals met the established criteria: 27 nPC and 27 PC, and 14 healthy controls were also included. The most frequent symptoms among people with post COVID-19 condition were thoracic (dyspnea, chest pain, cough) 59.25 %, general (asthenia, hair loss) 44.44 %, nervous (behavioral disorder, headache) 25.93 % and musculoskeletal (arthralgias, myalgias) 25.93 %. No significant differences between groups were observed based on clinical data.
TPX levels at baseline were significantly higher for nPC and PC individuals compared to HC (nPC: 899.61 [562.61-1073.56] and PC: 756.43 [575.78-976.46] vs HC: 440.09 [289.86-705.79]). From the acute phase, levels of TPX significantly decreased during the follow-up in both groups (44.45 and 39.06 % in nPC and PC, respectively). No differences were observed between nPC and PC at any of the three assessed time points.
Il-6, IL-18, and IP-10 levels at baseline were significantly higher for nPC and PC compared to HC. The levels of all cytokines significantly decrease during the follow-up, where the sharpest declines occurred for IP-10 (nPC: 87.16 %, PC: 76.00 %) and IL-6 (nPC: 83.32 %, PC: 80.02 %), without differences between nPC and PC.

Conclusion: These findings suggest that post COVID-19 conditions may not be related to persistent inflammation, since in both nPC and PC the levels of inflammatory molecules significantly decrease after 1 month of the onset of SARS-CoV-2 infection.

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Effect of Co-Infection With Intestinal Parasites on Covid-19 Severity: A Prospective Observational Cohort Study

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Background: COVID-19 symptomatology in Africa appears significantly less serious than in the industrialized world. We and others previously postulated a partial explanation for this phenomenon, being a different, more activated immune system due to parasite infections. We investigated this hypothesis in an endemic area in Africa.

Material and Methods: Ethiopian COVID-19 patients were enrolled and screened for intestinal parasites, between July 2020 and March 2021. The primary outcome was the proportion of patients with severe COVID-19. SARS-CoV-2 infection was confirmed by RT-PCR on samples obtained from nasopharyngeal swabs, while direct microscopic examination, modified Ritchie concentration, and Kato-Katz methods were used to identify parasites and ova from a fresh stool sample. Ordinal logistic regression models were used to estimate the association between parasite infection and COVID-19 severity. Models were adjusted for sex, age, residence, education level, occupation, body mass index, and comorbidities. Data were analyzed using STATA version 14. P-value <0.05 was considered statistically significant.

Results: A total of 751 SARS-CoV-2 infected patients were enrolled, of whom 284 (37•8%) had an intestinal parasitic infection. Only 27/255 (10•6%) severe COVID-19 patients were co-infected with intestinal parasites, while 257/496 (51•8%) non-severe COVID-19 patients appeared parasite positive (p<0.0001). Patients co-infected with parasites had lower odds of developing severe COVID-19, with an adjusted odds ratio (AOR) of 0•14 (95% CI 0•09–0•24; p<0•0001) for all parasites, AOR 0•20 ([95% CI 0•11–0•38]; p<0•0001) for protozoa, and AOR 0•13 ([95% CI 0•07–0•26]; p<0•0001) for helminths. When stratified by species, co-infection with Entamoeba spp., Hymenolopis nana, and Schistosoma mansoni implied a lower probability of developing severe COVID-19. There were 11 deaths (1•5%), and all were among patients without parasites (p=0•009).

Conclusion: Parasite co-infection is associated with a reduced risk of severe COVID-19 in African patients. Parasite-driven immunomodulatory responses may mute hyper-inflammation associated with severe COVID-19.

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Real-Life Data on Viral Variants and on Antiviral Treatment in Adult Patients With Mild COVID-19 Symptoms From December 2021 to January 2023

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Background: In the outpatient setting, guidelines recommend treatment with COVID-19-specific therapy for symptomatic adults who have mild to moderate symptoms and are at increased risk for progression to severe disease based on older age, immune status, COVID-19 vaccination history, and comorbidities. Aim of the present study is to characterize non-hospitalized patients with mild/moderate COVID-19 who received different treatments between December 2021 and January 2023.

Material and Methods: Data on 292 consecutive COVID-19 outpatients who received early treatment with antivirals (n=295) and/or monoclonal antibodies (n=103) at the Department of Infectious Disease at Sacco Hospital were collected. Follow-up was assessed by phone call

after treatment. Differences between treatment groups were assessed by t test/chi-square test or Wilcoxon Mann-Whitney/Kruskal-Wallis for parametric and no parametric data, respectively. Lineage assignment was performed by real time PCR and/or whole genome sequencing (n=284).
Results: Males accounted for 52.7% (n=154) and median age was 62 years (IQR:52-75). Of the 268 vaccinated subjects, 60.7% (n=156) had also received the 3rd dose. Median time from last vaccine dose to infection was 5 months (IQR: 2.3-6.2). Lineages and their descendants were distributed as follows: Delta 13%, BA.1 26.1%, BA.2 33.8%, BA.4 3.2% and BA.5 23.9%. No differences were observed among lineages distribution and patients age or vaccination status. Stratifying subject based on treatment, median age was significantly higher in subjects treated on molnupiravir (73.3 yrs SD 14, p<.001). Among patients >65 yo, a higher proportion was treated with mAbs (34.1%) or nirmatrelvir/ritonavir (31.9%) compared to molnupiravir (21.7%) or remdesivir (12.3%) (p=.002). A significant higher proportion of patients with cardiovascular disease (31.7%), COPD (34.9%) and primary or iatrogenic immunodeficiency (49.5%) received preferentially nirmatrelvir/ritonavir compared to other treatments (p<.0001, p<.02 and p<.0001, respectively). Globally, median duration of infection was 7 days (IQR:6-11), with no significant differences based on age or according to vaccination (and number of doses), lineage, therapy or related pathologies also considering a time of ≤ or >7 days. Median time of negativization was higher in males compared to females (9 vs. 7 days; p=.002). No differences were observed between negativization time and period from last vaccine dose and infection.

Conclusion: Our study by combining molecular and clinical data provides an accurate overview of patients with mild/moderate COVID-19. Despite the large population on study, no significant differences were observed in the efficacy of different treatments. No long persistent viral shedding was observed even if several patients were immunodeficient confirming the efficacy of antiviral and mAbs therapies.

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Viral Dynamics and Factors Favouring the Duration of COVID-19 Positivity: Evidence From the First-Three Epidemiological Waves in Cameroon

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Background: Coronavirus 2019 (COVID-19) disease progression evidence and viral clearance time remain limited in tropical settings. Understanding this is crucial for public health control measures at community-level. We evaluated the viral dynamics of SARS-CoV-2 infection and factors associated with positivity duration in COVID-19 cases in Cameroon.

Material and Methods: We conducted a prospective cohort-study of SARS-CoV-2 positive cases from the first to third wave (March 2020-October 2021) in Yaounde-Cameroon. RT-PCR was carried out on the participants using nasopharyngeal swabs. SARS-CoV-2 positivity duration was evaluated from the first to last positive PCR-test before a negative result. Epi-info

V.7.0 was used for data analyses with p<0.05 considered statistically significant.

Results: A total of 282 participants were enrolled. The mean age was 41±14 years, with male predominant (62.1%). We had 15.6% symptomatic participants of which 59% had cough. The overall median positivity duration was 15[IQR: 9-23] days with 15[IQR: 13-16] in the first, 17[IQR: 11-26] in the second and 8[IQR: 4-12] in the third wave (p= 0.007). Positivity duration was significantly higher in males (16 versus 14 days, p=0.03) and people aged >40 years (15 versus 14 days, p=0.02). Positivity duration was not affected by presence or absence of symptoms (p=0.80). No significant correlation was found with viral load (r=0.03; p=0.61). Considering baseline (24.7±7.2Ct) and last viral load (29.3±5.9 Ct), the ΔCt (4.6±1.3) and positivity duration (15 days) revealed a kinetic in viral decay of 0.3±0.087 Ct/day.

Conclusion: A median positivity duration of 15 days is in accordance with viral clearance around 2 weeks for optimal confinement at community-level. Men and/or the elderly stand at higher risk of prolonged infection. Given the viral decay (0.3 Ct daily), we suggest personalized confinement periods. The variability of positivity duration according to waves could be function of strains which could be a factor influencing positivity duration.

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Replication of Clinical Isolates of SARS-CoV-2 is Not Modified by Androgens

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Background: Expression of the transmembrane protease serine 2 (TMPRSS2), involved in SARS-CoV-2 cellular entry, is androgen-regulated. By using models of SARS-CoV-2 pseudoviruses, some groups have suggested that antiandrogens could reduce the SARS-CoV-2 entry into lung cells but these results have to be confirmed by live virus-based assays.

Material and Methods: Caco-2 cells were infected (MOI=0.25) by a wild-type (WT) or by a delta variant (B.1.617.2) clinical isolate of SARS-CoV-2 in the presence of dihydrotestosterone (DHT, 10 nM during 24h) or enzalutamide (ENZA, 10 µM), which activates or inhibits the androgen pathway, respectively. TMPRSS2 mRNA and SARS-CoV-2 RNA were extracted by the RNeasy kit (Qiagen) and the NucleoMag Pathogen kit (Macherey-Nagel), respectively, and quantified by an in-house RT-PCR technique and by the TaqPath COVID-19 RT-PCR Kit (Thermofisher), respectively.
Respiratory viral loads (VLs) were measured by RT-PCR in the nasopharyngeal swabs of adult patients as a routine practice in our institution from March 2020 to November 2022.

Results: Caco-2 cells strongly supported the viral replication of both isolates (+ 8.0 to 9.1 Ct at Day 4 post-infection). Stimulation of Caco-2 cells by DHT increased the expression of TMPRSS2. However, treatment of the cells by DHT (10 nM) or EZNA (10 µM) did not induce any significant variation in SARS-CoV-2 replication at 24h and 48h post-infection.
Moreover, mean respiratory SARS-CoV-2 VLs, obtained over more than 2 years in our institution, were not significantly higher in men (Ct=25.48) than in women (Ct=24.90).

Conclusion: Our results do not argue in favour of a biologically relevant role of androgens on SARS-CoV-2 replication.

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Mental Health of People Living With HIV During Pandemic SARS-COVID-19 in Ukraine

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Background: The spread of COVID-19 and measures related to the pandemic have a significant impact on population mental health, namely on the cognitive, emotional and volitional areas and social communication. The persons suffering from chronic

infectious diseases as well as individuals infected with HIV belong to a high-risk group. The aim of our work was to assess the impact of anxiety and depression in people with secondary immunodeficiency caused by HIV on consent to vaccination against the coronavirus disease during the COVID-19 pandemic in Ukraine.

Material and Methods: 67 individuals with a diagnosis of HIV/AIDS in "Lviv Regional Information and Analytical Center of Medical Statistics" were surveyed from December 2021 to April 2022. The average age of the respondents was 35.67±1.25 years and ranged from 18 to 68 years. All individuals are being treated with antiretroviral therapy at the time of the survey. The GAD - 7 (General Anxiety Disorder - 7) questionnaire was used to assess the level of anxiety, and the PHQ - 9 (Patient Health Questionnaire - 9) was used to diagnose the level of depression. The HIV Registry software was used for statistical processing of the results.

Results: 52.2% of individuals were diagnosed with minimal anxiety, 25.4% - mild anxiety, 14.9% - moderate anxiety, and 7.5% with severe anxiety. 53.7% of respondents denied signs of depression, 28.4% of individuals were diagnosed with mild depression, 13.4% - moderate depression, and 4.5% of individuals with moderately-severe depression. 3.0% of respondents showed a combination of moderately-severe depression with severe anxiety. Among people with severe anxiety 73.3% were aware of their HIV status within 3 years. And among people with moderately- severe and severe depression, this figure was 75%.
Regarding the data on the incidence of SARS-COVID-19, in the group of individuals with moderate and severe anxiety, the rate of laboratory-confirmed cases was 26.7%, and among individuals with moderately-severe and severe depression - 25.0%. At the time of the survey, 34.3% of people refused to be vaccinated against SARS-CoV-2. Among individuals with moderate and severe anxiety 40% of respondents refused vaccination, and among individuals with moderately-severe and severe depression - 50% of surveyed people refused to be vaccinated.

Conclusion: The mental state of persons, namely, anxiety and depressive symptoms, can significantly affect the disease acceptance by patients and information perception. This leads to rejection of preventive and therapeutic measures due to the SARS-COVID-19 pandemic in particular, the consent for vaccination, which should be taken into account by health care workers. Mental health changes caused by SARS-COVID-19 pandemic issues should be considered for medical care of people living with HIV.

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Association of the Genes Coding The Renin-Angiotensin System (RAS) Polymorphisms With the Severity of SARS Cov-2

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Background: RAS plays one of the key roles in the pathogenesis of COVID-19 life-threatening complications.
Aim of the study: to establish the association of genes encoding RAS polymorphisms with the severity of SARS Cov-2.

Material and Methods: Genotyping of 206 DNA samples isolated from the plasma of patients was carried out by real-time PCR for genes:
• ACE2 at 3 loci: rs2074192 (G/A), rs2285666 (G/A) and rs413031713 (T/C),
• type 1 receptor gene for angiotensin-II- AT1R rs5186 (A>C).
A set of reagents for DNA extraction from blood plasma by phenol extraction, reagent kits manufactured by NPO DNA-Technology LLC, Russia. Primer design was carried out using the GenBank annotated nucleotide sequence bank, using the global pairwise alignment method in the nucleotide BLAST service (http://www.ncbi.nlm.nih.gov/).
The patients have been divided into 2 study groups based on the severity of COVID-19: Group 1 - 99 patients with severe COVID-19 (age - 64.0 (54.0; 71.0) years; men - 51/ 52%, women - 48/48%); Group 2 - 107 with moderate and mild forms of the disease (age - 61.0 (57.0; 68.0) years; men - 48/45%; women - 59/55%).
Statistical processing of the results of the study was carried out using the R program (http://www.r-project.org/) for Windows using additional packages for the analysis of genetic data "SNPassoc" (version 1.9-2). The genotype frequencies were tested for compliance with the

Hardy-Weinberg equilibrium (RWE) using Pearson's χ2 test.

Results: A comprehensive analysis of two markers of the ACE2 gene loci rs2074192 and rs2285666 made it possible to identify 4 allelic combinations, the frequency of which in the study group was more than 3%, p = 0.043. For allelic combinations A-A, statistically significant differences were found in patients of groups 1 and 2: 7.9% and 1.2%, respectively, OR (95% CI) 3.1 (1.1-8.6), p=0.031. The most common allelic combination was G-G: 38.1 and 32.0%, respectively. No statistically significant association was found for G-A and A-G allelic combinations (p > 0.05 in all cases). The frequency of carriers of the heterozygous variant AT1R rs5186 A/C was significantly lower in the group 1 in compare with group 2: 35.8 and 56.0%, respectively (OR=0.5, p=0.01). Comprehensive analysis of three loci of 2 genes: ACE2 rs2074192 and rs2285666 and AGT rs699 made it possible to identify 8 allelic combinations, p = 0.015. For carriers an allelic combination of G-G-T the risk of a severe course of coronavirus infection has been significantly reduced (OR = 0.4, 95% CI 0.2-0.8, p = 0.018), as well as for carriers a combination of A-A-C the risk of a severe form of the disease increased significantly (OR = 4.2, 95% CI 1.1–9.4, p < 0.0001).

Conclusion:
Loci of genes encoding the renin-angiotensin system - ACE2 rs2074192 and rs2285666, AT1R rs5186, as well as allelic combinations between these loci are informative markers for predicting the severity of COVID-19.

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Logistics Management Strategy of Inputs for Molecular Diagnosis and Genomic Surveillance of COVID-19: Experience of the Chantal Biya International Reference Center (CIRCB) in Cameroon

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Background: The response strategy against COVID-19 relies on adequate supply of inputs. As the COVID-19 reference laboratory, the Chantal Biya International Reference Center (CBIRC) assessed the consumption of PCR, screening and sequencing inputs for better forecasting.

Material and Methods: This was a description of the use of inputs from August-2021 to July-2022. Delivery notes and reagent consumption sheets (extraction, amplification, screening, and sequencing kits) were collected and analyzed using the weighted average method.

Results: Overall, 37,008 extractions (35,280 in manual platform and 1,720 in automatic platform) and 37,248 PCRs (35,520 in manual and 1,720 in automatic) were performed for the molecular diagnosis of COVID-19 on 31,453 samples received; that is to say an average monthly consumption of 3,084 extractions, 3,104 PCRs and 2,622 samples for an estimate of 1,17 extractions/sample and 1,18 PCR/sample. Available usable stock (AUS) was 40kits of manual extraction (1,920 reactions) due to

expire in 5 months and 10kits of automatic extraction (960 reactions) expired 3 months ago. In PCR, the SDU was 1,056 tests in manual mode due to expire in 4 months and 10,560 in automatic mode due to expire in 7 months. Of the 2,238 (6.2%) positive cases diagnosed, 265 were screened and 200 were sequenced. This gives a total of 465 replicates out of 1773 positive cases that were not eligible due to low viral load. In screening, 288 tests were used, i.e., 1.08 tests/sample with a UDS of zero tests. In sequencing, 279 tests were used, i.e. 1.39 tests/sample, with an available usable stock (AUS) of 100 tests expiring in 7 months and 130 sequences already generated.

Conclusion: Estimates indicate an overuse of the manual platform at the expense of the automatic one, an understock of sequencing inputs, and a break in screening. Our logistical strategy would optimize input management and allow for efficient genomic surveillance in response to waves of COVID-19 or any future pandemic in Cameroon.

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Determinants of SARS-Cov-2 Infection Among Travellers In Cameroon: Toward Evidence-Based International Regulations for African Countries

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Background: In Cameroon, COVID-19 infection spread rapidly in the general population, with up to 122,375 confirmed cases and continuous monitoring. However, situation reports focused on travelers are lacking, which limit revision/standardisation of COVID-19 international regulations across African countries. We thus sought to update the burden of COVID-19 and its epidemiological, virological and clinical features among international travellers in the Cameroonian context.

Material and Methods: An laboratory-based study was conducted among international travellers tested for SARS-CoV-2 from January 2022 through June, 2022 at Chantal BIYA International Reference Centre, Yaounde-Cameroon. SARS-CoV-2 diagnosis was performed on nasopharyngeal swabs using Realtime qPCR. Statistical analyses were performed using SPSS and $p<0.05$ considered statistically significant.

Results: Out of 22,194 individuals (57.4% male) enrolled, 6.4% was symptomatic and 7.6% (1685/22,194) were vaccinated. The overall SARS-CoV-2 positivity was 0.7% (147/22194) from 0.2% (2/1047) in children (0-14 years) to 0.8% (11/1365) in elderly (>64 years), p=0.107. Positivity rate among symptomatic individuals was 2.5% versus 0.5% among asymptomatic, p< 0.001; and being symptomatic (aOR [95% CI]: 4.8 [3.2-7.1], P< 0.001) was a predictor of SARS-CoV-2 positivity. Positivity among vaccinated versus non-vaccinated individuals was 0.83% versus 0.58% respectively, p=0.21. The month of February had the highest positivity rate (7.6%), and the month May with the lowest positivity rate (0.8%). Regarding PCR cycle threshold (CT), 32.0% of positive individuals had a CT < 30. Among confirmed cases, those aged >40 years showed a non-significant higher proportion in high viral-load (CT< 20): 9.8% versus 7.8%, p=0.682; symptomatic travellers showed a higher proportion with high viral-load (22.2%) compared to asymptomatic (5.4%), P=0.003.

Conclusion: In the current state of low SARS-CoV-2 burden (< 1%) among international travellers in Cameroon, positivity is associated with symptoms and seemingly higher among the elderly. This evidence underscores the implementation of a symptom-driven “track-and-test” strategy focused on symptomatic and elderly travellers, regardless of vaccination status across Africa.

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The Sensitivity of HIV-1 gp120 Polymorphs to Inhibition by Temsavir Correlates to Temsavir Binding On-Rate

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Background: Temsavir (TMR) is a small-molecule HIV-1 attachment inhibitor that binds directly to the gp120 subunit within the HIV-1 envelope glycoprotein, gp160, and selectively inhibits the interaction between the virus and cellular CD4 receptors. Previous studies identified amino acid positions in gp120 where substitutions or polymorphisms have the potential to reduce phenotypic susceptibility to TMR. Seven substitutions (S375H/I/M/N/Y, M426L, M434K) have been associated with higher TMR IC50 fold-change relative to a reference strain. Biophysical analyses were performed to explore the mechanism by which TMR susceptibility was altered in these envelope glycoproteins.

Material and Methods: The 7 polymorphs were engineered, either alone or in combination, into the JRFL gp160 gene. Pseudoviruses that expressed these gp160 proteins were produced and tested for sensitivity to TMR and a control compound, efavirenz. Separately, 6xHis-tagged gp120 proteins containing these changes were expressed in 293HEK cells via a BacMam approach and purified by immobilized metal affinity chromatography. Binding studies on these purified proteins were conducted using a Creoptix WAVE Delta GCI system to determine the impact of these polymorphs on binding to TMR and soluble human CD4, as well as the ability of TMR to block gp120 binding to CD4.

Results: The 7 individual polymorphic pseudoviruses were found to have a range of altered susceptibility to inhibition by TMR from a low of 4-fold to a high of over 12,500-fold, relative to wild-type JRFL sensitivity. A dual polymorph, S375H/M475I, exhibited the greatest shift in susceptibility of over 29,700-fold. Kinetic parameters (on-rate, off-rate, and affinity) were measured for the binding of the gp120 proteins to a soluble, recombinant form of human CD4. The affinity of the gp120 proteins for CD4 varied between 0.4-fold and 3-fold compared to that of WT JRFL gp120. There was no correlation between the TMR sensitivity of the individual polymorphic pseudoviruses and either the affinity, on-rate, or off-rate for CD4 binding to the corresponding gp120 proteins. In contrast, the affinity of TMR for the polymorphic gp120 proteins varied from 0.7-fold to 74-fold compared to WT JRFL gp120. A strong correlation (p=0.0011) was found between the on-rate of TMR binding to the polymorphic gp120 proteins and the susceptibility of those polymorphs to inhibition by TMR in the pseudovirus assay. However, for all polymorphic gp120 proteins, TMR retained the capacity to fully block binding of gp120 to CD4, though some polymorphs required higher concentrations of TMR than others.

Conclusion: The loss of susceptibility to TMR observed for these HIV-1 envelope glycoprotein polymorphs was strongly correlated to reductions in the on-rate for TMR binding. This result may suggest that these polymorphic envelope proteins prefer conformations that are less amenable to initial TMR engagement. Nonetheless, TMR was still able to fully block these gp120 polymorphs from binding to CD4 given sufficiently high concentrations.

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Hepatitis B, Hepatitis C And HIV Infections and Associated Risk Factors Among Substance Abusers in Mekelle Substance Users Treatment and Rehabilitation Centers, Tigrai, Northern Ethiopia

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Background: Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) constitute a serious healthcare problems worldwide. Blood-borne pathogens HBV, HCV and

HIV are commonly associated infections among substance or Injection Drug Users (IDUs). The objective of this study was to determine the prevalence of HBV, HCV, and HIV infections among substance users, in Mekelle Substance users Treatment and Rehabilitation Centers.

Material and Methods: A cross sectional study design was used from Dec 2020 to Sep / 2021 to conduct the study. A total of 600 substance users was included. Data regarding sociodemographic, clinical and sexual behavior data were collected using a structured questionnaire. For laboratory analysis 5-10 ml of venous blood was taken from the substance users. The laboratory analysis was performed using Enzyme Linked Immunosorbent Assay (ELISA) in Mekelle University, Department of Medical Microbiology and Immunology Research Laboratory. The Data was analyzed using SPSS and Epi-data. Association of variables with HBV, HCV and HIV infections were determined using multivariate analysis and P value < 0.05 was considered statistically significant.

Results: The overall prevalence rate of HBV, HCV and HIV infections were 10%, 6.6%, and 7.5% respectively. The mean age of the study participants was 28.12 ± 6.9. The higher prevalence of HBV infection was seen in participants who were users of drug injection and those who were infected with HIV. HCV was comparatively higher in those who had a previous history of unsafe surgical procedures than their counterparts. Homeless participants were highly exposed to HCV and HIV infections than their counterparts. The HBV/HIV Co-infection prevalence was 3.5%. Those making unprotected sexual practice [P= 0.03], Injection Drug users [P= 0.03] those who had HBV infected person in their family [P=0.02], infected with HIV [P= 0.025] were statistically associated with HBV infection. HCV was significantly associated with Substance users and previous history of unsafe surgical procedures [p=0.03, p=0.04) respectively. HIV was significantly associated with unprotected sexual practices and being homeless [p=0.045, p=0.05) respectively.

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Characterization Of The Cerebrospinal Fluid Virome In Virological Suppressed Hiv-1 Infected Patients And Correlation With Hiv-1 Detection In Csf

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Background: Despite optimal virological control attained by antiretroviral therapy (ART), a significant proportion of people living with HIV (PWH) still displays HIV-RNA traces and inflammation in different body compartments, like the central nervous system (CNS). To date, it is unclear whether blood-brain barrier (BBB) or immunity impairment might allow virus passage, thus enriching CNS virome composition and thereby CNS inflammation.

Material and Methods: Eighty-one cerebrospinal fluid virome (CSFV) of adult PWH on three drug regimen ART with plasma HIV-RNA<200cp/mL and no active CNS disorders were analyzed. Prokaryotic and eukaryotic viruses (DNA+RNA viruses) were enriched and sequenced on Illumina NextSeq, following a modified version of QIAseq® Single Cell RNA Library Kit. After host decontamination and background removal, taxonomy and species abundance were estimated by Kraken2 and Bracken. CSFV composition analyses, including α (Observed, Shannon and Simpson) and β (Bray-Curtis and Jaccard) diversities and PCoA, were performed by phyloseq and vegan R packages, while viral reads were assembled with Spades. CSFV composition was compared to participants' demographics, viroimmunological status, neurocognition, and CSF inflammatory biomarkers by Spearman correlation and Mann-Whitney tests.

Results: Among the 81 participants (71.6% male, median age 49 years and CD4+ T cell count 455 cell/µL), 21(25.9%) had CSF HIV-RNA >20cp/mL (median[IQR]: 46[30-58] cp/mL). After background filtering, metagenomic analysis returned 289.89 million non-human reads (2.27[0.81-4.66] million/subject). When considering only viral reads, CSFV was detected in 58(71.6%) samples, with a median of 305(122-1035) viral reads. All CSFV+ samples presented bacteriophages (median[IQR] reads: 231[110-1,057]). The most abundant family was Siphoviridae with median (IQR) reads of 233(133-1,071), followed by Myoviridae (72[45-111]) and Podoviridae (72[42-172]).
Six (10.4%) samples carried eukaryotic viruses (median[IQR] reads: 122[79-303]). The most represented families were Herpesviridae (EBV, 1,467 reads, and HHV-6, 356 reads), followed by Papillomaviridae (HPV-96/201 with 72 and 145 reads, respectively). In 2 samples, HCV (98 reads) and TTV (27 reads) were also found. Read assembly confirmed the presence of all eukaryotic viruses as assembled contigs.
The within-specimen CSFV composition (α-diversity) was significantly higher in HIV-1 CSF samples with detectable HIV-1 RNA at RT-PCR compared to CSF samples with undetectable HIV-1, when measured by Shannon and Simpson indexes (median[IQR]: 1.55[1.03-2.37] vs 1.40[0.68-2.62],p=0.032 and 0.78[0.61-0.81] vs 0.65[0.37-0.89], p=0.027, respectively), while a trend could be detected by looking at Observed species (p=0.075). A positive correlation was also observed between all α-diversity indexes and CSF HIV-1 viral load (p<0.05). A higher CSFV α-diversity was also seen in patients with lower CD4+T cells and CSF glucose (p<0.05). All these associations were further confirmed by correlation tests (Rho= +0.264; -0.444; -0.223, respectively, p<0.05 for all). No associations were found with BBB permeability. Significant differences were not appreciated for β-diversity, likely due to the limited sample.

Conclusion: Thanks to refined metagenomic analyses, detecting both prokaryotic and eukaryotic viruses, we described the CSF virome profile in PWH. Our data suggest that virome composition may be associated with HIV-1 detectability in CSF, and may correlate with worse viroimmunological profiles. Further insights are needed to confirm these findings and address the interplay among HIV, CSFV and neuroinflammation.

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Ultrasensitive HBV-RNA Quantification as a Promising Biomarker to Optimize the Staging of Chronic HBV Infection and to Detect Minimal Viral Activity Under Prolonged Virological Suppression and Occult HBV Infection

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Background: Serum HBV-RNA quantification reflects the burden of virions containing pre-genomic RNA (pgRNA) and is used as surrogate marker of cccDNA transcriptional activity. Here, we define HBV-RNA levels across the natural history of HBV infection, including the under studied phase of occult HBV infection (OBI) and the levels after long-term NUC exposure.

Material and Methods: This study includes 106 treatment-naive patients (pts) categorized in 17 eAg+ with chronic infection (CI), 7 with eAg+ chronic hepatitis (CH), 50 with eAg- CI and 32 with eAg- CH according to EASL guidelines. 38 eAg- virologically suppressed pts (HBV-DNA <10 IU/ml) under long-term NUC treatment and 68 Anti-HBc+/HBsAg- pts (28 HIV coinfected, 18 HCV-viremic coinfected and 22 monoinfected under iatrogenic immunosuppression) are also included. Serum HBV-RNA is quantified by droplet digital PCR (ddPCR) targeting pgRNA (LOQ: 5 copies (cps)/ml).

Results: eAg+ CH and CI have elevated HBV-RNA levels (median [IQR]: 7.5 [5.7-8.3] and 7.1 [6.7-7.4] log cps/ml) in line with high HBV-DNA and HBsAg production (median [IQR] HBV-DNA: 9.2[7.4-9.8] and 8.9 [8.7-9.2] log IU/ml; HBsAg: 20,895 [10,696-67,693] and 52,518 [32,236-77,136] IU/ml).

HBV-RNA undergoes a significant decrease in eAg- phases, achieving the lowest levels in eAg- CI (median[IQR]: 1.3[0.8-2.0] in eAg- CI vs 2.5[1.9-3.9] log cps/ml in eAg- CH, P<0.001), paralleling the declining trend of HBV-DNA and HBsAg. In both eAg- phases, HBV-RNA correlates with HBV-DNA (Rho=0.49, P<0.001 for eAg- CI and Rho=0.33, P=0.06 for eAg- CHB) while no correlation with HBsAg is revealed (Rho=0.2 and 0.12, p>0.2), consistent with HBsAg production from integrated HBV-DNA. Notably, by AUROC, HBV-RNA <50 cps/ml shows the best accuracy in predicting the status of eAg- CI (sensitivity: 87.5%, specificity: 71%).
In virologically suppressed pts (median [IQR] NUC duration: 6.0 [4.1-9.1] years), HBV-RNA is positive in 78.9% of pts with a median of 1.7 (1.3-2.0) log cps/ml, despite HBV-DNA undetectability. Notably, the rate of HBV-RNA positivity and HBV-RNA levels remain stable independently from NUC duration, suggesting no decline in intrahepatic HBV activity over prolonged therapy (73.3%, 75% and 90% for NUC duration of <5, 5-9 and >9 years, with median [IQR] HBV-RNA of 1.7 [1.5-1.9], 1.4 [1.2-1.4] and 1.8 [1.6-2.3] log cps/ml).
Finally, HBV-RNA is also positive in 29.4% of Anti-HBc+/HBsAg- pts (median [IQR]: 0.9 (0.7-1.3) log cps/ml): 32.1% in HIV coinfected, 31.8% in monoinfected and 22.2% in HCV coinfected pts, supporting occult viral activity despite HBsAg negativity. Notably, in the setting of HIV coinfection, HBV-RNA positive pts are characterized by a lower nadir CD4 T-cell count (median [IQR]: 181 [69-242] vs 283 [94-441] cells/µl, P=0.08), highlighting the role of immune-compromission in modulating HBV replicative activity.

Conclusion: The production of pgRNA-containing viral particles predominates during the initial phases of chronic infection and decreases after HBeAg-seroconversion. In this context, HBV-RNA can enhance the categorization of chronic HBV infection including the eAg- infection status. By detecting minimal viral activity in the setting of long-term NUC treatment or OBI, the ultra-sensitive HBV-RNA quantification can contribute to discriminate patients achieving or not functional cure.

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Evaluation of a Standardized Dried Blood Device to Monitor SARS-CoV-2 and Hepatitis B Vaccine Response in Persons Living With HIV

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Background: Vaccine responses are often less robust among persons living with HIV (PWH), and may wane more quickly compared to those without HIV. Hence, it is of primary importance to collect data on vaccine response and long-term persistence of antibodies in PWH. Novel devices for the collection of capillary blood at home may contribute to develop an alternative to vaccine monitoring based on venipuncture at hospital.
Objective: In this study, we assessed vaccine response SARS-CoV-2 and hepatitis B virus (HBV) in PWH, and evaluate the performance of a standardized dried blood collection device (Ser-Col) to quantify SARS-CoV-2 receptor-binding domain IgG (RBD-IgG), and anti-hepatitis B virus (HBV) surface antigen antibodies (anti-HBs Abs).

Material and Methods: This cross sectional study is part of the EU-funded "SCAUT" research project (from finger to laboratory: personalized and automated blood collection for laboratory diagnostics). We included 256 PWH without prior SARS-CoV-2 infection, vaccinated or immunized against HBV. Samples were collected between October 2021 and January 2022 at Montpellier University Hospital, two to six months after the second dose of SARS-CoV-2 mRNA vaccine (Pfizer-BioNTech or Moderna) or adenovirus vector vaccine (AstraZeneca). The Ser-Col device (Labonovum, Netherlands) contains a microfluidic paper separating plasma from whole blood. RBD-IgG and anti-HBs Abs were quantified in plasma and Ser-Col eluate using fully automated

chemiluminescence immunoassays (Alinity i, Abbott, IL, USA).

Results: RBD-IgG response was higher in participants vaccinated with Moderna compared to Pfizer-BioNTech (p=0.021) and AstraZeneca (p<0.0001). We observed a lower RBD-IgG level when CD4/CD8 ratio was < 0.6 (p = 0.032). Anti-HBs Abs level was lower in the group of HBV vaccinated with < 350 CD4 T cells/mm3 (p=0.0062). The antibody levels quantified on the Ser-Col eluate were highly correlated to plasma levels: r2 = 0.98 and 0.99 for RBD-IgG and anti-HBs Abs, respectively).
Agreement between Ser-Col and plasma results were substantial or almost perfect for RBD-IgG: absence of RBD-IgG (<7 BAU/mL) Cohen's kappa: 1, low RBD-IgG level (7,1 – 266 BAU/mL) Cohen's kappa: 0.88, moderate RBD-IgG level (266,1 – 590 BAU/mL) Cohen's kappa: 0.72, high RBD-IgG level (> 590.1 BAU/mL) Cohen's kappa: 0,95. The agreement was almost perfect or perfect for anti-HBs Abs: anti-HBs Abs positive (> 10 IU/L) Cohen's kappa: 0.97, moderate HBs Abs level (10-100 IU/L) Cohen's kappa 0.91, high HBs Abs level (> 100.1 IU/L) Cohen's kappa: 0.97.

Conclusion: Our results support previous findings showing that antibody responses differ according to Covid vaccines and CD4 T cell count in PWH. The Ser-Col device facilitate collection of capillary blood, transport and testing of dried blood. Micro-sampling on standardized dried blood devices could help to enable decentralized clinical trials and improve access to vaccine monitoring.

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Hepatitis Delta Diagnosis: Do We Need Reflex Testing?

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Background: Hepatitis delta constitutes a global health problem affecting between 15 and 20 million people worldwide; with the recent introduction of Bulevirtide, the first antiviral for its treatment, and the imminent arrival of new antivirals against B virus, therapeutic options are expanded and may facilitate elimination strategies. Our aim has been to analyze the current status of undiagnosed hepatitis Delta in the South of Spain (Andalusia), and evaluate the efficacy and feasibility of hepatitis delta reflex testing.

Material and Methods: An ambispective (retro- and prospective) multicentre study in 17 hospitals in Andalusia is ongoing. In the retrospective phase, we have analysed hepatitis delta diagnostic cascade, searching for HBsAg-positive individuals, those in whom anti-delta antibody detection was performed, and those in whom HDV RNA detection was performed, extracting data from the laboratory information systems (LIS) of the participating centres (January 2018 to June 2022). From October 2022 to March 2023, all centres initiated the prospective phase in which reflex hepatitis delta

(testing for anti-HDV in all HBsAg positive individuals without a prior test) was implemented.

Results: Regarding the retrospective phase, a total of 17872 HBsAg positive individuals were analyzed; of those, anti-HDV was performed in 3287 (18%), and was positive in 178 individuals (5,4%); HDV RNA was tested in 131 individuals and, finally, 36 individuals (1,1%) were identified as HDV-RNA positive.
The prospective phase is ongoing. We present data from a single center (Hospital San Cecilio, Granada). From a total of 258 HBsAg positive individuals, 104 had already been tested for anti-HDV; from the remaining 154 individuals, reflex anti-HDV was performed in all of them (100%); eleven (7,1%) were anti HDV positive and subsequently tested for HDV-RNA, finding one viremic patient (0,7%). Final results from all the participating sites will be presented at the conference.

Conclusion: Our retrospective data indicate an infra-diagnosis of HDV: only a fifth of HBsAg positive individuals have been screened for anti-HDV through the years 2018 to early 2022, indicating there is a need for the implementation of strategies to overcome it. Despite the limitations of our study, and waiting for final results we show that reflex HDV testing is feasible and may result in an increase in the detection of individuals with HDV chronic infection. We believe this implementation will facilitate finding the "missing" hepatitis delta individuals.

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Detecting Hepatitis C Outbreaks Among Men Who Have Sex with Men: Findings from a Community-Based Screening Program in Barcelona

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Background: Hepatitis C Virus (HCV) outbreaks have been reported among Men who have Sex with Men (MSM), particularly in relation to People Who Live with HIV (PWLHIV) and Pre-exposure Prophylaxis (PrEP) use. BCN CheckPoint, a community-based center for MSM in Barcelona, began an intensive Hepatitis C screening program in 2021 for all clients attending routine HIV and sexually transmitted infection (STI) screening or PrEP controls.

Material and Methods: During the screening campaign, confirmed cases in our center were invited to complete an assessment form to identify sexual and other possible behaviors related to HCV transmission. The survey included sexual practices and places, drug use, ulcerative STIs, and dating apps.

Results: Between August 2021 and February 2023 a total of 9843 persons were screened and 22 HCV infections were reported, of which 14 (63.6%) were considered acute. The genotypes were distributed as follows: 12 cases (1a), 2 cases (1b), 1 case (3), and 6 cases of type (4). 8 (36.4%) cases were PrEP users, 9 cases (40.9%) were non-PrEP users, of which 2 were diagnosed with HIV at the same time, and 5 (22,7%) were PWLHIV. All cases were living in Catalonia, 50% were born in Spain, 13.6% were

born in other European countries, and 36.4% were born in South America. Regarding sexual behaviors, when comparing acute vs. chronic HCV infections, meeting via apps, especially Scruff (57.1% [28.9-82.3] vs. 0) and MachoBB (28.6% [8.4-58.1] vs. 12.2% [0.3-52.7]), appeared more frequent use among acute infections. Recent ulcerative STIs (50% [23-77] vs. 25% [3.2-65.1]) and sharing sexual toys (42.9% [17.7-71.1] vs. 25% [3.2-65.1]) showed a higher frequency among acute HCV cases. In general, 90.9% reported condomless receptive anal sex , 36.4% shared sexual toys, 45.5% had practiced fisting, 18.2% had practiced Slam, 0% shared needles, 40.9% shared sniffing rolls, 45.5% reported sex with somebody who slams, and 40.9% had previous ulcerative STIs. Only 9.1% reported condomless anal intercourse as a possible mode of transmission.

Conclusion: Understanding outbreaks and modes of transmission can help focus screening campaigns and public health agency interventions. Most of our cases showed at least one classic risk factor associated with HCV transmission, although condomless anal intercourse was the only risk factor reported by 9%. HCV screening must be extensively included in screening campaigns among the MSM community to achieve micro-eradication goals.

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Evaluation of Three Assays for Hepatitis Delta Virus RNA Detection

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Background: Hepatitis delta virus (HDV) infection increases the severity of chronic hepatitis B virus (HBV) infection, can lead more rapidly to liver failure, cirrhosis or hepatocellular carcinoma affecting younger individuals. Detection and quantification of HDV in these individuals is of vital importance for diagnosis and, very recently, for treatment management. Furthermore, eight HDV genotypes have been described worldwide, and the specific genotype could influence the severity of the disease and, also, the accuracy of viral load tests. The aim of this study was to evaluate and compare the efficacy of the three commercial assays for the detection and quantification of HDV RNA.

Material and Methods: Sixty-three HDV-RNA positive samples previously analysed with Hepatitis Delta RT-PCR system kit (Vircell, Granada, Spain) were tested in parallel with EurobioPlex HDV assay (Eurobio Scientific, France), and sixty-two of them with RoboGene HDV RNA Quantification kit (Roboscreen Diagnostics, Leipzig, Germany). In addition, 200 samples (100 serum and 100 plasma) from individuals never exposed to HBV were also analysed with Hepatitis Delta RT-PCR system kit. For genotyping, an amplicon-based sequencing strategy with overlapping primers was used to amplify the whole genome of HDV on a Nextseq 1000 (Illumina).

Results: Using the EurobioPlex HDV kit, 61 samples were positive and 2 were negative; with Robogene HDV 60 were positive and 2 negative. Considering the qualitative result, the concordance between the Vircell Hepatitis Delta kit and the EurobioPlex HDV kit was 96.9%, the concordance between the Vircell Hepatitis Delta kit and the RoboGene HDV RNA kit was 96.8%, while the concordance between EurobioPlex HDV kit and RoboGene HDV RNA kit was 100%. All 200 HBV-negative samples tested by the Hepatitis Delta RT-PCR kit (Vircell) were all negative.
Regarding the quantitative result of HDV RNA (log IU/ml), we found a correlation coefficient of 0,703 for the comparison of Hepatitis Delta RT-PCR/EurobioPlex HDV, of 0,833 for Hepatitis Delta RT-PCR/RoboGene HDV RNA Quantification and of 0,825 for EurobioPlex HDV / RoboGene HDV RNA Quantification. The Bland-Altman statistical analysis yielded a mean bias of 2,083 Log10 UI/mL and -1,283 Log10/mL between Eurobio/Vircell and Robogene/Vircell respectively, that showed significant differences in the quantitative performance of all the three kits compared.
Full HDV genome sequencing data is available in 45 samples: 38 were annotated as genotype 1 and 7 as genotype 5.

Conclusion: In terms of qualitative detection of HDV-RNA, we have shown a good concordance

between the three assays evaluated. However, as there are important differences at the quantitative level, we believe that these three assays are not interchangeable for monitoring HDV viral load during HDV treatment. All three systems were able to detect and quantify HDV-RNA from individuals infected by genotypes 1 and 5.

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Quantitative Hepatitis C Virus (HCV)-RNA Analysis Using the NeuMoDx System

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Background: Quantification of hepatitis C virus (HCV) RNA in serum or plasma is an important method for diagnosing active HCV infection and for monitoring disease progression and the efficacy of antiviral therapies. Various commercial kits and automated platforms are available for these quantitative PCR analyses. One of the latest platforms is the NeuMoDx (NMDx) system (Qiagen).
Our aim was to investigate the linearity and reproducibility of quantitative HCV analyses on the NMDx system for the most common HCV genotypes and to directly compare the results of the NMDx and the widely used cobas c6800 system (Roche).

Material and Methods: Linearity was analysed using 19 HCV serum/plasma samples from patients with the four most common HCV genotypes (1-4) in our cohort. Serial dilutions were prepared in HCV-negative plasma and tested in duplicate with the NeuMoDx HCV assay according to the manufacturer's instructions. For precision analysis, 7 plasma samples with different HCV RNA concentrations (range 0 - 1,000,000 IU/ml; provided by Qiagen) were quantified in triplicate in 10 different runs. The coefficient of variation (CV) within runs (intra-assay) and between runs (inter-assay) was calculated based on the Ct values. For comparison of the NMDx with an established reference platform, the results of 33 HCV plasma/serum samples were correlated with the HCV RNA concentrations determined on the c6800 reference system.

Results: Quantitative HCV RNA analysis with the NMDx system showed high coverage of HCV genotypes and subtypes and, in addition, very good linearity with patient samples across all HCV genotypes tested (R2 range: 0.9257-0.9991). We observed low intra-assay variation in the mean triplicate analysis of 1.650% (range 1.045-3.778%) and also adequate inter-assay variation from ten independent runs with a mean CV of 2.462% (range 1.385-3.142%), representing low variation in HCV measurements. A direct comparison of 33 clinical samples between the c6800 and NMDx platforms showed a strong correlation (R2=0.6254, p=0.0001), but with HCV RNA concentrations of the c6800 on average 3.3-fold higher compared to the NMDx results (p=0.0006).

Conclusion: This study illustrates high reproducibility, linearity and precision of the NMDx HCV RNA assay. The strong correlation with results obtained on the cobas c6800 system as an established assay for monitoring HCV RNA levels demonstrates the suitability of the NMDx HCV assay for routine diagnostics.

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Detection of Circulating SARS-CoV-2 Variants of Concern (VOCs) Using a Multiallelic Spectral Genotyping Assay

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Background: SARS-CoV-2 has continuously been evolving since the start of the COVID-19 pandemic. This resulted in new variants, some of which possessing increased infectivity, and immune evasion, posing a high risk to public health. Such variants have been denoted by the World Health Organization as variants of concern (VOC). Thus far, five VOCs have been designated, Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), including their sublineages.

Even though next-generation sequencing (NGS) can yield a high amount of information for the study of variants; it is time-consuming and inefficient during outbreaks, when rapid identification of VOCs is required. During these periods, fast and accurate methods are needed, such as real-time RT-PCR in combination with probes, which can be used for monitoring and screening of the population for these variants.

Material and Methods: Thus, we developed a molecular beacon(MB)-based real-time RT-PCR method to detect and discriminate between VOCs according to an adaptation of the principles of spectral genotyping. This assay uses a combinatorial pattern-based reporting system to achieve discrimination by using multiple molecular beacons at the same time, each targeting different deletions/insertion, of SARS-CoV-2. Targeting deletions/insertion for this assay was due to the high discriminatory power they inherently confer as a result of the larger nucleotide difference between genomes with and without the deletions/insertion. The assay focused on the ORF1a and S gene regions of SARS-CoV-2 VOCs: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (BA.1, BA.2, BA.4, BA.5). These regions contain the targets of the molecular beacons: the deletions ORF1a:ΔS3675/G3676/F3677, S:ΔH69/V70, S:ΔE156/F157, S:ΔN211, and S:ΔL242/A243/L244 as well as the insertion S:ins214EPE, with each of them reported to be highly prevalent in specific VOCs. Specifically, ORF1a:ΔS3675/G3676/F3677 is found in Alpha, Beta, Gamma, and Omicron (BA.2, BA.4, BA.5); S:ΔH69/V70 is found in Alpha and Omicron (BA.1, BA.4 and BA.5); S:ΔE156/F157 is found in Delta. S:ΔN211 and S:ins214EPE are found in Omicron (BA.1); and S:ΔL242/A243/L244 is found in Beta. Following the selection of targets, the molecular beacons and primers were designed de novo for this assay. The folding and structure of each MB along with their thermodynamic details were examined through the mfold DNA folding program. All MBs were labeled with HEX fluorophore at the 5'-end and DABCYL at the 3'-end. The stem section of each MB is 5-7 nucleotides (nts) with high-GC content, while the loop is 21-34 nts and is complementary to the gene fragment to be amplified. The thermodynamic compatibilities and dimer formation of primers and MB were examined through the IDT Oligo Analyser webtool. A melting curve analysis was performed to assess the thermodynamic characteristics of each MB. The analysis was completed by real-time RT-PCR using the 4× TaqPath™ 1-Step Multiplex Master Mix (No ROX) (Life Technologies, Frederick, MD, USA).

Results: The results of the melting curve analysis revealed that all molecular beacons can be used under the same real-time RT-PCR conditions (annealing temperature 53 °C), consequently improving the time and cost efficiency of the assay. The assay was then tested using a panel of reference samples derived from cultured SARS-CoV-2 virus (Vero E6 cells) obtained from European Virus Archive goes Global (EVAg, Charité, Berlin, Germany). These samples correspond to the first four VOCs, Alpha, Beta, Gamma, and Delta. In addition to the EVAg panel of reference samples, the assay was tested using clinical samples (Bioethical approval received) derived from the study of the Genomic Epidemiology of the SARS-CoV-2 Epidemic in Cyprus which were collected under the ongoing collaboration between our laboratory (Laboratory of Biotechnology and Molecular Virology of the University of Cyprus), the Ministry of Health, Medicover Genetics, and the other members of the Cypriot Comprehensive Molecular Epidemiological Study on SARS-CoV-2 (COMESSAR) Network. These samples were classified as Delta and Omicron (including their sublineages). Based on the results, it was shown that this assay was able to confirm the genotype of each of the reference samples and clinical samples from various VOCs. Furthermore, the molecular beacons used in this assay displayed high specificity in identifying their intended target and discriminating against the incorrect target, as the results show that there was no fluorescence with incorrect targets.

Conclusion: This assay constitutes an accurate and reliable uniplex molecular beacon-based real-time RT-PCR assay that can be used for detection and discrimination of VOCs. The assay was able to accurately identify the correct VOCs when tested using reference samples from cultured virus received from EVAg (Charité, Berlin, Germany), as well as clinical samples previously classified by NGS. Overall, this assay is a valuable tool that can be used for screening and monitoring the population for VOCs or other emerging variants, contributing to limiting their spread and protecting public health.

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Reverse Transcription of Plasma-Derived HIV-1 RNA Generates Artifacts Through tRNA(Lys-3) Priming

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Background: In order to keep up with the increasing amount of HIV drugs and the introduction of new antiretroviral classes, future genotypic drug resistance tests will need to include additional HIV genomic regions. This evolution rekindles the interest in developing a sensitive protocol for near full-length HIV RNA sequencing from plasma.

Material and Methods: HIV RNA was extracted from high viral load plasma samples from 3 individuals with an HIV-1 subtype B infection, using the QIAamp Viral RNA Mini Kit (Qiagen). Four enzymes were used to perform reverse transcription (RT): SuperScript IV (Thermo Fisher Scientific), PrimeScript II (Takara Bio), Transcriptor (Roche) and M-MuLV (NEB). Either random 6-mers or anchored oligo(dT) were used to prime RT. No-enzyme as well as no-primer control reactions were run simultaneously. RT performance was monitored by performing 2 real-time PCRs, one with primers/probe located in integrase (INT) and the other with primers/probe in the 5' untranslated region (5'UTR). HIV-specific nested PCRs were performed to confirm real-time PCR results.

Results: Real-time PCR outcomes for 5'UTR showed cycle threshold (Ct) values that correlated with the viral load of the individual samples. Remarkably, high amounts of 5'UTR cDNA were observed in the no-primer control RT reactions. No substantial differences in Ct values for 5'UTR were observed between random 6-mers, anchored oligo(dT) and no-primer RT reactions. Ct values for the INT real-time PCR, on the other hand, did alter depending on primer choice with, as expected, the lowest Ct values for random 6-mers reactions and slightly higher values for anchored oligo(dT) reactions. Overall, based on Ct values, only minor differences were observed in the performance of the different RT enzymes. A nested PCR targeting the 5'UTR region confirmed the generation of HIV 5' UTR cDNA in the no-primer control reactions. Sequencing of the obtained 5'UTR amplicons revealed clear individual-specific sequences, refuting a contamination problem. Furthermore, negative no-enzyme control reactions ruled out the presence of DNA contamination. The use of 5'UTR and tRNA(Lys-3) specific primers allowed us to amplify and sequence a HIV-UTR/tRNA(Lys-3) hybrid molecule from all reactions, including the no-primer controls. This confirmed the presence of high amounts of tRNA(Lys-3) in plasma-derived HIV RNA extracts and its function as initiator of strong-stop minus-strand cDNA synthesis during in vitro RT.

Conclusion: Commonly used silica-based nucleic acid purification methods for extraction of HIV RNA from plasma co-extract tRNA(Lys-3). Our findings show that a tRNA(Lys-3) molecule is bound to nearly every single viral RNA molecule in the extract. Moreover, the HIV RNA-tRNA interaction appears to be exceptionally stable, both the procedures for RNA extraction and the initial steps of RT do not wipe out existing interactions. During in vitro RT, the tRNA(Lys-3) functions as an efficient primer for 5'UTR minus-strand cDNA synthesis. This spontaneous generation of strong-stop minus-strand HIV cDNA must be taken into account as it may induce bias in certain applications.

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Whole Genome Sequencing of Hepatitis Delta Virus (HDV) Using Next-Generation Sequencing: A New Tool to Detect Nucleotide Variations and Phylogenotyping

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Background: Hepatitis Delta Virus (HDV), a satellite of Hepatitis B Virus (HBV) on which it depends for its amplification, is known to possess the smallest genome (1700 nucleotides) among viruses that can infect humans. HBV/HDV co-infections affect over

12 million people worldwide and Chronic hepatitis D (CHD) is the most severe form of viral hepatitis, characterised by the greatest increase in risk of cirrhosis, hepatic decompensation, and hepatocellular carcinoma. Among the new treatment approaches under clinical evaluation, Bulevirtide, an entry inhibitor, is the only one that has received conditional approval from the European Medicines Agency (EMA) in July 2020, and approval is pending in the United States. No selection of BLV resistance in HBV/HDV has been reported in vivo to date, but the implementation of an effective drug resistance surveillance system is becoming a priority. Today, many public and private laboratories are equipped with NGS sequencers because of the covid crisis we have just gone through. It is a real windfall to put this technology at the center of the surveillance device. The objective of this study was to evaluate the Whole Genome HDV assay using NGS and Sanger.

Material and Methods: HBV/HDV positive human plasma samples and one negative human plasma sample were prepared using MagNa pure 24 (Roche). The whole genome HBV DNA and HDV RNA were amplified using the DeepChek® HBV Assays Whole Genome HBV (ABL) and the DeepChek® HDV Assays Whole Genome HDV (ABL). HBV/HDV libraries were sequenced using the NGS iSeq100 (Illumina). Sequences were compared to HBV/HDV reference genome. DeepChek® HBV/HDV Whole Genome software (ABL) was used for the interpretation of subtype and drug resistance.

Results: Samples were accurately genotyped. The median coverage per sample for the whole genome sequencing of the HBV genotypes was 10.000 reads and the Q30 was 87% (2x150bp). HDV was detected in two HBV genotype B positive patients. The phylogenetic analysis carried out made it possible to classify the two sequences obtained as genotype 1, one of the eight genotypes known to date in HDV. NGS provides a cost-effective solution for identifying potential new or rare drug resistance variants.

Conclusion: This study is the first evaluation of the DeepChek® Assays Whole Genome HDV (ABL) using the iSeq100 system combined with an easy software. NGS should occupy a major place in HIV, HCV, and HBV/HDV resistance surveillance, thanks to its decreasing costs and ability to reveal resistant minority variants or new mutations and study their impact.

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Aichi Virus Infection as a Cause of Chronic Hepatitis in Patients With Primary Immune Deficiencies

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Background: Metagenomic Next Generation Sequencing (mNGS) was used to assess patients with primary or secondary Immune Deficiencies (PIDs and SIDs) presenting with immunopathological conditions related to immunodysregulation.

Material and Methods: 30 patients with PIDs and SIDs presenting symptoms related to immunodysregulation and 59 asymptomatic patients with similar PIDs and SIDs were enrolled. mNGS was performed on organ biopsy (liver, spleen, kidney). Specific AiV RT-PCR was used to confirm Aichi virus (AiV) infection and screen the other subjects. In situ hybridization assay (ISH) was done on AiV infected liver and spleen tissue to identify infected cells. Virus genotype was determined by phylogenetic analysis.

Results: AiV sequences were detected by mNGS in tissue samples of 5 patients and by RT-PCR in peripheral samples of another patient who all presented with PID and long-lasting multi-organ involvement, including hepatitis, splenomegaly in all and nephritis in 4. CD8+ T cell infiltration was a hallmark of the disease.
RT-PCR detected intermittent low viral loads in urine and plasma from infected patients but in none of the other subjects. Viral detection stopped after immune reconstitution obtained by hematopoietic stem cell transplantation. ISH demonstrated the presence of the AiV RNA in hepatocytes (n=1) and spleen tissue (n=2). AiV belonged to genotype A (n=2) or B (n=3).

Conclusion: The similarity of the clinical presentation, the detection of AiV in a sub-group of patients suffering from immunodysregulation, its absence in asymptomatic patients, the detection of viral genome in infected organs by ISH, and the reversibility of symptoms after treatment argue for AiV causality.

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Evaluation of HIV-1 Gag Gene Variability and Drug Resistance-Associated Mutations According to Viral Subtypes Among Drug-Naïve Individuals

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Background: HIV-1 Gag mutations contribute substantially to protease- and capsid-inhibitors resistance besides compensating for loss of viral fitness. However, little is known about the significance of these mutations according to viral clades among drug-naïve patients and their effect on the viral immune escape potentials. We sought to evaluate the natural variability of the entire Gag gene according to different subtypes, with a special focus on mutations associated with protease inhibitors (PI) and Lenacapavir (LEN) resistance. We further investigated the role of Gag drug-resistance mutations on the viral immune escape.

Material and Methods: A total of 2031 HIV-1 sequences from drug-naive patients were analysed for capsid amino acid modification and the prevalence of Portease inhibitors and lenacapavir DRMs. Amino acid positions with <5% variability were considered as conserved and variability was analysed by HIV-1 clades.

Results: Overall, 2031 HIV-1 Gag sequences were retrieved from the Los Alamos HIV database and analysed based on documented Gag mutations associated to PI and LEN resistance. Overall conservation analysis of the Gag gene revealed 255/500 (51.0%) amino acid positions were conserved (< 5% variability) in the entire study population and p24 (capsid) protein presented the highest conservation rate (156/231; 67.5%). The prevalence of Gag mutations associated to PI resistance was as follows: Y79F (46.28%), R76K (39.73%), P453L (16.0%), K436E (13.24%), V128I (6.79%), T81A (5.76%), L449F (2.80%), I437T (1.23%), R452S (0.54%), A431V (0.44%) and K112E (0.09%). Of these, apart from R76K, T81A and I437T, similar prevalences were observed between B and non-B subtypes. Conversely, Gag mutations associated to PI resistance were significantly higher in viral recombinant forms as compared to pure subtypes (72.3% [542] vs. 31.1% [398], p< 0.0001). Concerning LEN, the prevalence of overall resistance mutations was 0.14% (3/2031); with M66I (0.05%) and Q67H (0.05%) observed in subtype C and T107N (0.05) in CRF01_AE. Interestingly, four Gag mutations associated to PI resistance (K436E, I437T, L449F and P453L) and one LEN resistance mutation (Q67H) are significantly associated with a reduced binding affinity between T-cells'lymphocyte epitopes and MHC class II molecules.

Conclusion: Among antiretroviral-naïve patients, about half of amino acid positions within the Gag gene are conserved. Higher rates of Gag mutations associated to PI resistance are found with recombinant viruses. The low resistance to LEN (< 1%) suggests a high effectiveness of LEN-based regimens.

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DORAVIR: A French National Survey of People With HIV-1 Treated by an Antiretroviral Regimen Including Doravirine

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Background: Doravirine (DOR) is the latest NNRTI approved drug to treat HIV-1 and has a distinct resistance profile from first generation NNRTIs. We aimed to describe the factors associated with virological prior regimen failure (VF) in naïve and experienced-antiretroviral people living with HIV-1 treated with a DOR-containing regimen in clinical practice.

Material and Methods: Between November 2020 and December 2022, a national survey including people with HIV-1 either naïve or experienced receiving an antiretroviral treatment including DOR was conducted. VF was defined as 2 consecutive plasma viral load (VL) > 50 copies/mL or 1 VL > 200 copies/mL. Reverse transcriptase (RT) was sequenced at baseline. Mutations associated with resistance and the genotypic susceptibility score (GSS) of the current regimen with DOR have been studied according to the Stanford algorithm. Gender, viral subtype, nadir CD4 count, CD4 count at baseline, log zenith plasma HIV-1 RNA, log plasma HIV-1 RNA at baseline, presence of NNRTI resistance mutations at baseline (Stanford algorithm) and GSS were investigated as potential factors associated with VF.

Results: Among the 594 people with HIV-1 treated by any DOR-containing regimen, 8.6% were naïve and 91.4% were previously experienced (n=461 were virologically suppressed at time of switch to a DOR-containing regimen and n=82 had a VL > 200 cp/ml), 335 (56.4%) were infected with HIV-1 B subtype and 259 (43.6%) with non-B subtypes. The median duration of current DOR regimen was 12.1 months (IQR 6.9-19.0). The DOR co-treatment was principally 3TC+TDF (n=419, 70.5%), DTG (n=69, 11.6%), RAL (n=54, 9.1%) and 3TC+ABC (n=31, 5.2%). The GSS was distributed as follows: n=14 (3.4%) with GSS 0-0.5, n= 97 (23.7%) with GSS 1-1.5, n= 70 (17.1) with GSS 2-2.5 and n= 229 (55.8%) with GSS 3.
Overall, 537 (90.4%) and 517 (87.0%) participants were in virological success at M3 and M6, respectively. At M3 and M6, the virological success was as follows depending of the context of DOR-containing regimen initiation: naïve 86.3% and 90.2%, switch controlled 82.7% and 89.4% and switch failing (on BL regimen) 82.7% and 71.6%, respectively
In univariate analysis, the factors associated with VF at 6 months after initiation of DOR-containing regimen were the viral subtype (OR 2.91 for CRF02_AG and OR 1.31 for non-B vs. B, p=0.0007), CD4 count at baseline (OR 0.91, p=0.0227), log zenith plasma HIV-1 RNA (OR 1.42 p=0.0015), log plasma HIV-1 RNA at baseline (OR 1.41 p=0.0002) and the presence of the baseline NNRTI resistance associated mutations A98G (OR 6.41, p=0.0007) and V179D (OR 6.01, p=0.0293). In the multivariate analysis, the HIV subtype remained associated with VF (OR 2.81 for CRF02_AG and OR 1.16 for non-B vs. B, p=0.0086), as well as the presence of A98G (2.6%; OR 4.81, p=0.0274) and V179D (1.11%; OR 5.84, p=0.0439). The log plasma HIV-1 RNA at baseline tended to be associated with VF (OR 1.655, p=0.0593).

Conclusion: This study is one of the largest studies characterizing DOR-containing regimen virological efficacy in routine clinical care and reveals factors associated with VF that should be taken into consideration in clinical management.

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People With Pan-Drug Resistant HIV Infection Can Have Low Levels of Intact DNA

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Background: HIV reservoir has not yet been investigated in heavily treated-experienced people living with HIV (PLWH) with a pan-drug resistant infection (PanDR), defined as a reduced susceptibility to the 4 principal antiretroviral drug classes.
Aim of the study was to asses HIV-1 reservoir by measuring the frequency of CD4 T cells that harbor intact proviral HIV-1 DNA in people with a PanDR infection on virological suppression.

Material and Methods: We included PLWH with a documented 4-class drug-resistant HIV infection from the PRESTIGIO registry on antiretroviral therapy (ART) and with HIV-1 RNA <50 copies/mL (PanDR group) and PLWH under suppressive ART with no history of drug resistance (non-PanDR group). Intact proviral HIV-1 DNA assay (IPDA) was performed using digital droplet PCR (Bio-Rad QX200 AutoDG Digital Droplet PCR system). Characteristics were reported as median (interquartile range) or frequency (%) and compared using the Chi-square/Fisher's exact test or the Wilcoxon rank-sum test.

Results: We evaluated 35 PLWH: 14 (13 M; 1 F) in PanDR group and 21 (19 M; 2 F) in non-PanDR group; median age was 57 (54-62) and 48 (40-52) years, respectively (p=0.008). Years of HIV-1 infection were 31.0 (29.3-32.2) in PDR people and 13.0 (8.3-22.6) and in no PDR ones (p<0.001), while exposure to ART was 27.4 (25.5-29.3) and 8.5 (7.0-16.6) years, respectively (p<0.001).
Median number of ART regimens was 15 (10-32) in the first group and 4 (3-6) in the second one (p<0.01).
Median level of copy/years viremia was 12218 (8535-22318) and 5591 (611-12834) among people with and without PanDR infection (p=0.117), while median CD4 was 629 (287-720) and 768 (414-1026) cell/mcL, respectively (p=0.081)
Median time spent on undetectable viremia since last positive viremia was 3.5 (1.9-5.5) vs. 6.9 (4.5-7.5) years (p=0.023). All the participants included in the analysis had a documented subtype B infection. Percentage of naïve CD4 T cells was significantly greater in the non-PanDR group [27.1 (18.9-48.5) vs. 8.8 (6.1-15.3), p=0.002], while percentages of CD4 T effector memory and T transitional memory were higher in the PanDR group [32.1 (24.8-39.8) vs. 18.2 (13.0-26.2), p=0.017 and 3.8 (2.1-5.3) vs. 1.1 (0.6-1.9), p=0.004, respectively]. No differences emerged in the quantification of intact and defective proviruses: intact HIV-1 DNA was 11.9 (0-46.5) and 16.7 (0-127.0) copies/10^6 CD4 T cells among people with and without PanDR infection (p=0.481), while 5' deleted proviruses were 167.6 (63.2-796.6) and 202.9 (86.9-410.7) copies/10^6 CD4 T cells (p=1) and 3' deleted proviruses 278.1 (67.0-615.1) and 419.5 (27.4-674.4) copies/10^6 CD4 T cells, respectively (p=0.801). We checked whether the presence of specific mutations in the envelope (env) region of the HIV genome could have influenced the results of the IPDA and data obtained from genotypic resistance testing (Sanger sequencing) showed mutations in the env region for 5 PLWH.

Conclusion: People with a PanDR infection can have low levels of intact proviral HIV-1 DNA, despite a long exposure to ART and a shorter period of undetectable viremia, even though mutations in the env region might have led to underestimate the amount of intact copies quantified by IPDA.

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Antiretroviral Resistance in Individuals With HIV-2 Infection: 5 Years of Experience

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Background: HIV-2 infection is endemic in West Africa, and is present in some regions of the world, namely in Portugal, which has the highest rate of this infection in Europe. Treatment initiation in individuals living with HIV-2 differs from HIV-1. All individuals living with HIV-1 must be treated to achieve undetectable viral load. Many of individuals living with HIV-2 have no detectable viral load so they do not transmit the infection. Due to this and the fact that there are no specific antiretrovirals for HIV-2, the treatment of these individuals depends on CD4+ lymphocyte count, plasma viremia and comorbidities. HIV-2 is treated with HIV-1 antiretrovirals, however there are much less therapeutic options for HIV-2 due to natural resistance to some antiretrovirals such as NNRTIs or fusion inhibitors and resistance selection is also much easier in HIV-2 than in HIV-1.
Objective: Retrospective analysis of resistance tests to antiretrovirals used in the treatment of HIV-2 infection (nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI) and integrase inhibitors (INTI)) performed at Centro Hospitalar Lisboa Ocidental, Portugal, between January 2017 and December 2021, from samples received from several hospitals in the center and south of Portugal and from the islands of Azores and Madeira.

Material and Methods: In plasma samples from people who are treatment naïve and treated individuals with viral load above 100 copies/mL, viral RNA was extracted in the automatic EMAG® equipment. RT-PCR and nestedPCR reactions were used according to an in-house protocol, then were sequenced by the Sanger method. Mutations identified were analysed according to the HIV-2-EU-algorithmGRADE.

Results: 365 resistance tests (RT) were performed; 263 for NRTI/PI and 102 for INTI. Overall 28% of NRTI/PI tests and 17% of INTI were positive. In people who are treatment naïve only 5% of the samples had resistance associated mutations (RAM) in the RT/PROT region. In the INTI region no RAM were found in people who are treatment naïve. In treated individuals 65% of individuals had RAM for the NRTI/PI and 34% for INTI. Overall the most frequently found mutations were: M184V (27%), V111I (19%) and K65R (17%) for NRTIs; I54M and L90M (24%) and I50V (19%) for PI; N155H and T97A (27%) and E92Q for INTI. 24% of RT to NRTI/PI and to 31% of RT to INTI did not amplify mainly due to low level viral loads.

Conclusion: Albeit not be able to amplify all samples, RT are a very useful tool for the detection of HIV-2 resistance both in people who are treatment naive and in treatment failure, allowing to choose the best regimen to achieve HIV-2 suppression and avoid the accumulation of resistance, which can exhaust the few options available. We found a preteatment drug resistance (PDR) of 5%. The most frequently identified resistance mutations have impact on the few HIV-2 recommended options. So whenever possible, RT should always be performed before starting or restarting treatment.

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The First Case of Integrase Strand Transfer Inhibitor Resistance Found During Transmitted HIV Drug Resistance Surveillance in Estonia

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Background: Integrase strand transfer inhibitors (INSTIs) consumption has widely increased in first-line as well as salvage antiretroviral therapy in Eastern-Europe and Estonia in the last years. However, there is limited data on HIV-1 transmitted drug resistance (TDR) in the region, especially to INSTIs. We aimed to determine the level of TDR in protease (PR), reverse transcriptase (RT) and integrase (IN) regions among newly diagnosed HIV-1 positive individuals in Estonia in 2020.

Material and Methods: Study included individuals diagnosed with HIV-1 in Estonia in 2020 (n=125). HIV-1 genomic RNA was sequenced in PR-RT and IN regions. Socio-epidemiological data was obtained from the Estonian Health Board. Clinical data was provided by hospitals' laboratories. Surveillance Drug Resistance Mutations (SDRMs) in PR-RT and IN region were determined using Stanford HIV Drug Resistance Database Calibrated Population

Resistance Tool and Stanford HIVdb Program (v 9.4). HIV-1 subtypes were determined separately for PR-RT and IN region by REGA HIV-1 Subtyping Tool (v 8.1). In case of discordant results subtype was assigned as unclassified recombinant form (URF). The duration of HIV infection was determined by limiting antigen avidity enzyme immunoassay in combination with HIV-1 viral load (VL) data. Statistical analysis was conducted by the R-Studio (version 1.3.1093) (R version 4.1.1).

Results: We successfully sequenced PR-RT and IN regions in samples from 79 persons. Majority of them were males (59%) and the median age was 40 years (IQR 33-46). The most common transmission route was heterosexual contact (53%; 42/79) and 33% (26/79) of the individuals were tested because of clinical suspicion of HIV. Baseline CD4+ T-cell count and HIV-1 VL was 232 (IQR 147-420) cells/µL and 5.2 (IQR 4.5-5.9) log10 copies/mL, respectively. The duration of HIV infection was determined for 60 individuals, of whom 33% (CI 95% 22-47%) were recently infected. The overall TDR rate was 7.6% (6/79, 95% CI 2.8-15.8%). Three individuals had NNRTI mutation K103N. One person had dual resistance to NRTI (K219E) and NNRTI (Y181C). PR mutation L90M and major INSTI mutation Y143H were detected in single case each. Overall there were 11 persons with accessory mutations: G163K (n=2) in IN region, Q58E (n=2) in PR region and A62V (n=1), E138A (n=1), V179E (n=4), V179T (n=1) in RT region. The most common HIV-1 subtype was CRF06_cpx (42/79; 53%) followed by subtype A1 (16/79; 20%), URFs (15/79; 19%), subtype B (5/79; 6%), and CRF02_AG (1/79; 1%). Successfully sequenced samples had significantly higher VL compared to not sequenced (5.4 IQR 4.9-6.3 vs 4.7 IQR 3.2-5.4 log10 copies/mL; p=0.0007), however there were no significant differences in CD4+ T-cell count. No risk factors were associated with the presence of SDRMs.

Conclusion: Compared to the last TDR surveillance years (2013 [6.7%] and 2017 [7.9%]) TDR has remained relatively stable. INSTI TDR was discovered for the first time suggesting the impact of the use of low genetic barrier INSTIs and a high number of INSTI drug resistance mutations among treatment experienced individuals in Estonian population. Regardless of the stable overall TDR, the emergence of INSTI resistance emphasizes the importance of continuous monitoring of TDR.

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Characterization of Clinical Envelopes With Lack of Sensitivity to the HIV-1 Inhibitors Temsavir and Ibalizumab

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Background: Fostemsavir and ibalizumab are antiretroviral agents that target HIV entry, albeit with different MOA. Temsavir (active moiety of fostemsavir) has a unique and distinct MOA; it binds near the CD4 binding site in gp120 and inhibits envelope binding to CD4, therefore preventing attachment of HIV to CD4, while ibalizumab targets CD4 and inhibits entry at a post-CD4 binding step. Genotypic determinants of reduced susceptibility to temsavir and resistance to ibalizumab appear to be distinct. Reduced temsavir susceptibility has been primarily associated with specific amino acid substitutions at positions 375, 426, 434 or 475 in conserved regions of gp120, whereas the key determinant of reduced sensitivity to ibalizumab is loss of potential N-linked glycosylation sites (PNGS) in the gp120 V5 region. We have previously performed studies supporting a lack of cross-resistance between temsavir and ibalizumab. Here we report on a unique amino acid substitution that under certain circumstances can result in reduced susceptibility to both temsavir and ibalizumab.

Material and Methods: Two envelopes (sequences 21-116102 and 21-116108), derived from untreated individuals, from the Monogram Biosciences library, were previously shown to exhibit reduced susceptibility to both temsavir and ibalizumab. In this study, these envelopes were sequenced, and residues mapped onto the temsavir-bound gp120 structure. Although one of the envelopes contained a known temsavir-related polymorphism, no known polymorphisms were observed in the second envelope. Residues within 5.0 angstroms of the temsavir binding site were identified for further characterization and reverse genetics was used to explore the cause of reduced susceptibility to both temsavir and ibalizumab.

Results: For sequence 21-116102, M434T was identified as a known polymorph responsible for reducing temsavir susceptibility, and further analysis showed that in this envelope there was no link to resistance to ibalizumab. For sequence 21-116108, the polymorphisms identified that may impact reduced temsavir susceptibility, either individually or in combination, were T202E, I424V, N425R and K432Q. In the context of this envelope, E202 was identified as the key polymorphism, as back mutation to E202T restored phenotypic sensitivity to both temsavir and ibalizumab. However, in the original 21-116108 envelope with E202 present, swapping the V5 region with that from an ibalizumab-sensitive envelope (containing 3 PNGS) restored sensitivity to ibalizumab, but not temsavir. The effect of E202 on ibalizumab resistance therefore appears to be V5 context dependent. E202 was infrequent in the 2021 release of the LANL database, occurring in 1/8365 (0.01%) isolates, and was not present at screening in any participant in the fostemsavir Phase 3 BRIGHTE study. However, K202E was emergent at Week 16 in one fostemsavir-treated, ibalizumab-naïve participant. Reduced susceptibility to both temsavir and ibalizumab was rescued by E202K back mutation in this envelope. The importance of E202 for temsavir susceptibility and the context dependence for ibalizumab resistance was confirmed in additional envelopes.

Conclusion: E202 was identified as a novel substitution that results in reduced susceptibility to temsavir and can also result in resistance to ibalizumab. However, this substitution is context dependent with respect to susceptibility to ibalizumab, with dependence on the sequence of the V5 region.

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Low Prevalence of Resistance Mutations Associated With Integrase Inhibitors as Detected by Sanger and Next Generation Sequencing Among HIV-1 Patients Followed Up in Portugal

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Background: The widespread introduction of HIV integrase inhibitors (INSTs) into clinical care may result in appearance of drug resistance mutations (DRMs) affecting antiretroviral treatment (ART) outcomes. The aim of our study was to monitor the drug resistance patterns of INSTs beside protease (PR) and reverse transcriptase (RT) inhibitors among naive and antiretroviral (ARV) experienced patients in Portugal.

Material and Methods: This was a cross-sectional study conducted with 448 samples from HIV-1 infected patients, between 2005 to 2022, in Portugal. The PR, RT, and integrase were sequenced using Sanger and NGS approach. DRMs patterns were analyzed based on the Stanford Interpretation Algorithm 2009 list. Univariate logistic regression was conducted to examine any association between DRMs to INST with Clinical and sociodemographic factors.

Results: A total of 284 patients were naïve to INSTIs and 164 were under INSTIs therapy. Overall, 6% of the studied patients, presented HIV drug-resistance (HIVDR) to INSTIs. Dolutegravir-associated HIVDR was detected in two ART naïve patients (0.7%). From these, one patient was collected in 2018 and infected with sub-subtype F1 with N155H major

mutation while another sample from subtype G was collected in 2019 and presented R263K major mutation. Moreover, naïve patients presented 4.2% (12/284) accessory DRMs to INSTIs. From these, 6 (50%) patients presented the T97A mutation that was the most frequent, while the E157Q, G163R, T97A and G163K mutations were less frequent. Most of the INSTI accessory mutations were observed within non-B subtypes (11/12; 91.6%) with the majority associated with HIV-1 subtype G (n = 6) and subtype A (n = 3). Regarding patients under therapy, about 14% presented any Dolutegravir-associated HIVDRMs. From these, 15.2% harbored HIV-1 variants with major DRMs, such as N155H (52%), Q148H (24%), E138A (16%), G140S (16%), S147G, E92Q (8%), Y143H (8%) and R263K (8%) mutations. The E92Q + N155H mutations were observed in 2/25 samples (8%) simultaneously. It is noteworthy that 4 participants (16%) had 4 combined major mutations (E138K, S157S, Q148R and N155H) which compromise the effectiveness of therapy with all 5 available integrase inhibitors (Raltegravir, Elvitegravir, Dolutegravir, Cabotegravir and Bictegravir). In this population, females were more likely to harbor an HIV-1 strain with at least one major DRM to INSTIs (OR: 2.68; 95% CI: 0.95 – 7.30, p=0.053).

Conclusion: Our results support the implementation of a wide scale-up of dolutegravir-based regimes. However, the detection of polymorphisms contributing to INSTI particularly in non-B subtypes that prevail in Africa, warrants the continuous surveillance of INSTI resistance.

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Drug Resistance Mutations Conferring Resistance to Integrase Inhibitors Among HIV Patients in Tel Aviv, Prevalence and Risk Factors

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Background: Integrase Strand Transfer Inhibitor (INSTIs) are used as first-line treatment in HIV-1 naive patients and as a second-line treatment in patients who have failed treatment with other antiretroviral treatments (ART). These drugs have high anti-viral efficacy and safety profiles, however, the resistance profile is different among them. The aims of the study were to describe the prevalence of drug resistance mutations (DRM) to INSTIs in HIV-1 infected individuals between the years 2010-2020 and Identify risk factors for the emergence of INSTI resistance.

Material and Methods: A case-control study identified risk factors for DRM. The study was conducted by analyzing data on different treatment regimens, demography, HIV risk factors, and genotyping of HIV-1-infected individuals before and after treatment failure. Patients who had no genotyping and who did not come to follow up after starting ART or who died during the study period were excluded.

Results: The study population included 362 participants. The rate of DRM found was 6.9% (n=25). 68% of participants with viruses harboring DRM were treated with first-generation INSTIs (Raltegravir, Elvitegravir). Among participants who did not show resistance, 84.3% were treated with second-generation drugs (Dolutegravir, Bictegravir). The median time from HIV diagnosis to the beginning of INSTI was 46 months among those who had INSTIs DRM and 1 month among those who did not (p<0001). The median age among those who have DRM was 43 years old compared to 37 years old among those who did not (p<0.047). Among the risk group MSM and heterosexuals had more risk of having DRM to INSTIs compared to other groups (p<0.006).

Conclusion: Our study supports the evidence that the rate of INSTIs DRM is low among patients treated with second-generation INSTIs. In addition, we found characteristics of participants who are likely to be at risk for the development of drug resistance, such as a late start of treatment after diagnosis, older age, MSM, and heterosexuals.

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High-Level of Cross-Resistance to 2nd Generation Non-nucleoside Reverse Transcriptase Inhibitors Among Patients Failing Antiretroviral Therapy in Cameroon: Implications for Future ART-Regimens in Africa

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Background: Etravirine (ETR), rilpivirine (RPV) and doravirine (DOR) are second generation (2Gen) non-nucleoside reverse transcriptase inhibitors (NNRTI) approved for the treatment of HIV-1 infection. In Africa, there are limited data on the resistance profile of 2Gen-NNRTI. This study aimed to evaluate 2Gen-NNRTI resistance and their susceptibility in patients failing antiretroviral treatment (ART) in Cameroon.

Material and Methods: A cross-sectional study was conducted from 2021-2022 among 340 patients failing ART, received at the Chantal Biya International Reference Centre, Yaoundé-Cameroon. Treatment history and immuno-virological data were obtained from patients' files. Genotypic resistance testing was interpreted using Stanford HIVdb v8.7. The following variants were considered as resistance mutations to 2Gen-NNRTI: Y181CIV, Y188LC, V106AMI, M230L, K101EP, L234I, G190ASEQ, L100I. The penalty scores of drug resistance were ≥60 (high-resistance); 30–59 (intermediate-resistance); <30 (susceptible). Acceptable threshold for potential drug-efficacy was set at >50% at population-level.

Results: A total of 340 patients were enrolled, of which 230 were failing first-line (1Gen-NNRTI based) and 110 second-line (protease-inhibitors) regimens. Median [IQR] CD4 and viremia were respectively 184 [60–332] cells/µl and 82,374 [21,817–289,907] copies/ml; ART-duration was 18 [10–27] months. Overall rate of resistance to 2Gen-NNRTI was 79.70% [71.30–87.02], similar between first- vs second-lines. Prevailing mutations were: Y181C (23.52%), G190A (17.64%) and P225H (13.53%). Drug susceptibility rate was 52.05% (ETR); 43.23% (RPV), 36.17% (DOR). Following susceptibility profile, patients failing on EFV-based regimens were more susceptible to 2Gen-NNRTI (OR=0.42;95%CI:[0.24–0.74]; p=0.003), while those failing after receiving EFV and NVP were less susceptible to 2Gen-NNRTI (OR=4.4; 95%CI:[1.16–14.81]; p=0.02). Low viremia (≤4log10) was associated with susceptibility to 2Gen-NNRTI (OR=0.22; 95%CI:[0.12–0.41]; p<0.0001). CRF02_AG was the prevailing subtype (58.53%), followed by A1 (11.47%), G (7.35%); without any significant effect on 2Gen-NNRTI susceptibility (CRF02_AG vs non-AG; p=0.8).

Conclusion: After ART-failure in Cameroon, there is a high-level of cross-resistance to 2Gen-NNRTI. However, etravirine retains residual efficacy in half of the population. Thus, after ART-failure in African patients, the use of etravirine as 2Gen-NNRTI is possible, pending genotypic profiling.

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Prevalence and Phenotypic Susceptibility to Doravirine of the HIV-1 Reverse Transcriptase V106I Polymorphism in B and Non-b Subtypes

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Background: This study aimed to evaluate prevalence and the in vitro susceptibility to doravirine of the HIV-1 RT V106I polymorphism detected in samples collected among the MeditRes HIV consortium.

Material and Methods: MeditRes HIV includes ART naïve people living with HIV newly diagnosed in France, Greece, Italy, Portugal, and Spain during the years 2018-2021. We evaluated the impact of V106I on susceptibility to doravirine (a) in site directed mutants containing V106I, V106A, V106M & Y188L mutations in subtype B (NL4-3, HXB2) and CRF02_AG background and (b) in a subset of recombinant viruses with clinically derived RT-RNAseH coding region harboring V106I and no other major NNRTI RAMs. Phenotypic susceptibility to doravirine was determined through a TZM-bl cell-based assay and expressed as fold-change (FC) with respect to the reference wild type virus.

Results: MeditRes HIV includes 2705 patients. Viral subtypes were B in 1523 cases (56.3%), CRF02_AG 441 (16.3%), A 160 (5.9%), C 141 (5.2%), F 124 (4.6%), others 316 (11.7%). The prevalence of V106I was 2.9%, 3.2% and 2.5% in the overall dataset, in B and non-B subtypes, respectively. Among non-B subtypes, the prevalence of V106I was 3.1%, 0.7%, 8.1%, 3.6%, 14.3%, 0.9%, and 3.1% in subtype A, C, F, G, D, CRF02_AG and CRF06_cpx, respectively. FC values for site directed mutants in the NL4-3, HXB2 and CRF02_AG background were 0.7, 2.0 and 2.5 with V106I, respectively; 3.4, 19.9 and na (not available) with V106A; 9.4, 27.3 and 13.5 with V106M; >100, na, and >100 with Y188L. The panel of clinically derived viruses tested includes 22 subtypes B and 28 non-B subtypes (2 A1, 2 CRF02_AG, 4 CRF06_cpx, 1 CRF44_BF, 3 D, 14 F1, 1 G and 1 URF). The median doravirine FC values were 1.3 (IQR 0.9-2.2) in the whole data set, while the susceptibility in B subtype is slightly lower than non-B subtypes (1.2 [IQR 0.9-1.6] vs. 1.8 [IQR 0.9-3.0]), and particularly than F1 subtype (2.6 [IQR 1.0-4.0]). Eight out of 50 (16%) viruses showed FC values equal or higher than the doravirine biological FC cutoff (3.0), one subtype B (FC 3.0) and seven non-B subtypes (A1, FC 5.5; CRF06_cpx, FC 3.7; F1, FC 7.9, 6.5, 3.1, 3.0, 3.0).

Conclusion: The prevalence of the HIV-1 RT V106I polymorphism in the MeditRes database remains low and comparable to previous studies. V106I appeared to minimally decrease the susceptibility to doravirine in site directed mutants and most of clinical isolates. Reduced susceptibility has been observed with increased frequency in non-B subtypes, especially subtype F1, however the clinical impact remains to be investigated.

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Transmitted HIV Drug Resistance Mutations in Bulgaria Are Mainly Identified in Transmission Clusters Among Men Who Have Sex With Men (Preliminary Analysis 2012-2020)

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Background: Our study aims to determine transmitted drug resistance mutations (TDRMs), whether transmission clusters accelerate the spread of resistant clades and the genetic diversity of introduced HIV-1 in Bulgaria from 2012 to 2020.

Material and Methods: The prevalence of TDRMs, HIV-1 subtypes and transmission clusters were identified in antiretroviral therapy (ART) naïve individuals diagnosed with HIV-1 in Bulgaria from 2012 to 2020. HIV-1 pol gene was sequenced using Applied Biosystems 3130xl or OpenGene DNA sequencing systems. TDRMs in protease and reverse transcriptase were defined using the WHO HIV drug mutation list by using the Stanford University HIV DB. HIV-1 subtypes were determined by using a set of methods which include the automated subtype tool COMET v2.4, REGA HIV-1 subtyping tool version 3.0, jumping profile Hidden Markov Model as well as manual phylogenetic analysis. ClusterPicker software was implemented to analyze the phylogenetic clusters of the strains having TDRMs.

Results: The overall rate of TDRMs in the investigated population was 5.7% (60/1053), 23 (38.3%) with resistance to nucleoside reverse transcriptase inhibitors (NRTIs), 19 (31.8%) to non-NRTIs (NNRTIs) and 22 (36.7%) to protease inhibitors (PIs). Dual-class TDRMs were found in 4 (6.7%) patients. Phylogenetic analyses identified high HIV-1 diversity consisting of mostly subtype B (60.4%), subtype F1 (6.9%), CRF02_AG (5.2%), subtype A1 (3.7%), CRF12_BF (0.8%) and other subtypes and recombinant forms (23%). A significant proportion of TDRMs was spread by transmission clusters with the dominant presence of men who have sex with men (MSM).

Conclusion: We found a relatively low prevalence of TDRMs against a background of high HIV-1 diversity among ART-naïve patients in Bulgaria diagnosed from 2012 to 2020. A significant proportion of TDRMs was involved in transmission clusters that arose from the sub-epidemic in the MSM group. Our results provide data on TDRMs and transmission clusters and support continuous surveillance of high-risk populations in Bulgaria to better address treatment and prevention efforts.

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Genetic Diversity and Drug Resistance of HIV-1 Among Children in the Republic of Belarus

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Background: An important aspect is the monitoring of HIV drug resistance against the background of significant coverage of antiretroviral therapy (ART), which has saved millions of people with HIV infection. The problem is of particular importance for HIV-exposed children due to the prescribing of ART to mothers during pregnancy and childbirth to prevent mother-to-child transmission of HIV infection. This may lead to the transmission of drug-resistant HIV-1 variants to children and become a source of primary HIV-1 resistance in the general population. In addition, studies of resistance to antiretroviral drugs in children are important

because of the possibility of developing resistance due to the ineffectiveness of ART, poor adherence. The aim of the study is to assess the genetic diversity and nature of resistance to antiretroviral drugs in HIV-infected children in the Republic of Belarus.

Material and Methods: Serum/plasma obtained from 58 children: 11 (19.0%) ART-naive and 47 (81.0%) ART-experienced children in the Republic of Belarus during 2018-2022. Resistance mutations were detected by gag-pol site sequencing using the «Bel HIV-1 resistance-genotype» test systems produced by Republican Research. The mutations was assessed using the WHO drug resistance list mutation (MDRM) list 2009 and the Stanford HIVdb tool (https://hivdb.stanford.edu/hivdb/by-sequences/).

Results: Among the 58 patients included in the study, 60.34% (n = 35) were males and 39.66% (n = 23) were females. The dominant subtype is A, represented by the A6 sub-subtype, it was detected in 91.38% of patients (n = 53). Subtypes B were detected in 1.72% (n=1) and subtypes C in 3.45% (n=2) of HIV-infected individuals. 3.5% (n=2) of cases recombinant forms CRF 02_AG were detected.
In the group of naive children, no drug resistance was detected in any of the cases due to the presence of mutations that are significant for monitoring according to the WHO mutation list (SDRM 2009). In the group of children with treatment experience, in 32 (68.0%) cases, drug resistance mutations to any of the groups of ART drugs were detected due to the presence of MDRM mutations (major drug resistance mutations, 2019). One child (2,1%) had resistance to three classes of drugs - protease inhibitors (PI), nucleoside (NRTI) and non-nucleoside (NNRTI) HIV-1 reverse transcriptase inhibitors. In 12 (25.5%) HIV-1 sequences, resistance mutations were identified for two classes of drugs - NRTIs and NNRTIs; in one case only to NRTIs (2.1%) and in 5 (10.6%) cases only to NNRTIs.
The most common NNRTI mutations are K101E (12.7%, n=6), K103N (14.9%, n=7), Y181C (10.6%, n=5), G190S (21.3%, n=10) and H221Y (6.4%, n=3). The most common NNRTI mutations are M184V (21.3%, n=10), K219Q (8.3%, n=4), K70R (8.3%, n=4), D67N (6.3%, 3).
Conclusion: The obtained data indicate a high level of the prevalence of mutations in the children with treatment experience in the Belarusian cohort. Considering the large-scale use of ART in the country, further representative studies are required, according to WHO recommendations, at the national level, and the solution of the problem of optimizing first-line ART after drug resistance studies prior to treatment is essential.

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Low Prevalence of HIV-1 Integrase Resistance Among People Who Are Treatment Naïve From Poland

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Background: Integrase strand transfer inhibitors (INSTIs) are an important class which is currently used in the first line treatment of HIV-1. Despite high efficiency and faster reduction of virus replication compared to non-nucleoside reverse transcriptase inhibitors and protease inhibitors, resistance mutations may affect susceptibility to specific INSTIs, especially raltegravir and elvitegravir. The aim of this study was to analyze the prevalence of INSTIs drug resistance among individuals who are treatment naïve from Poland.

Material and Methods: Resistance to INSTIs was assessed in a dataset of 994 people living with HIV-1 without prior antiretroviral treatment. All sequences were derived from Polish individuals from 2011 to 2022 using bulk sequencing. Substitutions with score >10 were defined as INSTI-resistance mutations according to the Stanford HIV drug resistance database, major mutations were also based on the IAS 2022 list.

Results: Most of the people were male (834, 83.90%) with a median age of 35 (range, 15 – 75 years) and subtype B (728, 73.24%). The prevalence of other subtypes was as follows: subtype A – 207, 20.82% (A6 – 200, 20.12% and A1 – 7, 0.70%), D – 14 (1.41%), C – 12 (1.21%), G and F – 2 (0.20%, each) and RFs – 29 (2.92%). The median baseline HIV-1 viral load was 4.81 log10 copies/ml (ranged from 1.30 log10 to 8.64 log10 copies/ml). The number of late presenters (baseline CD4 count <350 cells/µL) was 418, 42.05% (the median baseline CD4 count was 350 cells/µL, data available for 838 individuals). Major INSTI-related drug resistance mutations (DRMs) were detected in 23 people (2.31%, 2 with

subtype A6, 21 with subtype B). Among them, 33 (3.32%) major DRMs were identified. The number of sequences with one, two or three major mutations were 16 (1.61%), 4 (0.40%) and 3 (0.30%), respectively. The most common major DRMs were E138K- 6 (0.60%), Q148H/R and N155H - 5 (0.50%, each), R263K - 4 (0.40%). Overall, 12 (1.21%) individuals were resistant to all INSTIs (12 to bictegravir and dolutegravir, 19 to cabotegravir, 23 to raltegravir and elvitegravir).
Additionally, minor mutations (with score 10) were observed in 175 sequences (17.61%, 173 sequences with subtype B, 1 – A6, 1 – CRF03_AB) of which 11 (1.11%) people had both major and minor mutations. The total number of minor mutations was 178 (17.91%) with the most prevalent being E157Q - 169 (17.00%), T97A - 4 (0.40%), Q95K and D232N - 2 (0.20%, each).

Conclusion: The frequency of major INSTI drug resistance mutations among individuals who are treatment naïve remains low, but it is worth noting the high incidence of the E157Q polymorphism. With the increasing use of INSTI-based regimens, the transmission of resistant variants poses an important threat, which justifies the surveillance of INSTIs resistance.

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Resistance of 3a Subgenotype of Hepatitis C Virus 3a to NS5A Inhibitors in the Republic of Belarus

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Background: In the past few years, the use of direct antiviral drugs for the treatment of hepatitis C virus (HCV) can completely cure hepatitis C in more than 95% of cases. At the same time, in the process of virus replication, the accumulation of nucleotide substitutions occurs, affecting both the outcomes of treatment and the progress of the disease. This phenomenon is mainly associated with the occurrence of mutations in the region of the HCV genome encoding the NS5A protein RAS (resistance-associated substitutions), which differ in different genetic variants of HCV. The identification of such substitutions is especially important for patients who fail to achieve a sustained virological response or who experience a relapse of the disease. To personalize treatment, it is necessary to conduct a test to detect RAS (resistance-associated substitutions), which was the purpose of this study.

Material and Methods: The study included 125 plasma samples from patients infected with 3a subgenotype of HCV who either did not achieve a sustained virological response or relapsed 3-6 months after the end of treatment. The material was collected during 2020-2022. Amplification and genotyping were carried out by analyzing the core/E1 region of the HCV genome and the NS5A region of the genome using nested in-house PCR. Fragment sequencing was performed on an ABI PRISM 3500-AVANT automated genetic analyzer (Applied Biosystems, USA). Bioinformatic analysis of sequences was performed using the SeqScape®Software v.3.0, BioEdit v.7.2.5, and SeqA6 programs. The Clustal W program was used to align the genetic sequences. Virus genotyping was performed by phylogenetic analysis, including reference nucleotide sequences from GenBank, using the maximum likelihood (ML) algorithm in the PhyML V 3.0 program. Resistance mutations were analyzed using the online program https://hcv.geno2pheno.org/.

Results: Among 125 patients were 99 (79.2%) men and 26 (20.8%) women. Drug resistance mutations were identified in 32.0% (40/125) of the sequences. According to the Geno2pheno database [https://hcv.geno2pheno.org/], there were significantly more double amino acid substitutions in the NS5A region of the virus genome, among which Y93H+A/E62Q/T/S/L/K predominated (46/125, 36.8%), A30K+A62L/T/S (14/125, 11.2%). In 25 (20.0%) cases, single substitutions A30K and Y93H were detected.
In two patients, injection drug users, who did not show the presence of drug resistance of the virus to antiviral drugs, genotyping and phylogenetic analysis of samples obtained during treatment and after relapse of the disease were performed. Although both sequences from each of the patients belonged to the 3a subgenotype, they did not cluster together phylogenetically. This made it possible in this case to differentiate reinfection from relapse after successful therapy.

Conclusion: Thus, we have developed a method for detecting RAS NS5A, the results of which not only allow predicting the genotypic efficacy of direct antiviral drugs, but also influence further treatment tactics.

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Preliminary Report of Transmitted Resistance to HIV Integrase Inhibitors in Treatment-Naïve Patients Prior to Antiretroviral Therapy

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Background: In Chile, HIV-1 infection is a serious public health problem. Antiretroviral therapy has drastically reduced morbidity, increasing the life expectancy of patients. In addition, it has significantly reduced vertical and sexual transmission. However, the increase in therapeutic coverage unfortunately leads to the development of genotypic resistance of the virus to drugs, causing treatment failure. Therefore, permanent surveillance of HIV resistance is necessary. Resistance can appear in a patient in the course of their treatment (acquired resistance) or it can exist in a newly diagnosed person who has not yet started their therapy (transmitted resistance). More comprehensive monitoring of both acquired and transmitted resistance is now recommended to achieve longer drug life.
Aim: Obtain a profile of HIV-1 resistance to integrase strand chain transfer inhibitors (INSTI) in newly diagnosed treatment-naïve patients.

Material and Methods: A total of 50 people newly diagnosed with HIV-1 infection who had never received antiretroviral treatment were recruited. The complete integrase gene was amplified by nested RT-PCR and the sequences obtained were analyzed with the ReCall and HIVdb v9.0 programs.

Results: In 4 patients, mutations were found that conferred some degree of resistance to INSTIs. Thus, the overall prevalence transmitted due to mutations with some impact on INSTI activity during the study period was 8%. The major E138K mutation was detected in only one patient and the secondary G163R mutation was detected in the other 3. Considering that both mutations conferred a low level of resistance only to RAL and EVG, the transmitted resistance for the first generation INSTI was 8% and for the second generation it was 0%.

Conclusion: Our results confirm that the resistance transmitted to INSTI is low. However, since it is not null, it is recommended to carry out a basal genotype independent of the INSTI that is going to be used.

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Trends of HIV-1 Transmitted Drug-Resistance in Italy Over the Period 2015-2021 According to Subtype and Transmission Clusters

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Background: We evaluated transmitted drug-resistance (TDR) in HIV-1 infected individuals confirmed in different reference centres in North/Central Italy from 2015 to 2021, according to subtype and transmission clusters (TCs).

Material and Methods: 2386 HIV-1 PR/RT sequences and 1831 INT sequences from drug-naïve individuals were analysed. TDR was evaluated over time by considering the list of Mutations for Drug-Resistance Surveillance (https://hivdb.stanford.edu/). Phylogeny was generated by GTR model and 1000 bootstrap with maximum-likelihood method (MEGA6). TCs

included small TCs (2-3 sequences, STCs), medium TCs (4-9 sequences, MTCs), large TCs (≥10 sequences, LTCs). Factors associated with TDR were evaluated by logistic regression, considering as confounders gender, age, subtype, risk-factor, nationality, year of diagnosis and sequencing, viremia and CD4-count at sequencing, type of infection (recent or chronic) and to be part of TC.

Results: Individuals were mainly male (79.1%) and Italian (56.2%), with a median (IQR) age of 38 (30-48) years. The main transmission route was sexual (MSM: 34.6%; heterosexual: 24.0%; transsexual/other unknown sexual behaviours: 8.1%). Non-B infected individuals accounted for 44.6% (N=1065) of the overall population (CRF02_AG=195; F=148; C=139; A=139; other=444) and increased over time (2015-2021: 42.1% to 51.0%, p=0.002). Overall, TDR prevalence to any class was 8.0% (9.5% in B subtype vs. 6.1% in non-B subtypes, p=0.002). TDR prevalence to NRTI was 2.6% (3.5% vs. 1.5%, p=0.003), to NNRTI 4.8% (5.5% vs. 3.9%, p=0.086), to PI 1.3% (1.7% vs. 0.8%, p=0.046), to INSTI 0.3% (0.2% vs. 0.5%, p=0.415). The most prevalent resistance mutations were: K103N for NNRTI (n=70, 2.9%); M41L for NRTI (n=26, 1.1%); M46L for PI (n=11, 0.5%). The 3TC/FTC mutation M184V was negligible (n=9, 0.4%). INSTI-resistance was related to the following mutations: T66I (n=1), E138K (n=2), Y143C/H/R (n=1), N155H (n=1), G140S+Q148H (n=1). No significant changes in the prevalence of TDR to any class were found over 2015-2021, though a slight increase was observed in 2020/2021 (2015: 6.4%; 2020: 11%; 2021: 8.8%, p=0.181). Similarly, no significant changes in TDR prevalence were found by stratifying for subtype and considering the specific drug-classes.

Overall, 300 TCs were identified (B subtypes: 131 STCs, 49 MTCs, 8 LTCs; non-B subtypes: 70 STCs, 31 MTCs, 11 LTCs). These TCs involved 1155 (48.4%) individuals, with a similar proportion in B and non-infected individuals (49.7% vs. 46.8%, p=0.148). A similar prevalence of TDR was found among individuals in TCs and those out of TCs (8.2% vs. 7.8%, p=0.707).

By multivariable analysis, year of diagnosis was the only factor positively associated with TDR detection, while subtypes A, F, and CFR02_AG were negatively associated with TDR. No other factors, including being part of TCs, were significantly associated with TDR.

Conclusion: Over the years 2015-2021, in Italy TDR prevalence was 8% and remained almost stable over time, even though a slight trend of increase was found in 2020-2021. TDR was mainly related to RTI. Resistant strains were found circulating regardless to be in TCs, but less likely in non-B subtypes. These results highlight the importance of a continuous surveillance of newly diagnosed individuals for evidence of TDR to inform clinical practice.

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HIV-1 Drug Resistance Among Recently Infected Individuals in Krasnodar Region, Russia, 2021

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Background: HIV incidence refers to the estimated number of new HIV infections during a certain period (e.g., per year), which is different from the number of people diagnosed with HIV in this year. For this reason, determining the time after infection is a very important epidemiological and prognostic parameter. Recent Infection Testing Algorithm (RITA) is an approach based on laboratory methods that allow to discriminate recent (up to 12 months after infection) and established HIV infection. The roll-out of antiretroviral therapy (ART) has been a major breakthrough in the global response to the HIV epidemic. Unfortunately, the success of ART is frequently limited by the onset of HIV drug resistance (DR), included DR among people who are treatment naïve as a result of the transmission of DR-variants of the virus. The aim of the study was to analyze the prevalence of surveillance drug resistance mutations (SDRMs) among people who are treatment naïve and received the status «recent infection» in one-step RITA.

Material and Methods: The prevalence of SDRMs was studied among individuals infected with HIV up to 9 months (identified by DS-EIA-HIV-Ab-TERM kit (Diagnostic Systems, Russia)). Nucleotide sequences of pol region including protease gene and fragment of reverse transcriptase gene (2253–3369 bp according to the HXB2, GenBank accession

number K03455) were obtained using AmpliSens HIV-Resist-Seq kit (CRIE, Russia). The Stanford HIV Drug Resistance Database (CPR Tool) was used to describe and interpret SDRMs.

Results: This study was conducted to determine the duration of HIV infection in newly diagnosed people living with HIV in Krasnodar region. Plasma samples were obtained from individuals with the first positive immune blot test during 2021 (n=1117). All samples were tested using DS-EIA-HIV-Ab-TERM kit, and 274 (24.53%) samples had a "recent HIV infection" result: the mean age of individuals was 38.02 and 165 (60.22%) of them were male. One hundred seventy-one samples were sequenced from this group and passed sequence quality control.
Subtyping results showed that the predominant subtype was sub-subtype A6 (83.04%, 142/171). CRF63_02A6 was detected for 9.36% (16/171), subtype B – for 5.26% (9/171). The other 2.34% (4/171) were circulating and unique recombinant forms.
SDRMs were detected in 14.04% of individuals (24/171): the mean age was 35.0 and 19 (79.2%) of them were male. These individuals harbored SDRMs to NNRTIs 8.19% (14/171), NRTIs 1.75% (3/171), and PIs 4.09% (7/171). The mutations were: NNRTI – K101E (2/171, 1.17%), K103N (11/171, 6.4%), G190S (1/171, 0.6%), P225H (2/171, 1.17%); NRTI – M41L, D67G, L74V (1/171, 0.6% of each mutation); and PI – M46I/L (6/171,3.5%) and I85V (1/171, 0.6%). Each individual was infected with HIV-1 resistant only to one drug class.

Conclusion: It should be noted that we analyzed the prevalence of SDRMs only among recent individuals; this group most accurately reflects the current HIV epidemic. A 14.04% prevalence in this group exceeds 10%-threshold mentioned in the WHO guidelines, which recommended as the threshold to trigger clinical baseline resistance testing. Therefore, it is necessary to continue studies in this field of identification and analysis of newly HIV cases.

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Prevalence of HIV1 Drug Resistance Mutations in Naïve Patients

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Background: In recent years, there has been a major advance in the treatment of HIV/AIDS patients with the use of low toxicity drugs, high genetic barrier, and lasting virological suppression. The appearance of primary resistance compromises the initial treatment of patients and future treatment options. The prevalence of primary resistance varies in different geographic regions, its study allows the creation of new guidelines and identification of transmission chains. Objectives: To study the profile of the resistance to protease (PI), reverse transcriptase (NRTI, NNRTI) and integrase (INSTI) inhibitors of HIV1 and the predominant subtypes, in naïve patients followed in outpatient clinics and clinical units of a Lisbon hospital center.

Material and Methods: A retrospective study was carried out in the period between January 2021 and December 2022 with 351 naïve patients, 275 men and 76 women, with an average age of 39 years old and average viral load of 525418 copies/mL (177-7,4 x 10^6). Mutations, drug-associated resistance, and subtypes were studied in each patient. Two sequencing genotypic tests where used (Sanger method and Next Generation Sequencing) based on genome amplification and sequencing of the HIV1 protease, reverse transcriptase and integrase. The Stanford University HIV drug resistance database was used for interpretation (http://hivdb.stanford.edu). TDR mutations were defined according to WHO list of “Drug Resistance Mutations for Surveillance of Transmitted HIV Drug- Resistance”.

Results: A prevalence of 11,7% of naïve patients with resistance-associated mutations to antiretroviral treatment was found. For each class: NNRTI – 7,1%, NRTI – 3,7%, PI – 3,4%, INSTI -0,6%. In each class, the most frequent mutations were: NNRTI - K103N, K101E, P225H; NRTI- M41L, M184V; PI – L90M, I54V, M46L; INSTI – R263K was the only

mutation found on two patients. There was a high prevalence of Efavirenz and Nevirapine resistance. The most frequent subtypes in the studied population were: B (43%); CRF02_AG (17%); G (11%); C (10%); A (9%); F (9%).

Conclusion: There is an increase in the prevalence of primary resistance to antiretroviral drugs of 10,4% to 11,7% when compared to our study carried out in 2017. The NNRTI was the class where there was more resistance (7,1%). There was a decrease in lamivudine resistance (0,8%), an important factor in the introduction of dual therapies in current guidelines. An higher primary resistance to INSTI was observed as a consequence of the increasing use of these drugs. The change in the distribution of subtypes concerning the 2020 study, with the increase of subtype A (<1% to 9%) and F (3% to 9%), and the decrease of subtype G (17% to 11%) may be related to the characteristics of the migrant population in Portugal.

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Viral Suppression in the Era of Transition to Dolutegravir-Based Therapy in Cameroon: Children at Highest Risk of Virological Failure

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Background: Transition to Dolutegravir (DTG)-based antiretroviral therapy (ART) may improve virological response (VR) in sub-Saharan Africa. Because VR may vary by age, understanding ART response across age-range may inform interventions on ART program. Our objective was to compare VR between children, adolescents and adults in the Cameroonian context.

Material and Methods: A comparative cross-sectional study was conducted from January 2021 through May 2022 amongst ART-experienced patients received at the Chantal BIYA International Reference Centre in Yaounde-Cameroon for viral load (VL) monitoring. VS was defined as VL<1000 copies/mL and viral undetectability VL<50 copies/mL. Chi-square and multivariate binary logistic regression model was used to identify factors associated with VS. Data were analyzed by SPSS v.20.0, with $p<0.05$ considered significant.

Results: A total of 9034 patients among whom 6526 (72.8%) were female were enrolled. In all, there were 8585 (95.0%) adults, 227 (2.5%) adolescents, and 222 (2.5%) children; 1627 (18.0%) were on NNRTI-based, 290 (3.2%) on PI-based, and 7117 (78.8%) on DTG based ART. Of those on DTG-based ART, only 82 (1.2%) were children, 138 (1.9%) adolescents, and 6897 (96.9%) adults. Median (interquartile range) duration on ART was 24(12-72) months (24 months on TLD, 36 months on other first line and 84 months on PI/r based regimen). Overall, VS was 89.8% (95% confidence interval, CI: 89.2-90.5) and viral undetectability was 75.7% (95% CI: 74.8-76.7). Based on ART-regimen, VS on NNRTI-based, PI/r-based, and DTG-based therapy was respectively 86.4%, 59.7%, and 91.8%, $p<0.0001$. Based on ART-duration, VS was respectively 90.1% (12 months), 87.6% (24 months), 87.9% (36 months), and 89.9% (≥ 48 months), $p<0.0001$. Also, there were 5929 (90.9%) virally suppressed females against 2183 (87.0%) in males, $p<0.0001$; Following with age group, 144 (64.8%) children, 169 (74.4%) adolescents, and 7799 (90.8%) adults were virally suppressed, $p<0.0001$. Following multivariate analysis, VS was associated to adults, females, TLD regimens, and cART duration >24 months ($p<0.05$).

Conclusion: In Cameroon, ART response indicates encouraging rates of VS (about 9/10) and viral undetectability (about 3/4), driven essentially by access to TLD-based regimens. However, ART response was very poor in children, underscoring the need for scaling-up pediatric DTG-based regimens.

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Impact of Retention Challenges for People Living With HIV in Border Areas on Their Survival Rate: The Case of Richard-Toll Health District, Senegal in 2022

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Background: Located in northern Senegal, Richard-Toll is a border area crossed by the Dakar-Nouakchott Corridor. It covers 236 villages and 26 HIV treatment facilities where health workers has always been challenging in retaining and monitoring people living with HIV, hence the need to study the case of the Richard-Toll Health District in 2022.

Material and Methods: A retrospective descriptive cross-sectional study was conducted in 2022 in Richard-Toll, respecting basic ethical and deontological rules. An analysis of the follow-up database of people living with HIV in the said border district was carried out to describe the flagship indicators monitoring the HIV programme and to measure survival rate at 12, 24, 36, 48, 60, 120 and 180th month using the following software: Excel2010® and Epi InfoTM7.2.4.0.

Results: The 2022 active cohort represented 52.5% of all the people living with HIV recorded since the beginning and 4% of whom were under 14 years old. The study revealed the feminisation of the infection with a female predominance (73.6%) including 2,8% of people working in the sex industry. Nevertheless, it was noted that 1,3% of intravenous drug users and 30,3% of men having sex with men were in the active cohort. The proportion of people living with HIV on antiretrovirus was 87.2%, 77% of whom were on the Tenofovir+Lamivudine+Dolutegravir protocol, with a high representation of HIV1 (91.6%), as opposed to 3.5% of dual-profile patients (HIV1+2). The 25-49 year old age group was the most represented (67.2%) followed by 5 years or older (24%) versus 5-9 years (1.6%) and 0-4 years (0.8%). The first line of treatment was more represented (97.8%) and according to the stability of the treatment follow-up, the frequency of antiretrovirus dispensation was less than 3 months in 26.6% of cases ; between 3 and 6 months in 65.6% ; and greater than 6 months in 6.3% with 1.6% of people living with HIV declared as stable. The viral load was undetectable in 73% of cases including pregnant and breastfeeding women for better prevention of transmission among children born to HIV-positive mothers. However, it was noted that 3% of people living with HIV were co-infected with tuberculosis without associated hepatitis B and 3% with diagnosed diabetes. Furthermore, in the end of 2022, the retention rate was 91.9% overall with a mortality rate of 4.4% and 3.7% of lost to follow-up. But a focus on adolescents (10-19 years) shows, despite the absence of lost to follow-up (0%), a lower specific retention rate (83.3%) in relation probably to a higher mortality (16.7%) for this target population. Thus the evolution of the overall decreasing survival rate was at 12 months 86.7% ; at 24 months 68.4% ; at 36 months 47.8% ; at 48 months 45.5% and at 120 months 25% with an improvement of the said rate at 60 and 180 months (respectively 50% and 55.6%).

Conclusion: Difficulties in retaining people living with HIV in the border areas of Richard-Toll has had a significant impact on them and consequently on their 2022 survival rate.

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Hepatitis C Virus Micro-Elimination in Georgian Plhiv; Reaching the 2025 Program Goals; Where Do We Stand?

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Background: Georgia's world's first national hepatitis C (HCV) elimination program set an ambitious goal to eliminate HCV infection by

nationwide screening, active case finding, linkage to care, decentralized care, and provision of treatment for all persons with HCV infection.
Since the launch in 2015 from estimated 150 000 HCV RNA positive Individuals, which is the 5.4% of the general population, 67% underwent treatment with sustained viral response (SVR) rates reached 99.0 % among treated. This strategy resulted drop of HCV RNA prevalence from 5.4 to 1.8%. Despite this achievements program result is still running behind the target goal for reaching 90/95/95 for 2025, 90% accounting for diagnosed, 95% for treated, and 95% cured respectively.
Micro- elimination within specific high risk groups is one of the strategies that can speed up the process before overall program goal is reached. Georgian PLHIVs (person living with HIV) have highest HCV prevalence (27%) among specific high-risk populations. Before the DAA era, due to the limited resources limited numbers of the patients were treated annually.

Material and Methods: Micro-elimination among PLHIV envisaged screening all persons for anti HCV antibodies during routine hospital visits, reflex testing for HCV RNA among HCV antibody positives and initiation of the DAA therapy during patient's subsequent visits. This approach shortened time and resources for patient enrolment in the treatment program.

Results: As of February 1, 2023 9869 PLHIVs were officially registered in four HIV/AIDS treatment clinics in the country. Of live PLHIVs individuals, 2101 were estimated to be chronically infected with HCV, of those 1705 are already treated and cured. Thus, resulting 6.6-fold decrease in HCV viremic infections (27% to 4.1%), and subsequently reducing infection by 85%. This approach among PLHIV resulted approaching final program goal of 90/95/95.

Conclusion: The analysis of Georgian micro-elimination in PLHIV demonstrates the importance of such approach for ultimate HCV macro-elimination. Eliminating HCV by targeting other at-risk specific populations (prison settings and dialysis clinics) has great potential for bridging the gap in the HCV care continuum, which ensures high rates of treatment uptake toward achieving the ultimate elimination targets.

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HIV DNA Sequences Analysis After 1 Year of DTG/3TC Maintenance Therapy (ANRS LAMIDOL Trial)

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Background: Hypermutated viruses induced by APOBEC3, a human protein contributing to the innate immune response, represent a part of defective viruses in HIV DNA reservoir. A decrease of intact proviruses has been reported with sustained viral suppression under triple-therapy. However, few data are available on HIV DNA sequence changes in the context of a dual-therapy. Here, we assessed the proportion of people living with HIV (PLWHIV) harboring APOBEC3-induced defective proviruses when receiving a DTG/3TC maintenance dual-therapy.

Material and Methods: PBMC of virologically-suppressed PLWHIV enrolled in the ANRS 167 LAMIDOL trial, which consisted in switching from a triple-therapy to DTG/3TC, were collected 8 weeks before DTG/3TC initiation (W-8) and at W48. Next-generation sequencing of Vif and RT regions was performed using Nanopore Oxford Technology. Three PLWHIV experienced a blip and one a virological failure during the trial, 2 of the 4 were still on DTG/3TC at W48. Raw reads were mapped to HXB2 strain using minimap2. Perl/R scripts were developed to produce consensus sequences and to identify defective sequences and APOBEC3-related drug resistance mutations (APOMut). All hypermutated reads and those containing at least one stop codon were considered as defective (threshold: 5%).

Results: 104 PLWHIV were enrolled in LAMIDOL trial (median virological suppression: 4.2 years, IQR: 2.0-9.1). Proviral defective reads at W-8 and W48 were detected in vif in 13/58 (22%) and in 17/58 (29%) patients, respectively, and in RT, in 21/55 (38%) and in 31/74 (42%) patients, respectively. No significant difference was observed between the two genomic regions regarding the proportion of patients with defective proviruses at both time-points. Similarly, no significant differences between W-8 and W48 were observed regarding the number of PLWHIV with APOBEC3-defective proviruses or the median number of APOBEC3 mutations. At least one APOMut was present in proviruses of 15 (27%) and 28 (38%) PLWHIV at W-8 and W48, respectively. The ratio of APOMuts/number of potential APOMuts sites was significantly higher at W48 (15.9%) than at W-8 (9.8%) (p=0.007). The presence of APOBEC3-defective viruses at W-8 was not associated with HIV total DNA level, nor the third drug class received prior switching to DTG/3TC, nor the virological suppression duration. A different hypermutation status of proviral sequences, between W-8 and W48, was observed in 11 out of the 49 paired PLWHIV' RT sequences (hypermutated to non hypermutated for eight PLWHIV and conversely for the three remaining), and in one out of the 38 paired PLWHIV' vif sequences (non hypermutated to hypermutated). These latter PLWHIV maintained virological suppression until W48, the last follow-up of the trial.

Conclusion: Whereas no significant changes of the proportion of patients with APOBEC3-defective proviruses was evidenced after one year of DTG/3TC maintenance dual-therapy, enrichment in APOMuts was observed between W-8 and W48, suggesting a potential association between duration of virological suppression and APOMut occurrence. Further longer-term studies are needed to assess the other forms of defective viruses with dual-therapy strategies.

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Evaluation of Blood Pressure Changes Among People Living With HIV-1 (PLWH) Without Baseline Hypertension Receiving Dolutegravir (DTG)-Based Regimens or Comparator Antiretroviral Therapy in Randomized Clinical Trials Through 96 Weeks

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Background: The RESPOND prospective cohort study found higher incidence of hypertension among PLWH who received INSTIs compared with NNRTIs but not with boosted PIs. However, the REPRIEVE prospective cohort study found no association with hypertension between INSTI-based vs non–INSTI-based regimens. We evaluated blood pressure (BP) changes among PLWH receiving DTG-based regimens or comparator ART (CAR) in pooled randomized clinical trials.

Material and Methods: Data from participants who were ART-naive and randomized to receive once-daily DTG with either ABC/3TC or TDF/FTC, or CAR with EFV/TDF/FTC, RAL + 2 NRTIs, or DRV/r + 2 NRTIs, were pooled from the SPRING-1, SPRING-2, SINGLE, and FLAMINGO clinical trials. Participants with BP and weight measurements at baseline and any post-baseline assessment (Weeks [W] 24, 48, or 96) were included. Data from participants with baseline systolic BP (SBP) ≥140 mm Hg and/or diastolic BP (DBP) ≥90 mm Hg measured after a 5-minute rest, history of hypertension, and/or baseline antihypertensive medication use were excluded. Adjusted mean changes from baseline in SBP and DBP through W96 were analyzed using mixed-models repeated-measures analyses adjusting for relevant baseline variables and pooled treatment (DTG or CAR). CAR included EFV, RAL, or DRV/r plus background therapy. Investigator-reported adverse events (AEs) of increased BP and

correlations between BP and weight changes were assessed.

Results: Among 2345 participants randomized, 23% (n=530) met exclusion criteria and were removed from analyses. Of the remaining 77% (n=1815), 927 received DTG-based regimens (56% [n=520] ABC/3TC; 43% [n=401] TDF/FTC) and 888 received CAR (41% [n=360] EFV/TDF/FTC; 37% [n=327] RAL + 2 NRTIs; 21% [n=189] DRV/r + 2 NRTIs, 1% [n=12] EFV + ABC/3TC). The majority of participants were from Europe (63%). Baseline median (range) age was 34 (18-85) years, 15% were female sex at birth, and median (range) weight was 73 (36-145) kg in the overall pooled population. At W96, adjusted mean (SE) change from baseline was 2.42 (0.398) vs 2.62 (0.437) mm Hg for SBP (treatment difference, −0.20 mm Hg; 95% CI, −1.36, 0.97; *P*=0.741) and 1.62 (0.624) vs 1.80 (0.629) mm Hg for DBP (treatment difference, −0.18 mm Hg; 95% CI, −1.05, 0.69; *P*=0.683) in the pooled DTG vs CAR groups, respectively. There was no evidence of heterogeneity between studies (*P*≥0.312), no differences in investigator-reported increased BP or hypertension AEs (2% per group), and <1% of each group initiated antihypertensives post-baseline through W96. A weak positive correlation between change in BP and weight was observed at W96 (*r* = 0.152).

Conclusion: Similar increases in BP were observed through W96 in both DTG and comparator groups in this pooled analysis of PLWH without history of hypertension, baseline antihypertensive use, and/or SBP ≥140 mm Hg and/or DBP ≥90 mm Hg at baseline. Observed BP increases were small and unlikely to be clinically significant in either group. Rates of increased BP and hypertension AEs and numbers of participants initiating antihypertensives post-baseline were very low. Clinical monitoring and appropriate management of increased BP and hypertension should be considered in all PLWH, especially those with hypertension and/or cardiovascular disease risk factors.

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Lamivudine Plus Dolutegravir Therapy for People Living With HIV-1 and Are Treatment-Naive – A Multicentric Real-Life Study

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Background: Dual therapy with Lamivudine (3TC) plus Dolutegravir (DTG) is one of the first-line treatment options for people living with HIV-1 and are treatment naive. It has proven non-inferiority when compared to triple therapy and presents several advantages such as good tolerability, low toxicity, favorable metabolic profile and availability as a single-tablet regimen. The lack of real-life data with 3TC+DTG in individuals who are treatment naive in Portugal led to this real-life multicentric study, which aims to characterize the Portuguese experience with 3TC+DTG in people living with HIV-1 and are treatment naive ever since it was made available at a national level.

Material and Methods: Multicentric retrospective cohort study involving 10 hospitals in Portugal. All consecutive cases of people living with HIV-1 and are treatment naive, aged ≥ 18 years and started antiretroviral treatment (ART) with 3TC+DTG from 01/01/2018 to 31/07/2022 were included. Exclusion criteria were pregnancy and breastfeeding, weight < 40kg, creatinine clearance < 30 mL/min, positive HBsAg, documented resistance to lamivudine or integrase strand transfer inhibitors and contraindication for treatment with lamivudine plus dolutegravir.

Results: A total of 213 participants were included, of which 82% were male, with a median age of 38 years. The majority of patients (n=141, 66%) were Portuguese; the remaining 34% were predominantly from Brazil (N=45) and Angola, Mozambique, São Tomé e Príncipe and Guinea-Bissau (N=14). Risk for HIV transmission was estimated to be MSM in 56% of cases, heterosexual in 34% and people who use intravenous drugs in 3%. Most common coexisting conditions were dyslipidemia (17%), psychiatric disorders (10%) and arterial hypertension (9%); five people living with HIV-1 had an active neoplastic disease and only one individual had chronic kidney disease (CKD). Approximately 12% of participants (N=26) were diagnosed at stage 3 of HIV infection, reflecting late diagnosis. At baseline, median HIV viral load (VL) was 23900 cp/mL (8 participants had VL > 500000 cp/mL) and median CD4+ T lymphocyte (CD4) count was 423 cells/mm3. In 63 participants (30%), treatment with 3TC+DTG was started before results of genotypic resistance testing were available. Virologic suppression was reached in 78% (of 182 tested participants) at weeks 4-6, 91% (of 200 tested) at 6 months, and 97% (of 152 tested) at 12 months. The 5 participants who did not meet criteria for virologic suppression at 12 months all had viral loads ranging between 53-68 cp/mL. Virologic failure was not described in this cohort. Median CD4 count rose consistently to 487 cells/mm3 at weeks 4-6, 639 cells/mm3 at 6 months and 691 cells/mm3 at 12 months. A total of 10 participants interrupted therapy with 3TC+DTG – 7 abandoned treatment and 3 switched due of pregnancy planning, CKD and weight gain. Eight participants were lost to follow-up.

Conclusion: This is the first real-life multicentric study of dual therapy with 3TC plus DTG in HIV-1 treatment-naïve patients in Portugal. It met similar results to previously published analysis of 3TC plus DTG people living with HIV-1 and are treatment naive, reinforcing that this is an effective treatment option, with good tolerability and safety profile. In the Portuguese setting, it can be safely started before knowledge of genotypic resistance testing.

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HIV-DNA Decay in ART-Naïve PLWH Starting Dolutegravir Plus Lamivudine vs Triple Therapy: 48-Week Results: In a Real-Life Setting

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Background: Data regarding dolutegravir (DTG) plus lamivudine (3TC), as an initial treatment strategy, showed high efficacy and safety in people living with HIV (PLWH). However, data are lacking regarding the HIV-DNA decay in ART-naïve PLWH starting a DTG plus 3TC dual regimen. Aim of this work was to compare the HIV-DNA dynamics in a real-life setting of ART-naïve PLWH starting a triple regimen vs DTG+3TC dual regimen.

Material and Methods: This was a prospective, longitudinal study enrolling participants who started a standard triple regimen with 2-NRTI backbone with an anchor drug (3-drug regimen group, 3DR), and participants who initiated a dual regimen with DTG and 3TC either two-tablet (DTG 50mg plus 3TC 300mg once daily) or single-tablet regimen (2-drug regimen group, 2DR). We quantified the total blood-associated HIV-DNA by droplet digital PCR using a home-made protocol targeting the HIV-1 LTR region (detection limit: 32copies/10^6leukocytes) at three time-points: before starting therapy (baseline, BL), at virological success (VS) (defined as the first HIV-RNA <50 copies/mL) and after one year (Week48, W48). Results were expressed as $\log_{10}$ HIV-DNA copies/10^6 CD4. GLM repeated measures ANOVA model was used to compare the levels of HIV-DNA over the study period within and between groups.

Results: We included 57 ART-naïve PLWH. Thirty participants started 3DR and 27 2DR: mostly males (84.2%), and Caucasians (82.1%), median age was 37 years (IQR 30-51). As compared to 3DR, participants in 2DR were younger (34 years, IQR 25-40 vs 40, IQR 35-52, p=0.012), with higher CD4

cell/mm³ (414, IQR 265-613 vs 232, IQR 71-511, p=0.008), and higher CD4/CD8 ratio (0.46, IQR 0.37-0.62 vs 0.25, IQR 0.12-0.46, p=0.003). No AIDS events were recorded in any group.
At BL, 2DR and 3DR groups displayed similar mean viral load [4.57 (4.24-4.90) and 4.87 (4.38-5.37) log_{10} copies/mL HIV-RNA (p=0.298)] and HIV-DNA levels [3.83 (3.57-4.08) and 4.12 (3.65-4.59) log_{10} HIV-DNA copies/10^6 CD4, p=0.283]; overall, BL HIV-DNA and pre-treatment HIV-RNA were correlated (r=0.530, p<0.001).
Time to reach VS was similar between groups (2DR: 49 days, IQR 27-130 vs 3DR: 76, IQR 26-119, p=0.699). The frequency of participants with undetectable HIV-RNA (0 copy/mL) was comparable between groups, about 60% at VS and 90% at W48 (p values 0.437 and 0.265, respectively). Whereas CD4 counts and CD4/CD8 ratio remained markedly higher in the 2DR group at VS (p values 0.011 and 0.015, respectively) and W48 (p values 0.008 and <0.001, respectively). Overall, a statistically significant reduction of HIV-DNA levels over 48 weeks was observed in both groups (both p values <0.001), with trends comparable between groups (p=0.385).

Conclusion: In this clinical practice setting, treatment-naive PLWH who start either dual regimen or triple standard therapy showed a similar marked decay in HIV-DNA at both VS and after 48 weeks. Our results add important new data that support the effectiveness of the dual therapy approach on the cellular reservoir, which needs to be confirmed in larger cohorts.

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Feasibility and Outcome of CAR-T Cells Therapy in People Living With HIV

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Background: Chimeric antigen receptor T (CAR-T) cells therapies have demonstrated efficacy in the treatment of relapsed or refractory Diffuse large B-cell lymphoma (R/R DLBCL) but remains a challenge for People Living With HIV. HIV/AIDS infection represents an obstacle to intensive cancer care and remains an exclusion criterion in most clinical trials, limiting the access to innovative therapies.

Material and Methods: We retrospectively analyzed clinical and virological outcomes of all People Living With HIV/AIDS treated for R/R DLBCL by approved anti-CD19 CAR-T cells at Institut Paoli-Calmettes (Marseille, France) between August 2019 and June 2022.

Results: Four participants were treated by Axicabtagene ciloleucel. Their mean age at DLBCL diagnosis was 51 (range: 44-57) years, and 50% were men. The first participant was diagnosed in April 2018 and received CAR-T cells in August 2019 after 4 lines of systemic therapy. At time of infusion, he was treated for HIV with Abacavir/Lamivudine/Dolutegravir, viral load was <50 copies/mL and CD4 cell count was 198 cells/mm3. Cytokine Release Syndrome (CRS) grade 1 and neurological toxicity grade 2 occurred after CAR-T cells treatment. At 6 months post-infusion, HIV load was <35 copies/mL. PET scans at 3- and 6-months showed complete metabolic remission. Hepatic relapsed occurred at 12 months. The second participantwas diagnosed in July 2020 and received CAR-T cells in June 2021 after 2 lines of systemic therapy. At time of infusion, he was treated for HIV with Emtricitabine/Tenofovir disoproxil/Dolutegravir, viral load was <20 copies/mL and CD4 count was <100 cells/mm3. CRS grade 2 occurred after CAR-T cells treatment. At 6 months post-infusion, HIV load was <20 copies/mL. PET scans at 1 months showed a stable disease. The participant died after 8 months due to DLBCL progression. The third participant was diagnosed in 2017 and received CAR-T cells in July 2021 after 4 lines of systemic therapy. At time of infusion, he was treated for HIV with Emtricitabine/Tenofovir disoproxil/Raltegravir, viral load was <20 copies/mL and CD4 count was 90 cells/mm3. CRS grade 1 occurred after CAR-T cells treatment. At 6 months post-infusion, HIV load was <20 copies/mL. PET scans at 1- and 12-months showed complete metabolic remission. The fourth participant was diagnosed in February 2022, shortly after HIV diagnosis that was treated with Bictegravir/Emtricitabine/Tenofovir alafenamide. Dolutegravir was added before leukapheresis to enhance antiviral efficacy. He was admitted in May for Pulmonary cryptococcosis and CMV viremia, which were treated by Fluconazole and vancomycin, respectively. He received CAR-T cells in June 2022 after 2 lines of systemic therapy

despite a detectable HIV load at 63 copies/mL, while CD4 count was 272 cells/mm3. CRS grade 1 occurred after CAR-T cells treatment. At 6 months post-infusion, HIV load was <40 copies/mL. PET scans at 3- and 6-months showed complete metabolic remission. Neither opportunistic infections after CAR-T cells treatment nor severe adverse effects (≥ grade 3) during the follow-up period were reported in these individuals.
Conclusion: CAR-T cells therapy is feasible in treating R/R DLBCL in People Living With HIV/AIDS. A large prospective study would be needed to validate these findings.

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Characterization of HIV-1 Reservoirs in Children and Adolescents: A Systematic Review and Meta-Analysis Toward Pediatric HIV Cure

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Background: The virostatic effect of antiretroviral therapies (ART) infers viral persistence in sanctuaries, with a high likelihood of reactivation off-treatment. This systematic review and meta-analysis aimed at estimating the global burden of archived drug resistance mutations (ADRMs), the size of reservoirs and their determinants in pediatrics.

Material and Methods: Were included, randomized and non-randomized trials, cohorts and cross-sectional studies of HIV reservoirs in vertically-infected participants, published in English/French between 2002-2022. As primary outcomes, we evaluated the prevalence of ADRMs and estimated the size of reservoirs (HIV-1 DNA copies/10 6 cells) in pediatrics. Subgroup analysis were performed to further characterize the data and the meta-analysis was done through random effect models.

Results: Overall, 50 studies from 17 countries worldwide were included encompassing 2569 vertically infected participants (aged 2-days to 19-years; 52.81% females). There were limited data on the quantitative characterization of viral reservoirs in SSA, and sensitive tool as ddPCR for characterizing viral reservoirs were not implemented in the most sub-Saharan Africa (SSA) countries. Overall prevalence of ADRMs was 37.80% [95%CI: 13.89–65.17], with 48.79% [95%CI: 0–100] in Africa, 42.08% [6.68-82.71] in America, 23.88% [95%CI: 14.34–34.90] in Asia, and 20.00% [95%CI: 10.72–31.17] in Europe; without any difference between infants and adolescents (p=0.656). Starting ART before 2 months of age limited the size of HIV-1 DNA (p=0.054). Participants with long suppressed viremia (>5years) had lower rates of HIV-1 DNA (p=0.027) whereas pre-/post-ART CD4 ≤29% and pre-/post-ART viremia ≥5Log were all found associated with higher rates of HIV-1 DNA (p=0.038, p=0.047, p=0.041 and 0.035 respectively).

Conclusion: Our findings underscore high levels of ADRMs in pediatrics worldwide, with a higher reservoir driven by delayed ART initiation, shorter period of viral suppression and immuno-virological failures. Thus, strategies for pediatric HIV functional cure should target adolescents/children with very early ART initiation, high immunity and long-term viral suppression.

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Adherence to Contemporary Antiretroviral Treatment Regimens and Impact on Immunological and Virological Outcomes in Newly Diagnosed People Living With HIV-1 in Israel, 2010-2020

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Background: Despite the progress in contemporary antiretroviral therapy (ART), virological failure (VF) is still on concern in new era of treatment therapy. The goal of this study was to identify the adherence patterns of newly diagnosed people living with HIV-1 initiating contemporary ART in 2010-2020 and their impact on immunological and virological outcomes.

Material and Methods: Individuals (n=947) diagnosed during 2010-2020 in Israel, were followed for at least two years post first-line therapy. All cases with VF (defined as not attaining virologic suppression (Viral load < 200 copies/mL), discontinuing ART or having virologic rebound) were recorded. Immunological (longitudinal CD4 levels), virological (treatment regimens, resistance tests, viral subtypes) and demographic data were collected. Cases diagnosed with CD4<200 and AIDS defining conditions as well as those with poor adherence (defined by missing medications, ART discontinuation or abusing treatments) were recorded. Descriptive statistics, mixed models' analysis for longitudinal CD4 levels and Cox model was applied to examine the hazard ratio (HR) and associations between the adherence pattern and all other parameters.

Results: The majority, 81%, were males and 61% of the study cohort were Israeli-born individuals. The most prominent risk groups were men who have sex with men (MSM, 54.6%) and heterosexual contacts (28%). Baseline resistance was assessed in 88% (837/947) and resistance mutations were recorded in 21.9%. Median baseline CD4 was 363 (IQR174-540) cells/mm³. Diagnosis with CD4<200 cells/mm³ and AIDS defining diseases was recorded in 13% (119/947) of the individuals. Median follow up comprised 7 (4-11) visits and 2.25 person years. Poor treatment adherence was recorded in 16% (154/947) of the participants. VF was recorded in 5.6% (53/947) of the study cohort, resistance tests were requested for 66% (35/53) and 34.3% (12/35) had acquired drug resistance mutations. Longitudinal CD4 analysis identified two distinct CD4 trajectories: "Class-1", accounting for 41.6% (394/947), included cases with median baseline CD4 counts of 252 (IQR 119-380) cells/mm³ and moderate increase of CD4 trajectory (mean change of -14.24 from the fixed effect, standard deviation, SD 15.71). "Class-2" comprised 58.4 % (553/947) and included all other cases with a median CD4 of 450 (IQR 260-625) cells/mm³ at baseline and mean change of +10.15 from the fixed effect, (SD 28.19). In multivariable analyses, poor adherence (HR=6.32, 95%CI:3.52-11.35, p<0.001) and diagnosis with CD4 <200 cells/mm³ and AIDS - defining condition (HR=5.58, 95%CI:2.48-12.54, p<0.001) were associated with increased VF risk. In contrast, belonging to CD4 "Class-2" group decreased the VF risk by 67% (HR=0.33, 95%CI:0.17-0.68, p<0.001) and with each additional visit the VF risk decreased by 22% (HR=0.78, 95%CI:0.72-0.85, p<0.001). Risk of VF was not significantly associated with ART initiation period (2010-2014 vs. 2015-2018) and resistance mutations at baseline.

Conclusion: Low baseline CD4 counts (<200) with moderate longitudinal increase and AIDS defining conditions are all factors associated with VF. Early detection of HIV-1 infection together with adherence to treatment and tight monitoring are recommended to reduce VF.

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Cardiometabolic Health in People With HIV: A Targeted Literature Review and Expert Consensus

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Background: Although many studies have evaluated cardiometabolic health in people with HIV (PWH), the findings have not translated into guidance aimed at improving their cardiometabolic health. Here, an international panel of experts developed comprehensive consensus data statements and clinical recommendations to address the lack of guidance.

Material and Methods: A targeted literature review identified 281 conference proceedings, peer-reviewed articles, and background references on cardiometabolic health in adult PWH published between January 2016 and April 2022 on topics including weight change, lipid abnormalities, insulin resistance and diabetes, fatty liver disease, cardiac health, and bone health. This literature review was used to develop draft consensus data statements and as a guide for a series of workshops. Using a modified Delphi method, a panel of 16 international experts convened in workshops and completed surveys to refine consensus data statements and generate clinical recommendations. Overall, 9 data statements, 4 data gaps, and 14 clinical recommendations were selected for inclusion.

Results: Data statements detailed increased risk of cardiometabolic health concerns in PWH compared with the general population, known risk factors that contribute to cardiometabolic health outcomes, and the potential impact of antiretroviral therapy on cardiometabolic health. Additionally, data gaps were identified to help inform future cardiometabolic health research in PWH. Clinical recommendations emphasized promoting awareness of increased risk of cardiometabolic health concerns in PWH, including access to cardiometabolic health services as part of comprehensive care, counseling on potential changes in weight after initiating or switching antiretroviral therapy, and encouraging lifestyle choices that help reduce cardiometabolic health risk. Furthermore, clinical recommendations advocated for regular assessment of cardiometabolic health parameters, including weight, body mass index, waist-to-hip and/or waist-to-height ratio, blood pressure, lipids and markers of glycemic control, cardiovascular health, bone health, and indicators of sarcopenia among older adults with HIV. Promoting lifestyle choices for improved cardiometabolic health was also encouraged, including smoking cessation and decreased alcohol consumption as well as offering advice related to healthy eating, physical activity, stress management, and sleep quality.

Conclusion: Cardiometabolic health remains an essential concern in the care of PWH. Based on the available data and expert consensus, clinical recommendations were made to address the increased risk of cardiometabolic disorders in PWH and data gaps were identified. A holistic approach to comprehensive care, including consideration of cardiometabolic health in PWH, is encouraged. Healthcare providers must be mindful of these recommendations and existing clinical guidelines to ensure appropriate cardiometabolic health management in PWH.

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Study of the Determinants of New Infections in Vulnerable Populations in the Health District of Richard-Toll, Senegal

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Background: Richard-Toll is located in the north of Senegal, about 400km from the capital. It has a population of 200698 and covers an area of 2912km2. Access to primary health care is not sufficient. The district follows a cohort of 284 people living with HIV. The district, like the national public health policy, aims to achieve zero new infections by 2030 by implementing several strategies, including facilitating access to screening and antiretroviral treatment, as well as all preventive material and methods available to date. Over the past 5 years, an increase in new infections among young people has been noted. The objective of the study was to analyze the cohort of people living with HIV followed and to identify the causes of this new wave of contamination.

Material and Methods: Survey forms on the socio-demographic characteristics of people living with HIV, contacts, profession, lifestyle, environment, level of knowledge on HIV, clinical and paraclinical characteristics, level of education, sexual orientation and practices were distributed according to the study protocol. Screening activities were also conducted during the study. Individual and focus group interviews were held. The statistical software R studio was used.

Results: Thus 79% of the people living with HIV in the cohort were considered to be in key populations (sex workers 27%), (injecting drug users 12%), (6% were young children who had experienced mostly gender-based violence such as rape in childhood, often unpunished and kept secret in a poor family with an income of less than 1 euro per family member per day). 55% of the participants were in the age group of 15-30 years and were pupils, students in the majority and others were engaged in art activities or were unemployed. The analysis of the survey forms showed that nearly 75% of these young people had same-sex sexual orientations and stated that they could not live their sexuality fully because of society's homophobic reticence and did not find the reception satisfactory to be able to attend health structures in order to benefit from care in case of illnesses and to obtain the available prevention methods. The majority of these young people said they needed proctology consultations but did not have access to them and feared gender-based violence.

Conclusion: The results of this study identified an urgent need to develop strategies for these key populations to provide better access to screening, treatment, and prevention.

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The Role of Peer Support on Adherence to Antiretroviral (ARV) Therapy in People Living With HIV (PLWH) : A Meta-Analysis Study

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Background: Adherence to antiretroviral (ARV) therapy is the key to successful treatment in people living with HIV (PLHIV), characterized by suppressed HIV viral load. To ensure that people living with HIV are obedient and prevent loss to follow-up, WHO (2016) proposes several ways, one of which is peer support. Peer support is considered effective to help people living with HIV overcome psychosocial and internal barriers to achieving a quality life. This need cannot be provided by health care.

Material and Methods: A meta-analysis study was conducted to obtain conclusion about the effect of peer support on adherence to ARV therapy in people living with HIV. Database searches were carried out in March – June 2022 through MEDLINE (PubMed), DOAJ (directory of open access journals), PLoS ONE, and Google Scholar. Inclusion criteria included: peer support on ARV therapy, published in Indonesian and English, observational design study, published in the period 2002 – 2022, adult participants, and available in full-text. Exclusion criteria included: having different operational definitions and pregnant women participating. The systematic review was carried out using PRISMA (preferred reporting items for systematic review and meta-analysis).

Results: A total of 8 (eight) studies were included in the meta-analysis and analyzed separately using aRR and aOR risk estimation. Both risk estimates resulted in heterogeneity index (I2) of 65% and 82% so the analysis used was a random effect model. In both risk estimates, peer support affects adherence to ARV therapy by aRR = 1.27 (95% CI = 1.13 – 1.44; P = 0.0001) and aOR = 1.97 (95% CI = 1, 16 – 3.34; P = 0.01) and statistically significant. Both funnel plot of risk estimation shows a potential for publication bias, characterized by an asymmetric distribution between plots.

Conclusion: This finding indicates that peer support affects adherence to ARV therapy in people living with HIV. It suggested that peer support be integrated with health care so that their existence is sustainable and in line with the treatment of PLHIV.

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Frequency of Allele HLA-B*57:01 in the Republic of Belarus (Research 2018-2023)

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Background: Currently for the treatment of HIV infection in first-line regimens abacavir, a nucleoside reverse transcriptase inhibitor of HIV-1, is used. One of problems with the use of this drug is the occurrence of a reaction delayed-type hypersensitivity in some people living with HIV. This requires not only the abolition drug, but in some cases can lead to serious consequences. It is known that such the effect is observed only in individuals who are carriers of the allele HLA-B * 57: 01 regardless race, and therefore, before starting treatment with abacavir, it is necessary screen for the presence of the HLA B*57:01 allele. The prevalence of this allele in different regions of the world varies from 3-8% depending on the population. In the Republic
Bleraus, such studies have not been conducted. Purpose: to study the prevalence of the HLA-B * 57:01 allele among people living with HIV in the Republic of Belarus (2018-2023) based on the method developed in Republican Scientific and Practical Center for Epidemiology and Microbiology.

Material and Methods: Whole blood samples from HIV-infected patients, received from different regions of the Republic of Belarus in the period from 06/01/2018 to 03/24/2023. To determine the presence of the HLA-B*57:01 allele, the method described in
instructions for use “Method for detecting the 57:01 allele of the B locus of the main complex human histocompatibility (HLA B*57:01)” (No. 024-1221), development The Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, Republic of Belarus.

Results: A study was carried out on 1368 samples, obtained from people living with HIV collected from different regions Republic of Belarus: 15 (1.1 ± 0.3%) samples were received from the Brest region, from the Vitebsk region - 2 (0.1±0.1%) samples, from Grodno - 10 (0.7±0.2%) samples, from Mogilev - 18 (1.3±0.3%). The largest number of samples came from three regions – Minsk region (n = 1123; 82.1±1.0%), Minsk (n = 130; 9.5±0.8%) and Gomel region (n =70; 5.1±0.6%). The age range was from 1 to 75 years, with a median age of 41 years. The following results were obtained: out of 1368 samples, in 92 (6.7±0.7%) cases there was discovered pharmacogenetic marker, allele 57:01 of locus B of the main complex human histocompatibility. In Minsk, and Gomel regions, the frequency the prevalence of this allele among the participants included in the study was 6.2±2.1% (8/130), 7.0±0.8% (79/1123) and 5.7±2.8% (4/70), respectively. One participant in anamnesis indicated clinical manifestations of allergy, another had the use of abacavir in the antiretroviral therapy regimen.

Conclusion: The results of the study obtained on the basis of the use of the method, developed at the Republican Scientific and Practical Center for Epidemiology and Microbiology, demonstrated that prevalence of the 57:01 allele of the B locus of the human major histocompatibility complex averages 6.7%. Despite the relatively low prevalence of the allele HLA-B*57:01, the introduction of this method can reduce the incidence of delayed-type allergic hypersensitivity reaction when prescribed the antiretroviral drug abacavir.

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Molecular Characterization of SARS-CoV-2 Omicron Clade and Clinical Presentation in Children

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Background: Since its emergence, SARS-CoV-2 Omicron clade showed marked degree of variability and different clinical presentation compared with previous clades. Here, the genomic characteristics and impact on COVID-19 presentation of this clade were evaluated in children.

Material and Methods: 657 Omicron whole sequences were obtained from participants aged ≤12 years referred for SARS-CoV-2 diagnosis at Bambino Gesù Children Hospital from December 2021, to early November 2022. Whole SARS-CoV-2 sequences, obtained by Multiplex PCR system (Illumina MiSeq) were analyzed by Maximum Likelihood and Bayesian coalescent methods to define Phylogenetic structure of the paediatric epidemic and evolutionary rate of Omicron clade. Constitutive (intra-participant prevalence >70%, according to https://covariants.org/) and non-constitutive (intra-participant prevalence 2-70%) mutations defining Omicron lineages were evaluated. Multivariate logistic regression analysis was performed to assess factors associated with disease severity.

Results: 347 (52.8%) children were male, with a median age of 0.56 (Interquartile range, IQR: 0.23-1.66) years. Mild infections were the most prevalent (82.0%), followed by asymptomatic (9.8%), and moderate/severe infections (8.2%). One-hundred and twenty-three (18.7%) participants had comorbidities. At least four Omicron lineages circulated widely in children: 40.5% of sequences belonged to BA.2, followed by BA.1 (33.6%), BA.5 (23.7%) and BQ.1 (2.1%). The Omicron mean evolutionary rate (subs/site/year) was 9.8x10^-4 (95%HPD, 8.8x10^-4 -1.1x10^-3) and no differences were observed among Omicron lineages.
Constitutive mutations increased from 46 (45-46) of BA.1 to 66 of BQ.1 (P<0.001). Of note, 4 non-constitutive mutations characterized by a median intra-participant prevalence of 7.6% (IQR: 6.2-11.5) (Nucleocapside-L221F, nsp6-L260F, RdRp-G678G, Spike-A694S) also increased their frequencies across lineages, with the exception for Spike-S686R, close to furin-cleavage-site, that decreased from 82% of BA.1 to 10% of BA.5 (P<0.001).
Multivariate logistic regression analysis showed that BA.5 and age <1 year were negatively associated with moderate/severe COVID-19 presentation (adjusted odds ratio, AOR: 0.26 [0.07-0.90] P=0.034; 0.45 [0.23-0.89] P=0.021), while positive association were observed with BA.1 and comorbidity (AOR [95% CI]: 2.2 [1.2-4.1] P=0.014; 2.6 [1.3-5.1] P=0.006).

Conclusion: These results highlighted the extensive SARS-CoV-2 Omicron circulation in children, mostly aged <1 year, and provided insights on non-constitutive mutations and their role in evolutionary processes. Our findings also suggested a milder phenotype of BA.5 compared to other Omicron lineages, letting suppose the potential contribution of viral diversification in affecting disease severity.

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Amino Acid Variability of Small-Molecule Inhibitors and mAbs Target Sites in Globally Circulating RSV Strains

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Background: Respiratory Syncytial Virus (RSV) causes high morbidity and mortality in high-risk population. Currently, the EMA-approved drugs for RSV are two monoclonal antibodies (mAbs) against fusion (F) protein (Pavilizumab and Nirsevimab) and the nucleoside analogue ribavirin. The efficacy of small molecule inhibitors (SMIs), targeting Large polymerase (L), Nucleocapsid (N), F and Glycoprotein (G), is under evaluation. Here, we characterize the extent of amino acid (aa) variability in target sites of both SMIs and mAbs by analyzing globally circulating RSV deposited sequences.

Material and Methods: 1367 full-length RSV sequences from participants who are drug-naïve were downloaded from NCBI database, considering one sequence/participant. RSV type was inferred by Maximum likelihood phylogenetic analysis. Genetic distance (Maximum Composite models) and aa variability (Entropy) were evaluated in SMIs and mAbs targets against RSV type, while selective pressure (dN/dS) by Fubar and MEME tools, retaining only positions under positive selection with both methods Statistical analyses were performed by T-test and Kruskal-Wallis test.

Results: Retrieved sequences included 845 (61.8%) type A and 522 (38.2%) type B RSV, mostly (1214, 88.8%) isolated before 2020. Sequences mainly came from Europe (47.5%) and North- and South-America (33.9%), followed by Asia (6.7%) and other Countries (11.9%).
The genetic diversity was the lowest in N protein, followed by F, G and L in both RSV types ($p<0.001$). Of note, within-type genetic diversity of G, F, and N was lower in sequences collected after 2020 ($p<0.001$, $p=0.013$ and $p=0.056$, respectively), probably because of the limited RSV circulation during COVID-19 pandemic. In line with these results no N positions were under positive selection, while 2 F and 6 G positions in type A, and 2 F and 1 G positions in type B were under positive selection. Even more, only 6 aa N positions (localized in N-ntd and N-ctd domains) showed different entropy between A and B types (delta-H>0.1, $p<0.05$), while 26 F (8 localized in antigenic sites Ø,I,II and V) and 105 G aa positions were characterized by different entropy in A and B types (delta-H>0.1, $p<0.05$), confirming divergent evolutionary pathways of these proteins between RSV types. Finally, L protein was characterized by 76 aa positions (32 localized in RdRp) showing different entropy between A and B types (delta-H >0.1, $p<0.05$) and no aa positions under positive selection. Notably, none of the aa positions characterized by different entropy and selective pressure corresponded to drug-resistant sites. Only 32 (3.3%) sequences carried aa mutations conferring resistance to at least one mAb or SMI. One B strain carried a combination of F mutations (K272N+F488L) conferring full-resistance to Pavilizumab and SMIs against F, respectively.

Conclusion: The genetic characterization of RSV strains circulating worldwide showed a lower degree of genetic variability of N protein compared to the other drug targets and little residue variability at positions associated to drug-resistance. Continuous efforts in monitoring RSV genomic evolution, especially regarding mAbs and SMIs targets, are needed to guide clinical decisions and future therapeutic and preventive strategies development.

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Neutralizing Antibodies Response to Novel SARS-CoV-2 Omicron Sublineages in Long-term Care Facility Residents After the Fourth Dose of Monovalent BNT162b2 COVID-19 Vaccination

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Background: The dynamics of SARS-CoV-2 variants has transitioned from complete long-term dominance of a successful variant to a dynamic swarm of genetically related lineages carrying convergent aminoacidic mutations located in the spike region. In particular, the BA.2 and BA.5 lineages have recently evolved into many sub-lineages, also known as omicron soup. Aim of this work was to quantify the neutralizing antibodies titre (NtAb) against the recently predominant sublineages BA.2.75.2, BQ.1.1 (BA.5 derived) XBB.1 and CH.1.1 (BA.2 derived) in a fragile population

naïve for SARS-COV-2 infection and vaccinated with four doses of monovalent BNT162b2 COVID-19 mRNA vaccine.

Material and Methods: Plasma samples were collected from 40 residents at Pio Albergo Trivulzio, the largest Italian long-term care facility, 71 [68-75] and 89 [80-91] median [IQR] days after the third (T3) and fourth (T4) BNT162b2 vaccine dose. The study group had median age of 91 [84-94] years, included 5 males and no subject with positive anti-nucleocapsid serology at first vaccine dose. Participants were weekly screened by swab analysis to exclude subsequently SARS-CoV-2 infection. NtAb titers were measured at T3 and T4 against the wild type (WT) lineage B.1 and at T4 against five Omicron sublineages (BA.5, BQ.1.1, BA.2.75.2, XBB.1 and CH.1.1). Live virus microneutralization was performed in VERO E6 cells quantifying the cell viability by luminescence. The NtAb titer was defined as the reciprocal value of the sample dilution showing 50% protection of virus-induced cytopathic effect (ID_{50}). Antibodies with ID_{50} titers < 10 were defined as negative and scored as 5 for statistical analysis. SARS-CoV-2 IgG II Quant assay (Abbott) was used to quantify the anti-spike protein Ab at T3, T4 and after the first and the second vaccine dose administration (T1, T2).

Results: Thirty-four and 13 patients had at least 1 and 3 comorbidities respectively, and polypharmacy was common. As expected, NtAb titers to WT variant significantly increased ($p < 0.001$) at T4 (1094 [612-3252] ID_{50}) with respect to T3 (518 [60-1515] ID_{50}). A significant increase was also observed when comparing the anti-spike Ab median titres at T3 and T4 (2724 [35-4542] vs. 9939 [4168-12,500]; $p<0.001$). One participant never responded to the full vaccination cycle showing negative anti-spike Ab titers and negative NtAbs at each time point analyzed. Overall, at T4 median NtAb titres to the WT strain correlated with those to each omicron variant ($p<0.001$ for all comparisons) but absolute values expressed as ID_{50} were significantly lower: BA.5, 287 (38-533); BA.2.75.2, 30 (15-67); BQ.1.1, 22 (11-58), XBB.1, 13 (5-24) and CH.1.1, 13 (5-19) ($p<0.001$). At each time point analyzed the anti-spike Ab and NtAb titres against WT and different variants, were not correlated to the different comorbidities when evaluated individually or stratified for increasing number ($p>0.05$).

Conclusion: In this fragile elderly long-term care residents, recently circulating omicron sublineages BQ.1.1, BA.2.75.2, XBB.1 and CH.1.1 showed greater escape from monovalent BNT162b2 COVID-19 mRNA vaccine with respect to the previously dominant BA.5 variant. It remains to be established whether the reduced NtAb titers still protect from incident infection with these and future variants.

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Geno2pheno: Recombination Detection for HIV and HEV Subtypes

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Background: The Human Immunodeficiency Virus 1 (HIV-1) endemic is still a major burden on society. Therapeutic success is still not ensured, especially in low-income countries. The identification of the correct HIV subtype can support the choice of a successful therapy. Recombination events, known to happen in HIV, give more insight in the epidemiology of HIV and could become more prominent. Like HIV, hepatitis E virus (HEV) is distributed world-wide, occurring in different genotypes (1-4) and subtypes. Genotype 3 is endemic in animals (pork, rabbit) in Europe. HEV genotype 3 causes in most cases a mild disease and a non-chronic infection. However, individuals who are immunocompromised can suffer from severe and from chronic courses. HEV subtyping has so far not been a major focus of interest. Contrary to HIV, recombinations of different subtypes of the Hepatitis E Virus (HEV) are considered rare. The majority of subtyping tools focus on either known subtypes or known circulating recombinant forms (CRFs). However, novel recombination events are hardly considered or just in specific observed and scrutinously analyzed cases. Our objective was therefore to develop a subtyping tool that not only identifies known subtypes or recombinants, but also detects new recombinant virus variants.

Material and Methods: We are using a new implementation of the computational method recco to detect de novo recombination of known subtypes, independent of and additional to CRFs. Our method moves along the positions of the

aligned reference sequences and penalizes mutations on the sequence of interest (SOI) with a cost factor alpha in (0,1). Once too many mutations (cost alpha) are detected on the current reference compared to the SOI, the method considers a recombination (cost 1-alpha) event as cheaper and jumps to a different reference with the recombination of the two sequences yielding fewer mutations. The optimal path(s) are considered. That path either follows just on one subtype or combines different subtypes to a recombinant form. The method efficiently identifies the optimal path with dynamic programming.

Results: Besides novel recombinations, our tool can successfully detect known recombination events given only the full references (without CRFs) of the participating subtypes. However, the recombinant sequences are given in more detail with explicit break points. E.g., if a CRF states that one part of the sequence is of subtype A, our method differentiates among all subtypes from A1 up to A8. Recco is the default tool in our geno2pheno (https://geno2pheno.de) software suite used for subtyping of HIV. In addition, the tool can be applied to other viruses, i.e HEV. By this, we could also detect several previously unknown recombinations in HEV. E.g., from the unidentified genotype 4 sequences (HEV-GLUE: hev.glue.cvr.ac.uk), the longest one is a recombination with large parts from 4g (56%) and 4h (26%). The rest is identified as 4a-c (5%,5%,8%).

Conclusion: The new version of our subtyping tool, which is integrated in the geno2pheno interpretation tool, now also offers the possibility to identify new HIV subtypes (A6) and new recombinations. In addition, this tool can also be adapted to other viruses, such as HEV.

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Molecular Analysis of Non-B Subtypes in Greece: Low Onward HIV-1 Transmission Among Migrants

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Background: The last few years, a substantial proportion of people diagnosed with HIV-1 in Europe are migrants. We aimed to estimate the proportion of infections within migrants in molecular transmission clusters (MTCs) and parameters associated with clustering for the most prevalent non-A1 and non-B clades in Greece, using molecular epidemiology methods.

Material and Methods: Our study sample consisted of 408 sequences: C (n=146, 35.8%), CRF02_AG (n=139, 34.1%), CRF01_AE (n=58, 14.2%), G (n=33, 8.1%), F1 (n=32, 7.8%). We used the first available pol gene sequence for all people living with HIV (PLHIV) diagnosed during 1999-2015 in Greece. HIV-1 subtyping was carried out using automated subtyping tools (COMET, REGA). Phylogenetic analysis was performed on sequences from migrants (C:78; CRF02_AG:61; CRF01_AE:8; G:25; F1:19) along with all the available sequences from non-migrants (C:68; CRF02_AG:78; CRF01_AE:50; G:8; F1:13). A random set of globally sampled sequences (C:972; CRF02_AG:858; CRF01_AE:1,993) or all the available global sequences (G:1,147; F1:1,385) and the most closely related sequences identified by BLAST tool (C:50;

CRF02_AG:33; CRF01_AE:50; G:30; F1:34) were used as references. Phylogenetic trees were estimated by maximum likelihood (ML) method (RAxML, FastTree). Phylogenetic clusters including sequences from Greece at proportions >70% and receiving bootstrap value >75% and SH-support >0.9 were defined as MTCs. Robustness of MTCs was confirmed by ML transfer bootstrap observation (TBE). Parameters associated with clustering were estimated by multivariable logistic and exact logistic regression models (STATA 13).

Results: For CRF02_AG we found 15 MTCs consisting of 2 to 8 sequences and one large MTC including 49 sequences. For CRF01_AE 2 MTCs consisting of 2 sequences each and two large ones including 17 and 26 sequences were identified. The size of MTCs ranged between 2 and 12 sequences for C (21 MTCs), 2 and 3 sequences for F1 (6 MTCs) and was 2 sequences for G (3 MTCs). The proportion of sequences from migrants clustered within MTCs ranged between 20% and 42.1%. It was <35% for all subtypes/CRFs [C: 27 (34.6%); CRF02_AG: 21 (34.4%); CRF01_AE: 2 (25%); G: 5 (20%)] apart from F1 [8 (42.1%)]. The majority of migrants was heterosexuals [C: 56 (71.8%); CRF02_AG: 39 (63.9%); CRF01_AE: 5 (62.5%); G: 20 (80%); F1: 12 (63.2%)]. Parameters associated with clustering were year of sampling for C [Odds Ratio (OR): 1.25, 95%CI: 1.13-1.38], MSM risk group for CRF01_AE (MSM vs heterosexuals; OR: 19.1, 95%CI: 2.31-Inf) and Greek origin for C (non-migrants vs migrants, OR: 3.95, 95%CI: 1.65-9.48), CRF01_AE (non-migrants vs migrants, OR: 21.1, 95%CI: 2.04-Inf) and CRF02_AG (non-migrants vs migrants, OR: 38.11, 95%CI: 8.09-79.44).

Conclusion: We showed that for the most prevalent non-A1 and non-B clades in Greece, the proportion of infections within migrants that was found in MTCs was low. For 3 out of 5 clades under study (C, CRF01_AE, CRF02_AG), Greek origin was found to be associated with infections within MTCs. Although the number of newly diagnosed cases among migrants showed an increasing trend, we provide evidence that HIV-1 infections among migrants were not associated with onward transmission and migrants didn't impact the HIV epidemic in Greece.

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Novel Robust Near Full Length Genome Deep Sequencing and Characterization of HIV-1 Recombinant Forms in Israel

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Background: The high genetic diversity and recombination events of the HIV-1 resulted over the years in the creation of multiple subtypes and novel recombinant forms. These changes are posing a challenge on viral near full length genome (NFLG) sequencing analysis from clinical samples. The currently available methods require multiple steps and several separate PCR reactions in order to generate DNA fragments spanning the HIV-1 genome. Here, we present a novel NFLG approach that is applicable for various HIV-1 sub-types, sub-subtypes and recombinant forms (RFs), which is based on the SARS-CoV-2 Artic network amplicon protocol. Multiple PCR fragments spanning the entire genome of HIV-1 are simultaneously generated in two parallel multiplex PCR reactions. This new HIV-NFLG approach was successfully employed for the characterization of different HIV-1 RFs circulating in Israel.

Material and Methods: A set of 118 diverse, full length genome HIV-1 sequences extracted from LANL HIV sequence database (pure subtypes and RFs) were used for primer design. Overlapping fragments, spanning the entire HIV-1 genome were generated. Appropriate primers were defined based on primal-scheme and manually edited on Geneious Prime software. Overall, 64 primers divided into 2 primers pools (each containing 32 primer pairs) were generated and used for PCR amplification. Sequencing was performed using Miseq Nextera XT protocol (20 samples per run). Samples from 35 people living with HIV (33 of which were suspected as RFs based on pol gene Sanger sequencing) were selected for this NFLG analysis. To determine recombination events phylogenetic analysis as well as known tools for recombinant analysis such as RIP, SimPlot++, jpHMM were used.

Final verdict was reached by a combinatory result of the different tools.

Results: The average coverage obtained (with a minimum of 5 reads per position) was 84.5% (range 73.25-98.55 %) of the entire HIV-1 genome. Coverage of CRF02_AG containing recombinants was at the lowest range. Overall coverage was similar between the different samples. While the coverage of the gag and pol gene was high in all samples analyzed the highly variable env gene region was not fully covered. Results of the four different tools used herein defined three sequences initially suspected to be recombinants (based on pol Sanger sequencing) as pure subtype B. All other samples were identified as RFs based on NFLG analyses. The most common (n=15) was recombination of CRF02_AG with either A or B subtypes. Additionally, three CRF01_AE and two BF1 RFs were identified. The rest of the samples displayed a recombination of subtypes B and G, C and A, B and A.

Conclusion: The approach described here is a robust method that simplifies the process of HIV-1 NFLG sequencing. This pan-HIV-1 method is applicable for a variety of subtypes, and can be useful for determination of recombination events across the viral genome.

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Analysis of HIV-1 Genetic Variants Among MSM Living in Moscow, Russia

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Background: According to statistics data, the proportion of men who have sex with other men (MSM) in HIV-1 epidemiological process is small. For instance, in 2020-2021, only 2.90% of HIV-infected were MSM. Partly it is related with stigma of MSM in Russia and closeness of this group. The same problems are existed for migrants and people working in the sex industry (PWSI) as well.
HIV-1 sub-subtype A6 is historically dominating in Russia (more than 70% in 2010-2019). However, other HIV-1 variants are circulating in Russia such as recombinant CRF63_02A6, CRF03_AB, rare genetic variants and unique recombinant forms (URFs).
HIV-1 subtype B (characteristic for Western Europe and USA) dominated in Russian MSM in the past. But recently the increasing of A6 proportion in MSM was shown. Finally, in 2019 was published the data about circulation of BG-recombinants in MSM in Russia.
The aim of the study was to analyze the HIV-1 genetic variants circulating in HIV-positive MSM living in Moscow, including migrants and PWSI.

Material and Methods: Plasma samples (n=123) from MSM were obtained in 2020-2023 with collaboration with «Steps» fund (http://stepsfund.ru/) and «LaSky» center (https://lasky.ru/). Viral load, CD4 counting and sequencing were carried out. Subtyping of gene region (2253–3369 bp according to the HXB2, GenBank accession number K03455) was done using HIVdb (https://hivdb.stanford.edu/), HIVBlast (https://www.hiv.lanl.gov/) and phylogenetic analysis in MEGA 6.0.

Results: In 123 samples, 93 (75.61%) were obtained from MSM migrants and 30 (24%) – from Russian citizens. Migrants' citizenship was: Uzbekistan (n=28), Kyrgyzstan (n=20), Tajikistan (n=18), Cuba (n=11), Kazakhstan (n=4), Ukraine (n=3), Armenia (n=2), Belarus (n=2), Turkmenistan (n=2), Congo (n=1), Peru (n=1) and Columbia (n=1).
9 individuals were PWSI. The middle age was 29 years (95%CIs 27.29–29.86), average HIV-infection duration (from HIV-positive date) was 90 days (95%CIs 52–129), middle viral load was 5.28 Log10 copies/ml (95%Cis 5.08 – 5.42), middle CD4 count – 477 cells/mcl (95%CIs 431 – 522). The total 105/123 (85.37%) of persons supposed were infected in Moscow. The same information was indicated by 81/93 (87.10%) of migrants. Middle number of sexual partners in last 6 months before including in study was 8 (95%CIs 6 – 11). HIV-1 sub-subtype A6 was found in 70/123 (56.91%) samples. 25 (20.33%) samples harbored subtype B, 9 (7.32%) – BG-recombinants, 7 (5.69%) – URF_0263, 5 (4.07%) – CRF19_cpx, 4 (3.25%) – CRF63_02A6. Finally, 2 samples harbored CRF01_AE and CRF56_cpx correspondingly and only one – CRF01_AE. 5/28 persons from Uzbekistan and 7/11 individuals from Cuba (who said that were infected in Moscow) were infected by URF_0263 and CRF19_cpx/CRF20_BG, correspondingly. URF_0263 is endemic HIV-1 variants in Uzbekistan, as well as CRF19_cpx and CRF20_BG in Cuba. It may indicate that these persons were infected in groups of persons, including their fellow citizens.

Conclusion: Our results clarify the genetic diversity of HIV-1 in MSM living in Moscow, including migrants from different countries and people working in the sex industry. It is important to expand HIV-monitoring in MSM for a better understanding of the epidemiological process and development of preventive programs in this vulnerable group.

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Identification of Four New HIV-1 Circulating Recombinant Forms, CRF129_56G, CRF130_A1B, CRF131_A1B, and CRF138_cpx in Cyprus

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Background: Continuous global transmission of HIV-1, together with fast viral turnover and high mutation frequency has led to an increased rate of molecular evolution. As such, HIV-1 has acquired broad genetic diversity. Accordingly, HIV-1 was classified into four groups (M, N, O and O) with ten distinct phylogenetic subtypes (A, B, C, D, F, G, H, J, K, and L) within the major group M. In polyphyletic HIV-1 epidemics, adequate prevalence of several HIV-1 subtypes together with the highly recombinogenic nature of the virus could lead to the occurrence of recombination events between these subtypes and hence generation of new HIV-1 recombinant strains.

Material and Methods: Molecular studies of the HIV-1 pol region (2253-5250 in the HXB2) sequences derived from consenting individuals infected with HIV-1 in Cyprus (2017-2021) revealed four transmission clusters of HIV-1 recombinants, which are not classified as previously established CRFs. In order to characterize the unique mosaic structure of these recombinant sequences belonging to the four recombinant transmission clusters, the near-full-length HIV-1 genome sequences (790-8795 on HXB2 genome) were obtained for the 18 recombinant query sequences. For this, we have utilized our recently published near-full-length HIV-1 genome RT-PCR assay. The acquired sequences were later aligned against a reference dataset of all known HIV-1 subtypes and CRFs obtained from the Los Alamos HIV Sequence Database (RIP Alignment 2020), where a different phylogenetic tree was constructed for each of the identified recombinant transmission clusters. Specifically, a multiple sequence alignment (CLUSTALW algorithm) and a maximum-likelihood tree were constructed (GTR model with gamma distribution, 1000 bootstrap replicates) using MEGA X software. It was followed by phylogenetic clustering analyses using Cluster-Picker (genetic distance ≤0.045, bootstrap support value ≥70%). Phylogenetic analyses were followed by detailed recombination analyses through similarity plot and bootscan analyses using SimPlot v3.5.1 to identify the putative intersubtype recombination breakpoints. The recombination analyses were conducted against a reference dataset of HIV-1 group M pure subtypes (A, B, C, D, F, G, H, J, K, and L) and CRF56_cpx acquired from the Los Alamos HIV Sequence Database, which was supplemented using BLAST results with the top BLAST hits for subtype B, F2, CRF22_01A1 and CRF56_cpx. A sliding window of 400 nucleotides overlapped by 40 nucleotides, with 1,000 bootstrap replicates were utilized as the optimal parameters. Consequently, sub-region confirmatory neighbor-joining tree analyses were performed using MEGA X software to confirm the recombination breakpoints and the subtype origin of each fragment (Kimura two-parameter model, 1000 bootstrap replicates, ≥70% bootstrap-support value was considered as definitive).

Results: The phylogenetic analyses revealed that the HIV-1 recombinant sequences did not cluster with any of the pure subtypes or CRFs but they exclusively clustered together, revealing their uniqueness. Seven samples were included in the first recombinant transmission cluster. The bootscan and similarity plot analyses demonstrated the same mosaic pattern for all seven query sequences, revealing two intersubtype recombination breakpoints; 1758 ± 112 and 2037 ± 25 (in the HXB2 genome). The subregion confirmatory analyses displayed that the three fragments clustered with CRF56_cpx (100%), G (72-98%) and CRF56_cpx (100%), respectively. Three samples were included in the second recombinant transmission cluster. The bootscan and similarity plot analyses demonstrated the same mosaic pattern for all three query sequences, revealing seven intersubtype recombination breakpoints;

2285, 3535, 4260, 4899, 6070 ± 15, 6322, and 8486 (in the HXB2 genome). The subregion confirmatory analyses displayed that the eight fragments clustered with A1 (100%), B (93-98%), A1 (100%), B (86-93%), A1 (97-98%), B (83-86%), A1 (100%) and B (83-93%), respectively. Four samples were included in the third recombinant transmission cluster. The bootscan and similarity plot analyses demonstrated the same mosaic pattern for all four query sequences, revealing seven intersubtype recombination breakpoints; 2285, 3827, 4251, 4823, 6085, 6524, and 8486 (in the HXB2 genome). The subregion confirmatory analyses displayed that the eight fragments clustered with A1 (100%), B (96-100%), A1 (100%), B (86-96%), A1 (96-100%), B (94-98%), A1 (99-100%) and B (81-92%), respectively. Finally, four samples were included in the fourth recombinant transmission cluster. The bootscan and similarity plot analyses demonstrated the same mosaic pattern for all four query sequences, revealing five intersubtype recombination breakpoints; 1138 ± 6, 2062, 3024, 4261 ± 49, and 4382 ± 24 (in the HXB2 genome). The subregion confirmatory analyses displayed that the six fragments clustered with F2 (96-97%), CRF22_01A1 (99%), F2 (100%), CRF22_01A1 (100%), U (8-22%), and CRF22_01A1 (100%), respectively.

Conclusion: In conclusion we have characterized the unique mosaic structure of the four novel HIV-1 CRFs identified in Cyprus using the 18 recombinant query sequences. In this regard, the four novel HIV-1 CRFs were named in accordance with the standards of HIV nomenclature as CRF129_56G, CRF130_A1B, CRF131_A1B and CRF138_cpx, respectively. Through further BLAST analyses, we have identified an additional HIV-1 sequence sampled in USA (2018/2019), which was identified to belong to CRF130_A1B strain, and three additional sequences that belonged to CRF138_cpx strain sampled in Belgium (2019) and Cameroon (2011), which can be attributed to these strains circulating among the human population for some time. Additionally, we have also identified a URF of CRF138_cpx, with two additional recombination sites caused by recombination of subtype F2 into the genome of CRF138_cpx.

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Genomic Epidemiology of the Main SARS-CoV-2 Variants Circulating in Italy in 2020 and 2021 Period

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Background: Since the beginning of the pandemic, SARS-CoV-2 has shown a great genomic variability, due in part to a high mutation rate and driven by the widespread and rapid circulation in the human population. The continuous emergence of SARS-CoV-2 new variants has made their global monitoring and the study of their characteristics a priority. Aim of this work was to study the genomic heterogeneity, the temporal origin, the rate of viral evolution and the population dynamics of the main circulating variants (20E.EU1, Alpha and Delta) in Italy, in the period August 2020-January 2022.

Material and Methods: The Whole Genome sequences of SARS-CoV-2 have been collected at the centres of the collaborative group SCIRE (SARS-CoV-2 Italian Research Enterprise). For each variant, we analyzed two datasets, the former comprising international genomes and the latter focusing on Italian sequences. Phylogenetic trees were estimated using IQ-TREE v.1.6.12. The Italian clusters were analyzed using BEAST v.2 in order to estimate their tMRCA (time of the Most Recent Common Ancestor) and main epidemiological parameters.

Results: In 20E.EU1 clade and Alpha variant, only mutations specific to the variant were observed; differently, Delta variant sequences showed a high number of additional mutations, especially in the ORF1a region. 20E.EU international dataset showed 26 clusters characterized by at least one Italian sequence of which 23% pure Italian, 23% Singleton, and 54% mixed. Among 40 clusters of Alpha variant, 60% were mixed, 37.5% Italian and 1 singleton. Likewise, 85.7% of Delta clusters were mixed, 9.5% singleton and 4.8% pure Italian. International clusters, including more than 70% of Italian genomes (11, 17 and 8 for 20E.EU1, Alpha and Delta, respectively), presented tMRCAs between 13/06/2020-28/09/2020, 10/11/2020-20/02/2021 and 13/03/2021-27/07/2021 for 20E.EU1 clade, Alpha and Delta variants, respectively. The annual growth rates estimated for each variant were 3.79, 2.85 and 5.9; considering a duration of infectivity of 7 days, the R0 resulted 1.07, 1.05 and 1.11. Re values above the unit were observed from the beginning of the epidemic in 20E.EU1 clade, and during the 2 phases of exponential growth (June and September 2020). Starting from February 2021, it was observed a reduction of Re around the unit and the joining of the plateau. Alpha variant showed an increase in the Re in December 2020, when the highest mean value was estimated (1.15), remaining above 1 until March 2021, when it started to decrease around 1 until June 2021. For Delta variant, we observed two peaks: the first between March and May 2021 (1.34), and the second between June and July 2021 (1.14), while the decrease of Re to 1 matched with the achievement of the plateau in August remaining stable until 2022.

Conclusion: Our work highlighted a different evolutionary dynamic of studied lineages. A high concordance was observed between epidemiological parameters estimation and phylodinamic trends. When the Skyline Plot displayed an exponential increase in the viral population (indicating an increase in transmission events) the birth-death analysis showed Re values above 1, while Re periods below 1 corresponded to a decrease in the size of the epidemic.

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Long Gone Unnoticed Highly Replicative Hepatitis B Virus Reactivation in the Presence of High Levels of Anti-HBs Antibodies

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Bacground: Hepatitis B virus (HBV) reactivation is not uncommon in a context of immunosuppression. It usually occurs as overt infection, but sometimes HBsAg can be undetectable (occult infection). Moreover, a lack of significant transaminase elevation is common during HBV reactivation. The diagnosis can thus be challenging especially in individuals with anti-HBs antibodies (Ab), because in routine clinical practice, HBV DNA is not usually tested in such situation. Herein, we report a case of HBV reactivation which went unnoticed for 7 years in a KT recipient, presenting with negative HBsAg but an active viral replication despite high levels of anti-HBs Ab.

Material and Methods: A 67-year-old woman presented in 2022 to the emergency department for a right-sided hemiplegia, with a marked motor deficit in the lower limb. She underwent kidney transplantation (KT) 31 years ago, and was known with a serological profile of past HBV infection. On admission, liver function tests showed few mild abnormalities and prompted to a systematic screening of common viruses associated with chronic hepatitis. HBV serology found a positive result for anti-HBc antibodies and the presence of high levels of anti-HBs antibodies, suggestive of resolutive past infection. However, the signal of HBsAg was close to the cut-off. In addition, IgM antibodies were detected for hepatitis E virus (HEV). In such context of immunosuppression, further investigation was initiated. HBe antigen was strongly positive, and a negative reaction found for anti-HBe antibodies. Both HBV DNA and HEV RNA were detected, with plasma viral loads at 7.68 Log

IU/mL and 5.59 Log IU/mL, respectively. Several serum samples collected in the past (from 2009 to 2019) were obtained and tested in order to date the reactivation. Evidence of high viral replication was found since 2015. Anti-HEV IgM and HEV RNA were not detected in a previous sample, suggesting a recent superinfection on chronic HBV infection.

Results: Viral genome sequencing showed a high number of mutations especially in the PreS/S region. The PreS sequence displayed 2 amino acids substitutions in the B-dependent epitope (positions 124 and 128). More interestingly, analysis of the HBsAg amino acid sequence (226 amino acids) revealed several amino acid substitutions (11 in total, at positions 111, 112, 123, 124, 125, 137, 144, 145, 149, 159 and 220) in the major hydrophilic region (MHR, position 99-169) including 5 substitutions in the "a" determinant (positions 124-147).

Conclusion: We described in a individual with KT known with HBV past infection since 31 years, a highly replicative HBV reactivation which went unnoticed for 7 years, due to the negativity for hepatitis B surface antigen (HBsAg) and high levels of anti-HBs antibodies. Several mutations found in the S region of HBV genome and especially the "a" determinant are known to be associated with reduced affinity of antibodies, immune escape, a decreased sensitivity of several HBsAg immunoassays and a drastic decrease of HBsAg production. This case highlights the usefulness of frequent HBV viral load testing in individuals at risk of reactivation, with anti-HBc Ab, regardless of HBsAg detection, transminases and anti-HBs Ab levels.

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False-Positive HIV Molecular Testing in Individuals Treated by Lentivirus-Based Chimeric Antigen Receptor T-Cells (Car T Cells): Beware of Analytical Interference With HIV Viral Load Monitoring

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We report the case of a 65-year-old woman followed for multiple myeloma diagnosed in 2007. After multiple lines of therapy and relapses, she received idecabtagene vicleucel (a lentiviral vector based CAR T-cells immunotherapy). At day 9 post infusion, HIV-1 molecular testing was prescribed. A positive signal was detected by 2 commercial RT-PCR assays (Alinity m HIV-1, Abbott; 2.8 log cp/ml and Xpert HIV-1 Viral Load XC, Cepheid; 3.3 log cp/ml). Pretherapeutic HIV serology (fourth and fifth generation EIAs) and molecular testing were negative and no risk factor for HIV infection was reported by this individual. At day 11 post infusion, HIV serology remained negative while HIV-1 RNA was still detected with Alinity m HIV-1 assay (3.5 log cp/ml) on a control sample. At day 21, HIV-1 RNA was quantified at 2.18 log cp/ml (Alinity m HIV-1 assay) and was no more detected 3 months after infusion. Finally, we concluded to a false HIV-RT-PCR positive result, both assays targeting the long terminal region (LTR), due to an interference with the lentiviral based vector LTR sequence used to manufacture idecabtagene vicleucel therapy.

Eleven individuals have received idecabtagene vicleucel therapy in our center between March 2022 and January 2023. To better investigate this interference, we retrospectively tested available frozen samples. In total, prior to CAR-T infusion, negative HIV serological status and/or negative HIV-1 RNA were documented for 9 (82%) and 5

(45%) patients, respectively. HIV-1 viral loads ranging from 1.72 to 4.59 log cp/ml were detected in plasma taken 2 to 9 days post CAR-T infusion for 8 out of 8 participants, with Alinity m HIV-1 assay. For 3 tested samples, similar quantifications were obtained with the Xpert® HIV-1 Viral Load XC assay. Interestingly, HIV-1 RNA was not detected in 2 samples from 2 participants using an assay targeting only the integrase region (Abbott RealTime HIV-VL), while HIV-1 RNA was detected but not quantified for 2 samples from 2 participants with the transcription-mediated amplification method Aptima® HIV-1 Quant Dx Assay assay, targeting the LTR region. HIV-1 DNA (Generic HIV DNA cell, Biocentric) was quantified from 5 whole blood from 4 participants, and showed a weak correlation with the HIV-1 RNA quantification (rs 0.781, p=0.118). No correlation was found between HIV DNA or RNA loads and CAR-T and non-CAR-T cells cell-flow cytometry quantification.

Chimeric antigen receptor T –cell immunotherapies are promising therapeutic options in oncology. Interference between tisagenlecleucel, a lentivirus-based CAR-T, and different HIV-1 molecular assays, targeting LTR, has been previously described. Cross-reactions between idecabtagene vicleucel, another lentivirus-based CAR-T, and assays targeting the LTR region (as Alinity m HIV, Xpert® HIV-1 Viral Load XC or Aptima® HIV-1 Quant Dx Assay) are expected but have not been reported to date. Molecular HIV testing should not be performed for non-HIV infected patient receiving lentiviral based CAR-T. These participants should have only serological screening. For efficiently treated people living with HIV and eligible for this therapy, performing molecular HIV testing during therapy may lead to virological failure misdiagnosis.

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Case study: The Therapeutic Role of Human Papilloma Virus Vaccine in Individual with Human Immunodeficiency Virus and Giant Condyloma Acuminata

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Background: HIV coinfection changes the course of Human Papilloma Virus (HPV) infection into more invasive growth of benign giant condyloma acuminata (also known as Buschke-Lowenstein Tumor/BLT). Albeit its benign nature, invasive BLT removal require surgeries with potential complications when invading underneath organs which further reduce the quality of life of people living with HIV.

Case: A 37 years old man who has sex with other man/men was diagnosed with advanced stage of HIV infections and difficulty to defecate due to perianal giant cauliflower-like mass (see Figure). The inmoveable mass clinically diagnosed as giant condyloma with several small growth surrounding it. Upon further CT-scan evaluation, the mass sized 16x8x5 cm and has invaded to anal sphincter muscles. He was treated with antiretroviral with excellent adherence after other opportunistic infection medications. Initially the surgeon planned for total proctectomy with permanent anal closure and colostomy, but this individual refused since colostomy would have reduced the quality of life. He was given two intramuscular injections of quadrivalent HPV vaccine with two months interval. After the first injection he felt low grade pain surrounding the tumor and the small surrounding lesions start falling-off.

Results: Upon six months after the first injection, clinical evaluation found that the mass did not reduce in size, but the mass was moveable. The CT-scan evaluation had shown no more anal sphincter muscle invasion. The surgeon was able to perform complete removal while preserving the anal sphincter. There was no recurrence of lesion after 2 years of follow up.

Conclusion: HPV infection has pathogenesis that involving immune evasion which further worsen with HIV coinfection. Currently, the HPV vaccine widely available indicated as prevention of infection. In this case, two intramuscular quadrivalent HPV vaccine injections (proven to have comparable immune response with three injections) gave positive result in reducing perianal tumor size and making it possible to be completely removed without damaging anal sphincter which preserving patient's quality of life. This case describes potential therapeutic role of HPV vaccine and antiretrovirals in individual living with HIV having a giant condyloma.

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Are All People Living With HDV RNA Viremic and Have Compensated Cirrhosis Eligible for Bulevirtide Monotherapy? A Real-Life Experience

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Background: People with chronic hepatitis D (HDV) have a higher risk of developing cirrhosis and hepatocellular carcinoma (HCC). Bulevirtide (BLV), entry inhibitor drug, was recently approved by the European Medicines Agency (EMA) to cure HDV infection. The new antiviral demonstrated significant rates of virological and biochemical response with good safety profile. Since March 2023, BLV monotherapy is available for people with detectable viremia and compensated cirrhosis. Herein, we evaluated microbiological and clinical parameters of five Hepatitis B virus (HBV)/HDV viremic idividuals for eligibility to BLV monotherapy.

Material and Methods: The observational study was approved by the Ethical Committee of "Mater Domini" University Hospital. HBV serological markers were tested using commercial chemiluminescent assays (COBAS 6000, Roche Diagnostics). HBV DNA was quantified using real-time PCR, detection limit of 10IU/mL (COBAS 4800, Roche Diagnostics). HDV serological markers were tested by enzyme-linked immunosorbent assay (ELISA, Dia.Pro. Diagnostic Bioprobes s.r.l). HDV RNA was quantified by RealStar® HDV RT-PCR, detection limit of 9IU/mL (Altona Diagnostics). Hepatitis Delta virus antigen (HDAg) sequences were performed by Sanger method. Sequences were aligned with MAFFT and manually edited in MEGAv.11. Genotype/subtype was determined by phylogenetic analysis, including 30 reference sequences according to Karimzadeh and colleagues (2019). Tree was estimated by General Time-Reversible (GTR) nucleotide substitution model with gamma distribution by PhyML. The reliability of clusters was evaluated using 1000 bootstrap replicates. Bootstrap values higher than 70% were considered significant. Genetic distance was 0.06 nucleotide substitutions per site.

Results: The individuals included in this study were three females and two males with median age 55 years old, 2/5 were born in Italy. All of them presented the following serological pattern: anti-HBc IgG and anti-HD IgG reactive, HBeAg non-reactive. One person carried HBV pre-core mutant virus. HBsAg quantitative values ranged from 3.2 to 4.3 Log10 IU/ml. HBV DNA level was <10IU/ml for all people, except for one (450IU/ml). The median HDV viral load was 5.5 Log10 (ranging from 4.7 to 6.9 Log10) IU/ml among the four individuals with dominant HDV infection, while the only with predominant HBV replication displayed HDV RNA 20IU/ml. Viral strains were classified as HDV1c (2/5) and HDV1e (2/5) subtypes. One HDV and the five HBV isolates were not genotypable due to low viral loads.

Conclusion: Overall, our five individuals have compensated cirrhosis (5/5) and altered biochemical parameters (3/5). HCC developed in 2/5 people, who are scheduled for liver transplantation. Even if BLV treatment duration is currently unknown, all individuals are eligible for BLV monotherapy plus entecavir (ETV) to treat both viruses. ETV treatment is necessary to reduce liver damage due to co-infection. Identification of Delta virus subtypes (such as 1c and 1e) may help to understand pathogenesis, therapy response and to estimate their prevalence in specific geographical areas. In this study, we described the clinical portrait of people living with HBV/HDV, who may have significant benefits in terms of liver-related mortality after BLV treatment, increasing data in real-life setting.

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Spontaneous HBsAg Loss With Seroconversion in Individual With Chronic HBV Who Is Treatment Naive Following COVID 19 Infection

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Spontaneous HBsAg loss is considered a rare event in untreated Individuals with chronic hepatitis B (HBV) infection. Whether it is triggered by viral factors, or individual-related health or environmental influences, is still unknown. However, studies reported that low HBV-DNA and HBsAg levels, genotype B and male gender is related to higher likelihood of spontaneous HBsAg loss.

In this study we are reporting the 5 year follow up of an individual with chronic HBV, who is treatment naive and has experienced spontaneous HBsAg loss and ultimate seroconversion after the severe Covid infection in 2022.

The 56 years old male has been diagnosed and followed up since 2018. Based on the virologic and biochemical markers he was considered to be in an inactive carrier state by having very low serum HBsAg and HBV DNA levels and normal serum aminotransferases. This individual was followed up for every 6 month for liver panel and HBV virologic markers having all biochemistry panels including AST, ALT, GGT normal, HBsAg quantity always under 30 IU/ml and HBV DNA below the quantitation limit of the assay (<6 IU/ml).

In 2021, during the follow up visit the HBsAg was increased to 15 000 IU/ml and HBV DNA to 980 IU/ml, aminotransferases mildly elevated, however HBV markers remained same. After the follow up visit in the following months the loss of the antigen was observed, followed by the seroconversion (Anti HBs- 12 Iu/ml).

Abrupt rise of aminotransferases as well as viral indices, can be considered to be the result of a flare, caused by human leukocyte antigen-I restricted, cytotoxic T lymphocyte mediated immune response against HBV.

Due to limited clinical studies, we still do not know what triggered the cascade of events that lead to a flare. The only clinical factor that might have been the possible explanation of this flare coincides with prior COVID 19 infection. The possible role of the COVID 19 infection in this event with subsequent loss of the HBsAg and seroconvertion has to be further confirmed by additional studies.