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Abstracts

1

Infection with subtype D adversely affects the live expectancy among people living with HIV – data from Northwestern Poland

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Introduction: We have shown previously that in the Western Poland exists an expanding local cluster of subtype D infections characterized by the predominantly heterosexual transmissions, high percentage of women and delayed diagnoses as well as transmitted drug resistance. In this study we aimed to analyse the survival disparities between patients infected with the most common subtype B and subtype D.

Methods: For the study data from 455 Caucasian non-immigrant patients of Polish origin infected with subtype B (n=395, 86.81%) or subtype D (n=60, 13.18%) followed-up up from January 1996 to 31 January 2019 at Pomeranian Medical University, Szczecin, Poland were analyzed. Time zero was defined as date of positive screening HIV test if later confirmed by Western-blot, immunoblotting or positive serum HIV-RNA. End of observation date was defined as either death date, last recorded date of visit (cases lost to follow-up) or 31 January 2019 for the patients remaining under care (termination of data collection). When it was impossible to determine the exact date of death the median date between the last recorded visit and information on death was assumed as the death date. Survival was censored at 20 years of observation.

For survival statistics the following datasets were analysed: overall survival probability from date of diagnosis until death or end of observation, and on-treatment survival from the date of cART initiation until death or end of observation (on-cART). Unadjusted and multivariate Cox proportional hazards models were used to assess the effect of analyzed parameter on the risk of death and to calculate the hazard ratios (HR). P-values of 0.05 were considered significant.

Results: Mean observation time in the analysed cohort was 109.15 (±85.31) months, with overall all-cause mortality in the cohort being 13.66% . Mortality for the antiretroviral treated individuals was 12.71%. Subtype D infected patients were older (median 31 (IQR:25-38) years vs. 45 (IQR: 31-56) years, p<0.0001 for subtype B), more commonly female (65.0% vs 23.29%, respectively), p<0.0001, heterosexually infected (93.1% vs 25.36%, p<0.0001), diagnosed with AIDS (47.37% vs 25.42%, p<0.0001), with higher median baseline HIV-RNA, [69500 (IQR: 14000-230000) log copies/ml vs. 360025 (IQR: 87047-834233), p<0.0001] and lower median lymphocyte CD4 count [331 (IQR:110- 532) cells/µl vs. 119 (IQR: 30-315), p<0.0001]. In the group infected with subtype B 43 (10.89%) deaths were observed, with significantly higher mortality among subtype D (n=18, 30.0%) infected subjects compared to subtype B, p<0.0001. Increased risk of death among subtype D cases remained significant after introduction of cART with on-treatment mortality of 10.73% (n=38) among subtype B infected compared to 25.45% (n=14) in subtype D (p=0.0023). In unadjusted analysis subtype D was associated with decreased 20-year survival likelihood both in the overall survival analysis [HR: 3.3 (95%CI: 5.92-1.88)], p<0.0001 and on-treatment [HR:2.51 (95% CI: 4.65-1.35)]. In multivariate model adjusted for age, gender, HCV and AIDS status, lymphocyte CD4 count, transmission route and HIV viral load in overall analysis only age and subtype D significantly affected mortality [HR: 1.08 (95%CI: 1.03-1.14, p=0.002) and HR: 7.91 (95%CI:2.33-26.86), p<0.001, respectively] with only subtype D remaining notable mortality-associated factor in on-cART model [HR: 4.24 (95%CI:1.31-13.7), p=0,02]

Conclusions: Subtype D has an independent deleterious effect of survival, even in the setting of antiretroviral treatment. Observed effect indicated higher clinical vigilance for patients infected with this subtype.

2

HIV TRACE VS PHYLOGENETIC ANALYSIS: UNRAVELING TRANSMISSION CLUSTERS IN SPAIN

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Background: The HIV-1 TRACE (TRAnsmission Cluster Engine) is a new computational tool to identify molecular transmission clusters in large databases. This approach is based on viral genetic relatedness to a reference sequence in order to construct and visualize the connections among clusters. Our objective was to identify transmission clusters in CoRIS cohort (2018 update) by using HIV-1 TRACE computational tool focusing on B-subtype patients. Further, we compared those results with other phylogenetic approaches.

Methods: We used the RT available regions from newly HIV diagnoses in 2018 in >CoRIS. HIV-1 TRACE (http://hivtrace.datamonkey.org/hivtrace) was used to estimate transmission clusters in 589 subtype B antiretroviral-naïve patients enrolled in the CoRIS cohort. Phylogenetic analysis was conducted by maximum likelihood method (ML) with bootstrap using the GTR+G as nucleotide substitution model. Sequences were analysed along with all the most similar sequences as identified by a BLAST search. Local transmission networks (LTNs) were defined as phylogenetic clusters including sequences from Spain at proportions >70%, receiving bootstrap value >70%.

Results: HIV-1 TRACE results showed that 440 patients (74.7%, n=440/589) were not enrolled in any cluster and 149 patients (25.3%, n=149/589) were grouped in 62 clusters: 38 clusters with 2 nodes, 11 clusters with 3 nodes, 3 clusters with 4 nodes, 1 cluster with 5 nodes and 1 cluster with 6 nodes (range 2-6). Classical phylogenetic analysis revealed that 457 (77.6%, n=457/589) and 132 patients (22.4%, n=132/589) were grouped in 54 clusters: 47 clusters with 2 nodes, 8 clusters with 3 nodes, 4 clusters with 4 nodes and 3 clusters with 5 nodes (range 2-5). Overall, the concordance among classic phylogenetic approaches and HIV-1 TRACE tool is 87.1%. The discrepancies are not observed only in the number of clusters, as previously described, but also in the distribution, since HIV-1 TRACE identified 7 clusters with more than 3 nodes and classical phylogenetic identify only 5 of these clusters.

Conclusion: The implementation of HIV-1 TRACE as new computational tool is a feasible and allows to identify transmission clusters. Our results revealed that HIV-1 TRACE identified more clusters among B-subtype patients than traditional phylogenetic approaches.

3

Prevalence and evolution of transmitted HIV drug resistance in Belgium between 2013 and 2019

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Introduction: Baseline testing for transmitted drug resistance (TDR) is standard of care for patients newly-diagnosed with HIV infection in Belgium. A total of 3713 HIV-1 patients, newly-diagnosed between 2013 and 2019, were included in this study to assess TDR prevalence and evolution over time. Phylogenetic analysis was performed to improve insight on the origin of the TDR.

Material and Methods: HIV-1 pol sequences generated in the seven Belgian Aids Reference Laboratories were collected. Because monitoring of transmitted integrase resistance was only introduced in 2018, the study focussed on protease (PR) and reverse transcriptase (RT) mutations. Drug resistance was scored using the Stanford algorithm version 8.9-1 and the 2009 World Health Organisation (WHO) and 2019 International Aids Society-USA (IAS-USA) mutation lists. For subtyping a consensus was made out of the results of the Stanford (version 8.9-1), COMET (version 2.3) and Rega (version 3.0) subtyping tools. Statistical analyses were performed using SPSS Statistics (version 26). Trends over time were assessed using linear regression. Phylogenetic analysis was constructed using the maximum likelihood approach implemented in PhyML 3.0 with automatic selection of the best fit evolutionary model of DNA substitution.

Results: The overall prevalence of TDR did not change significantly over time (p-value 0.225) and was 17.9% on average when all PR and RT mutations with a Stanford score ≥ 15 were taken into consideration. If only mutations with a Stanford score ≥ 60 were retained, the average prevalence was 6.3%. Using the major mutation lists of WHO and IAS-USA the prevalence was 10.9% and 13.8% respectively.

The majority of observed mutations with a Stanford score of ≥ 15 impacted non-nucleoside reverse transcriptase inhibitors (NNRTI) (11.4%), followed by nucleoside reverse transcriptase (NRTI) (6.2%) and protease inhibitors (PI) (2.4%). Multi-class resistance was observed in 2.37% of the patients. When only mutations with a Stanford score of ≥ 60 and thus high presumed impact on resistance against at least one drug, were considered, the NNRTI mutations K103N, Y181C, G190A and Y188L, the NRTI mutation M184V and the PI mutations N88D and L90M were the most represented, detected in respectively 125, 19, 19, 10, 32, 12 and 12 individual patients. A slight increase over the years in the prevalence of K103N and M184V and a decrease in prevalence of L210W and M41L was seen.

A significant contribution of clustered transmission was demonstrated for the NNRTI mutations K103N and E138A/K, the NRTI mutations L210W and T215E/S and the PI mutations Q58E and N88D. Some subtype specificity was observed for the NRTI mutation T215E, only detected in subtype B, the NNRTI mutation A98G primarily observed in subtype F, and the PI mutation L90M most prevalent in subtype G.

Conclusions: In Belgium, the overall prevalence of TDR remained stable between 2013 and 2019 and is comparable to the prevalence observed in other Western-European countries. The high frequency of NNRTI mutations requires special attention and follow-up. TDR prevalence is to a large extend driven by clustered transmission.

4

Factors associated with the emergence of resistance mutations in patients failing dual or triple-integrase inhibitors-based regimen in a French national survey

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Background: Successful 2 drug regimens (DR) were made possible by the availability of drugs combining potency and tolerability with high genetic barrier to resistance. How these approaches would deal with resistance development/re-emergence, compared with 3DR, is thus of paramount importance.

Material and Methods: A national survey including patients failing either naïve or experienced (2 consecutive plasma viral load (VL) > 50 copies/mL) to any 2DR or 3DR integrase inhibitors (INI)-containing regimens was conducted between 2014 and 2019. Genotypic resistance tests were interpreted with the last ANRS algorithm.

Results: 1104 patients failing to any INI-containing regimen (2DR = 207 and 3DR = 897) were analysed. 577 (52.3%) patients were infected with a B subtype and 527 (47.3%) with non-B subtypes. Among the 897 3DR, the INI was dolutegravir (DTG) in 288 cases, elvitegravir (EVG) in 323 case and raltegravir (RAL) in 286 cases. Among the 207 2DR, the INI was DTG in 63 cases and RAL in 144. Among the 2DR, 110 were in combination with 1 PI, 73 with 1 NNRTI, 14 with 1 NRTI and 10 with another antiretroviral class. Overall, 644 (58%) patients showed no known INI resistance mutations at failure. Among the patients with at least 1 INI resistance mutations at failure, 286 (26%) had 1, 110 (10%) had 2, 44 (4%) had 3, 17 (1.5%) had 4 and 3 (0.5%) had at least 5 INI resistance mutations. In multivariate analysis, factors associated with the emergence of at least one INI mutation were high VL at failure (OR = 1.24 per 1 log10 copies/mL increase), non-B versus B subtype (OR = 1.75), low genotypic sensitivity score (GSS) (OR = 0.10 for GSS ≥2 versus GSS = 0-0.5), DTG versus RAL (OR = 0.46). Although 3DR versus 2DR reach statistical significance in univariate analysis (OR = 0.59, p value=<.001), the variable is not retained in the final model due to correlation with other variables retained (GSS and DTG).

Conclusions: This study is one of the largest studies characterizing INI resistance in patients failing to any INI-containing 2DR or 3 DR regimen in routine clinical care and reveals factors associated with emergence of INI resistance that should be taken into consideration in clinical management.

5

HIV DNA DRUG RESISTANCE GENOTYPING, BASED ON ILLUMINA SEQUENCING, AS DECISIONAL MARKER FOR cART OPIMISATION IN CLINICAL PRACTICE: ADDED VALUE, GAPS AND FUTURE DIRECTIONS

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Background: Even not yet validated, UDS of proviral DNA (DNA-UDS) may be an interesting alternative tool to detect archived HIV-1 drug resistance-associated mutations (RAMs) when RNA genotyping by Sanger sequencing is not informative (e.g. low RNA viral load, treatment interruption). Here, we report our feedback to address answers about hypothetic (not yet) integration of DNA-UDS as decisional marker tool in clinical practice.

Methods: Samples from 111 HIV-1 patients experiencing virological failure were derived from routine genotyping resistance testing sent to our laboratory for routine drug resistance genotyping from 5 reference centers involved in AIDS care in Burgundy. Patients were prospectively included between April 2015 and June 2018. In addition of routine genotyping, UDS was performed on HIV-1 PR, RT and INT fragments from PCR products amplified according to ANRS recommended procedures. Paired-end indexed libraries for MiSeq, using the Nextera DNA sample preparation kit (Illumina, San Diego, CA). All multiplexed libraries were spiked with PhiX to improve cluster detection by increasing base complexity. The generated reads were then processed in the bioinformatics pipeline developed in-house (Bioinformatics team; CHU Dijon - FHU TRANSLAD - Team GAD). Only reads with high-quality bases (Qscore > 30) were considered and aligned against the HIV HXB2 reference sequence. The archived DNA RAMs identified by DNA-UDS with a 1% frequency threshold. Reads with stop codons were excluded and not considered in the calculation of RAMs.

Results: Among the 111 HIV-1 infected patients. The median age was 52 years [IQR: 43-60]. Of the 111 patients, 32 (29%) were women, 94 (85%) were on cART (median duration 168 months) and 17 (15%) were treatment-naïve patients at time of sampling. As part of routine monitoring, HIV Sanger RNA genotyping was available for 91 (82%) patients and HIV Sanger DNA genotyping was available for 80 (72%) patients. UDS-DNA genotyping was obtained for all patients. For both NRTI and NNRTI, the magnitude of archived RAMs was: (i) affected by the duration RNA VL undetectability (p=0,04) ; (ii) affected by the time duration of virological failure under selection pressure of cART [NRTI (0,04) and NNRTI (0,01)]. Finally, based on GSS, both Sanger cumulated RNA genotypes and DNA-UDS showed the same prediction for virological response at one-year follow-up. Indeed, when the GSS is ≥ 2, the UDS-DNA assay is able to predict virological success in 97,7% of the cases and the Sanger cumulated RNA genotypes in 98,6%. Finally, in virological failure situations (RNA viral load >50 Cp/mL on two successive samples), when the GSS is <2, the UDS-DNA assay is predictive of failure in 40% of the cases and the Sanger cumulated RNA genotypes in 33% of the cases.

Conclusions: Our preliminary results showed that UDS-DNA HIV genotyping could be a relevant decision marker tool for antiretroviral therapy optimization when previous virological failures were not investigated or missing. More data is needed to determine, for each drug classes, the percentage thresholds of archived RAMs in proviral DNA that may help to take a therapeutic decision.

6

High efficacy after switching to integrase strand transfer inhibitors (INSTI) in PLWH with undetectable viremia and past virological failure and/or resistance

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Background: In PLWH with previous virological failures (VF) or resistance, data on optimisation of antiretroviral therapy (ART) with INSTI-regimens are limited. High virological efficacy was demonstrated with DTG+2NRTI in patients with residual NRTI activity and with BIC/TAF/FTC in patients suppressed; few data are available for other INSTI-based options.

Methods: In this retrospective multi-centre study we analyzed ART-treated PLWH with virological suppression (<50 copies/ml) and at least a previous VF, followed by a genotype, who had switched to an INSTI-containing regimen. Primary endpoint: viral rebound (VR, confirmed HIV-RNA >=50 c/mL). We estimated incidence ratio (IR) of VR according to a) genotypic susceptibility score (GSS); b) high resistance level to NRTI [K65R/E/N, >= 3 TAM, insT69]. Weighted Cox regression model was fitted to estimate HR of VR, after controlling for confounding variables (CD4 at nadir, duration of viro-suppression, number of previous genotypes and mode of HIV transmission).

Results: 654 patients included: 30% females, age 52 years (IQR 47-56), nadir CD4 116 cells/mmc (40-227), years of viro-suppression 3.2 (1.3-7.4). Ongoing ART regimens were: 1° generation INSTI + 2NRTI (33%), 2° generation INSTI + 2NRTI (26.6%), INSTI+ boosted PI (21.1%), INSTI + NNRTI or NRTI (19.3%).

INSTI used ere raltegravir in 32.9% of patients, elvitegravir in 18.7% and dolutegravir in 48.5%.

VR was detected in 120 patients over 1,387 person-year-follow-up (PYFU). IR of VR was comparable throughout the stratifications: a) 8.2 x 100 PYFU (95%CI 5.9-11.3) in patients with GSS <2 and 8.9 x 100 PYFU (7.1-11.0) in patients with GSS >=2; b) 8.8 x 100 PYFU (7.2-10.8) in patients with high resistance level to NRTI and 8.2 x 100 PYFU (5.7-11.8) in patients with no/low resistance to NRTI. Patients in 1° generation INSTI-containing regimens had IR of VR of 10.1 x 100 PYFU (8.1-12.6), those in 2° generation INSTI 6.5 x 100 PYFU (4.7-8.9). By multivariate analysis, patients with GSS>=2 had a lower risk of VR (aHR 0.60, 0.31-1.14), but not statistically significant in the overall population, except for INSTI+NRTI or NNRTI (aHR 0.18 [0.04-0.93], p=0.041). Similar results were obtained with the other definition of resistance.

Conclusions: After a switch during virological suppression guided by genotype, INSTI regimens maintained high rate of virological success even in heavily treatment-experienced patients and with low GSS and/or pre-existing NRTI resistance.

7

Emergence and Persistence of Integrase Mutation T97A Selected by Dolutegravir

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The relative contribution of a major accessory mutation T97A of integrase strand transfer inhibitors (INSTIs) to resistance remains unclear. Recently, we reported two treatment experienced patients with INSTI primary mutations G140S and Q148H who initiated dolutegravir (DTG) therapy, and experienced viral rebound with emergence of T97A, resulting in a >10-fold increase in phenotypic resistance to DTG (Kuriakose et al., 2018). In one of these patients, T97A was not detected prior to DTG by next generation sequencing (NGS), but emerged within 24 weeks after initiating DTG, suggesting T97A variants were present pre-DTG, but below the limit of detection of NGS, or that this mutation emerged after DTG was started. To investigate the population genetics of emergence of resistance to DTG we used single genome sequencing and ultrasensitive single genome sequencing (uSGS) to analyze HIV populations prior to, during, and following DTG therapy for one of the patients.

The study participant was enrolled in a clinical study investigating antiretroviral therapy failure (NCT 01976715). Plasma samples at 9 time points were obtained prior to (n=2), during (n=4), and following (n=3) DTG-therapy. Viral populations were analyzed by obtaining single genome sequences (SGS) of the entire HIV-1 integrase region (HXB2 nt 4168 - 5190). SGS (total number of sequences = 206) were aligned, and subjected to phylogenetic (MEGA) and population genetics analyses. uSGS was carried out using primer-ID with NGS. Plasma RNA from two time points from pre-DTG therapy were sequenced in reverse transcriptase region (HXB2 nt 2704-2943 and 3046-3253). In total, 2597 sequences were aligned and used to calculate HIV replicating population size based on allele frequencies as described previously (Maldarelli et al., 2013).

Prior to DTG, all SGS possessed G140S + Q148H, but none had T97A. After initiating DTG-containing therapy, viremia remained <50 copies/ml from week 12 to 20, but rebounded to 1,683 copies/ml by week 24 with emergence of T97A. The genetic diversity of the rebound population (average pairwise distance, APD=0.2%) was 6-fold lower than pretherapy (APD=1.2%). At that time, all SGS contained G140S and Q148H with two distinct populations – the first (86% of SGS) contained T97A, and a second contained V151A. Within 7 weeks of rebound, however, only the T97A lineage was detected. DTG was discontinued, and more than a year after INSTI discontinuation, G140S + Q148H persisted in all SGS, but T97A had declined from 100% to 5% (1/21 sequences). Population genetics analyses (panmixia) revealed that the pre-DTG, the DTG-therapy and the post-DTG populations were distinct from each other. Pre-DTG maximum replicating population sizes were large and exceeded 105. T97A emerged quickly in this large population, suggesting a strong selection for this variant. Viral populations were highly polymorphic, but the majority of polymorphisms were in linkage equilibrium, suggesting recombination was common.

Rapid DTG resistance with T97A emerged from a single variant after DTG failure, and emerged quickly from large replicating population. V151A, only infrequently reported in vivo, emerged at the same time, but was outcompeted by the T97A lineage. Pre-DTG populations undergo substantial shifts even after transient DTG therapy.

8

Evaluation of HIV-1 RNA and DNA mutational load in HIV-1 infected heavily treatment-experienced patients harbouring multi-drug resistant strains

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Background: Heavily treatment-experienced (HTE) people harbouring multi drug-resistance (MDR) HIV-1 need careful assessment to plan an effective treatment. In this study, we characterized drug-resistant variants in plasma and PBMCs of MDR PLWH and the impact of mutational load (ML) on virological outcome.

Material and Methods: We analysed 20 HTE persons with resistance to NRTI/NNRTI/PI/INSTI, failing ART, enrolled in the Italian PRESTIGIO registry. Resistant and APOBEC-related variants (Stanford HIVdb version 8.9-1) in HIV-1 RNA and DNA at current failure were evaluated through next generation sequencing (NGS, Illumina MiSeq) and compared to those present in the historical Sanger genotypic resistance test (GRT). Gp120-V3 NGS was performed to infer tropism through the geno2pheno[coreceptor] algorithm. Total HIV-1 DNA was measured with a standardized in-house digital droplet PCR assay. ML was calculated by multiplying the mutant frequency in plasma and proviral DNA for the HIV-1 DNA and RNA levels detected in the same sample, respectively. Levels of DNA and RNA ML were compared among patients who achieved, or not, plasma HIV-RNA <50 copies/mL after a therapy switch following sample collection.

Results: Patients had a median (IQR) age of 47 (37-53) years and a long treatment history. In particular, the median time (IQR) from diagnosis was 23 (19-26) years and patients have been exposed to ART since 19 (17-22) years, with a median (IQR) number of 11 (6-32) failed regimens. Median (IQR) plasma HIV-1 RNA, proviral DNA and CD4 counts were 4.5 (4.1-5.0) log_{10} copies/ml, 4.5 (4.0-5.2) log_{10} copies/10^6 CD4+ T-cells and 204 (97-329) cells/mm^3, respectively. By NGS, 4-class resistance was detected in 26% and 68% of patients in plasma and PBMC, respectively. Among 255 resistant variants detected in either compartment, 182 (71.4%; 148 in plasma and PBMC; 33 only in PBMC; 1 only in plasma) were already present in historical GRT. Thus, NGS detected 73 additional variants (28.6%).

Complex resistance patterns were detected in all individuals and 12 (60%) persons harboured an X4-tropic virus in at least one compartment. Median (IQR) DNA ML was 3.9 (3.1-4.6) log_{10} copies/10^6 CD4+ T-cells, while median (IQR) RNA ML was 4.5 (4.0-4.9) log_{10} cps/ml. APOBEC-related proviral resistance mutations (PI: M46I, D30N; NRTI: D67N, M184I; NNRTI: E138K, G190E) were found in nine patients (45%, median [IQR] ML: 2.7 [2.2-2.9] log_{10} copies/10^6 CD4+ T-cells), while substitutions at enzymatic catalytic sites (PR: G27E; RT: M184I, D186N; IN: E152K) were present in eight patients (40%, ML [IQR]: 2.8 [2.3-3.1] log_{10} copies/10^6 CD4+ T-cells). Fourteen people (70%) showed variants with stop codons in proviral DNA with intra-patient frequency <17% (median [IQR] frequency: 2.4% [1.4%-3.4%]; median ML [IQR]: 2.9 [2.4-3.2] copies/10^6 CD4+ T-cells).

Fourteen individuals modified ART after sample collection: 5/14 (35.7%) experienced virological failure and, compared to responders, had higher resistant ML in both proviral DNA (4.1 [3.0-5.4] vs. 3.5 [3.0-4.1], log_{10} copies/10^6 CD4+ T-cells, $p<0.001$) and in plasma (4.7 [4.0-4.9] vs. 4.1 [3.9-4.5], log_{10} copies/ml, $p<0.001$). Moreover, a higher proportion of failing people presented all resistant variants with a ML >3 log in both compartments (60% vs. 11%, p=0.095).

Conclusions: APOBEC activity can be detected in HIV-1 DNA in most HTE people with MDR, with a low viral burden. HIV-1 RNA/DNA ML might help to identify individuals more prone to experience virological failure and guide salvage therapy optimization.

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Long-term Bictegravir and Dolutegravir Resistance Selections Initiated with HIV-1 Containing M184V in Reverse Transcriptase

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Background: M184V is one of the most commonly observed NRTI resistance substitutions after treatment failure with emtricitabine- or lamivudine-containing ART and can be transmitted to other individuals. M184V becomes archived in the latent viral reservoir but reverts to wild-type in circulating HIV in the absence of drug pressure where it may not always be detected by standard genotyping assays or even proviral DNA genotyping assays. Previous in vitro studies suggest that M184V prevents the emergence of resistance substitutions against dolutegravir (DTG). Here, we assessed the evolution of drug resistance in HIV-1 containing M184V under long-term in vitro selective pressure by INSTIs.

Materials and methods: M184V was introduced into the reverse transcriptase (RT) of HIV-1 xxLAI by site-directed mutagenesis. In vitro dose-escalation resistance selections were performed on M184V mutant virus using DTG, bictegravir (BIC), elvitegravir (EVG), or raltegravir (RAL). HIV-1 nef and pol from culture supernatants were genotyped by population and/or deep sequencing. Viral pools and site-directed mutants representing viral genotypes observed at the end of selection were phenotyped for drug resistance.

Results: Two sets of parallel selections were performed for BIC and DTG and progressed for >200 days. M184V persisted in RT throughout all four selection experiments with no reversion to wild-type. In the first set of DTG and BIC selections, the INSTI resistance substitution S153Y was initially acquired followed by other integrase (IN) substitutions. The second set of DTG and BIC selections utilized the R263K pathway, with or without acquisition of other IN substitutions. For EVG and RAL, one selection experiment was performed for each and progressed for ~180 days. In the EVG selection, M184V was maintained and the INSTI resistance substitution T66I was acquired. The RAL selection utilized the Q148R pathway with subsequent acquisition of other IN substitutions and reversion of M184V to wild-type. Maximum fold-changes in susceptibility to DTG or BIC were 2.7- and 2.4-fold, respectively, for all viral pools and 2.8- and 2.3-fold, respectively, for all single and double site-directed mutants (SDM) tested. An SDM containing V72I, G140E, and S153Y showed high-level resistance to all INSTIs and had a replication capacity of 0.2% compared to wild-type. In the nef gene, 2 mutations in the 3' polypurine tract (PPT) were detected as minority variants in the first DTG selection.

Conclusions: HIV-1 containing M184V in RT acquired INSTI resistance substitutions S153Y or R263K plus other IN substitutions and maintained M184V under long-term selective pressure with DTG or BIC. There was little to no phenotypic resistance to either drug for all viral pools and most SDMs tested, though BIC has been shown to be the most broadly potent INSTI against a large cross-section of IN resistant mutants. The triple mutant V72I/G140E/S153Y demonstrated high level resistance to all INSTIs but low replication capacity suggesting possible nonviability in vivo. Two 3' PPT mutations emerged at low level under DTG pressure. These results confirm that DTG or BIC resistance can develop after M184V and that M184V is not fully protective against continued resistance development.

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Molecular analysis of HCV subtype 3a dispersal patterns among inmates in Greece: HCV transmissions are not related to incarceration

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Background: The most common mode of hepatitis C virus (HCV) infection in Greece is through injection drug use. Although HCV infection is more prevalent among inmates, to date it remains largely unknown whether incarceration contributes to the spread of HCV. Our aim was to investigate the HCV transmission patters among inmates with prior intravenous injection history in Athens, Greece, using molecular epidemiology methods.

Materials and Methods: The study population was consisted of 121 anti-HCV(+) inmates at Korydallos Prison Hospital "Agios Pavlos" and Korydallos Detention Facility, who participated in a test and treat program for viral hepatitis, HIV and tuberculosis, conducted between 10/2017 and 03/2018. HCV genotyping was performed in 117 samples in partial NS5B region by HCV Typing Tool. We analysed phylogenetically the subtype 3a sequences from inmates (N=51) along with subtype 3a sequences from HCV chronically infected patients in Greece (N=519). Specifically, phylogenetic analysis was performed in two steps: First, we analysed all the subtype 3a sequences from inmates (N=51) along with the most closely related sequences sampled globally (HCV BLAST) (N=73) and from Greece (NCBI BLAST) (N=87). The BLAST search was run against global and Greek dataset using as a query the sequences from inmates. Identification of inmate-specific clusters was based on phylogenetic analysis performed by the approximate maximum likelihood method, as implemented in FastTree v2.1 program. Second, inmate-specific clusters were confirmed by Bayesian analysis performed separately for each cluster along with the most closely related globally sampled sequences after using the BLAST tool available at NCBI. Bayesian analysis was performed by MrBayes v3.2 program, using the GTR+G as substitution model. Inmate-specific clusters were phylogenetic clusters including sequences from inmates at proportion > 50%, receiving posterior probability support > 0.9. Phylodynamic analysis was performed in the largest cluster using BEAST v1.8 program.

Results: Genotyping analysis revealed that 3a (n=51; 43,6%) and 1a (n=41, 35,1%) were the most prevalent HCV subtypes in our study population. Initial phylogenetic analysis on subtype 3a sequences showed that 10 sequences from inmates clustered in 4 separate groups and 12 sequences from inmates were found within a cluster of 26 sequences from Greece. Bayesian analysis confirmed the existence of 3 small inmate-specific clusters consisting of 2 sequences each. Within the largest cluster of the 26 sequences, no significant clustering was found for the sequences from inmates. Overall, 6 out of 51 (11.8%) sequences from inmates were found within 3 clusters. The time of the most recent common ancestor of the largest cluster was estimated in November 2013, suggesting that most HCV transmissions within this cluster occurred recently.

Conclusions: Our study showed that only 11.8% of the 3a sequences from inmates clustered together, suggesting a common origin of HCV infection. Given that the possibility of unsampled intermediates cannot be excluded, the estimated percentage should be interpreted as the upper limit of HCV transmissions among the inmates. Moreover, in the largest group of the 26 sequences, no clustering was found among the inmates and thus provides further support to our finding for low level HCV transmissions within this population. These results are in accordance with our previous findings for the subtype 1a, and therefore showing that incarceration is not associated with HCV transmissions in Greece.

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Characterization and Resistance Profile of "Unusual" Hepatitis C Virus (HCV) Subtypes in Italy

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Background: Recent data has shown that specific "unusual" hepatitis C virus (HCV) subtypes had a lower response rate to direct-acting antivirals (DAAs) compared to the other most prevalent subtypes. Our aim was to investigate the prevalence and resistance profile of "unusual" HCV subtypes in Italy. We also evaluated the potential presence of phylogenetic clusters in genotype (GT) 2c, which is the second most prevalent GT in Italy after GT1b.

Methods: Clinical and virological data of "unusual" HCV GT/subtypes, defined as GT1 non1a/b, GT2 non2a/b, GT3 non3a, GT4 non4a/d, and GT5, collected within the Italian Resistance VIRONET-C database, were retrospectively analyzed. Subtype assignment was confirmed by phylogenetic analyses on NS3±NS5A±NS5B sequences. Prevalence of resistance-associated substitutions (RASs) was evaluated at key positions (Sorbo et al 2018). Cluster analysis was performed on GT2c NS5A sequences by Bayesian analysis (posterior probability ≥0.98).

Results: A total of 311/3358 (9.3%) individuals with an available NS3±NS5A±NS5B sequence were found to be infected with "unusual" subtypes (GT1c/g/i/l=1/5/1/2; GT2c/i/j=267/1/2; GT3b/g/h/k=2/1/9/1; GT4c/i/l/m/n/o/r/v=1/1/1/1/3/5/5/1; GT5a=1). 245 patients (78.8%) were DAA-naïve, while 66 were DAA-failures (21.2%; Table 1). A different geographic distribution of these "unusual" subtypes was observed considering the patient's ethnicity: in particular, GT2c and GT3h were mainly found in Italians (91% and 100%, respectively), the other "unusual" GT3 subtypes were found mainly in patients from Asia (75%), while "unusual" GT1 and GT4 in patients from Africa (77.8% and 77.8%, respectively).

Phylogenetic analysis identified 11 transmission clusters (7 with 2 sequences and 4 with >3 sequences) among GT2c infected patients. In particular, 9 involved 23 patients from north Italy, 1 pair from central Italy and 1 pair from north-south Italy (both DAA-failures).

Overall, patients failed several DAA regimens such as glecaprevir/pibrentasvir (n=22),

sofosbuvir/velapatasvir+/-ribavirin (n=13), grazoprevir/elbasvir (n=2), or sofosbuvir/daclatasvir (n=14), sofosbuvir/ledipasvir+/-ribavirin (n=2), 3D/2D (paritaprevir/ombitasvir+dasabuvir)+ribavirin (n=6/2) or other DAAs (n=5). Analysing the 60 NS5A-failures, all patients (100%) with the "unusual" subtypes GT1l, GT3b/g/h/k and GT4n/o/r/v had combinations of multiple NS5A RASs (from 3 to 6). A similar pattern was also confirmed in DAA-naïve patients. Differently, in GT2c, only 53.5% of failures harbored >2 NS5A RASs, and this prevalence was decreased in DAA-naive patients (40.1%; p=0.12). Substitutions at position 93 (H/F/S) were detected only at failure in GT3h/k and GT4o/v. Considering NS3-RASs, few patients showed resistance only at failure (12.5%: 1 GT3h:80R; 3 GT2c:56H±168V/A), while NS3 polymorphisms were observed in some "unusual" subtypes, from both naïve and failures (54S in GT1g; 36L±80G in GT2c; 36L in GT3h and GT4n/r). The NS5B 316H+321I pattern occurred in 66.7% of GT4r failures (2/3). No other SOF RASs were found at failure, with the exception of 282G in one GT2c patient.

Conclusions: Overall, our study provided a characterization of the "unusual" subtype circulating in Italy. The majority of DAA-failures, with the exception of patients infected with GT2c, carried complex NS5A RAS patterns, some conferring high level of resistance. Further studies are needed to better characterize the impact on DAA efficacy of these "unusual" HCV subtypes, particularly for those with natural complex RASs or polymorphisms at baseline.

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Highly sensitive digital droplet PCR assay allows to reveal cryptic HBV replication in anti-HBc positive/HBsAg negative patients with HIV infection.

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Background: Occult HBV infection (OBI) is frequent and associated with poor survival in the setting of HIV infection. Here, we investigate cryptic HBV replication and factors correlated with its detection in anti-HBc positive/HBsAg-negative HIV-infected patients by applying a highly-sensitive (HS) droplet digital (dd) PCR assay for serum HBV-DNA quantification.

Material and Methods: This study includes 81 anti-HBc-positive/HBsAg-negative patients with serum HBV-DNA <10 IU/ml by a commercial Real-Time PCR. All patients are HIV-infected, treated with an antiretroviral therapy including >1 anti-HBV drug: TAF/FTC (N=37), TDF/FTC (N=25), or LMV (N=19) (median [IQR] duration: 35[17-61] months). Serum HBV-DNA is quantified by an in-house HS-ddPCR (Bio-Rad) whose linearity, reproducibility and sensitivity are assessed by testing serial dilutions with known concentration. FujiRebio assay is used to quantify anti-HBc titer (proposed to parallel HBV replication). Factors correlated with the detection of cryptic viremia (serum HBV-DNA >1 IU/ml) are defined by Fisher exact test. Population-based sequencing of HBsAg major hydrophilic region (MHR, aa 99-169) is used to analyse immune-escape mutations with cryptic HBV viremia.

Results: ddPCR shows excellent linearity in the range of HBV-DNA from 1 to 10'000 IU/ml (R2=0.997), good intra- and inter-run reproducibility (coefficient of variation: 7.8% and 18.6%) and high sensitivity (limit of quantification: 1 IU/ml).

Overall, median (IQR) anti-HBc is 4.2 (2.4-11.6) IU/ml. 29.6% of patients are isolated anti-HBc and 70.4% anti-HBc/anti-HBs positive (median [IQR] anti-HBs titer: 278[90-957] mIU/ml). Median (IQR) HIV-RNA and CD4+ cell count are <20(<20-38) copies/ml and 541(331-727) cells/ul, respectively.

Notably, by ddPCR, cryptic HBV viremia is detected in 29.6% of patients with a median (IQR) of 4 (1-15) IU/ml, more frequently in patients an advanced CDC stage (85.7% with cryptic HBV-DNA versus 54% without cryptic viremia have been diagnosed in B2/3 or C2/3 stage, p=0.01)

No impact of different anti-HBV drugs on cryptic HBV viremia is observed (% of patients with serum HBV-DNA >1 IU/ml: 27% for TAF, 28% for TDF and 37% for LMV, p=0.7). Moreover, a positive correlation is found between serum HBV-DNA and HIV-RNA (Rho:0.26, p=0.02).

By analyzing serological markers, anti-HBs <50 mIU/ml combined with Anti-HBc >15 IU/ml is predictive of cryptic HBV viremia (63% of patients with anti-HBs <50 IU/ml + AntiHBc >15 IU/ml have HBV-DNA >1 IU/ml vs 26% without this combination, p=0.046, OR: 4.7[1.1-21.7]). The sequences of HBsAg MHR are obtained for 5/24 patients with cryptic HBV viremia, showing immune-escape mutations in two of them.

Conclusions: ddPCR is a valuable assay for detecting cryptic serum HBV-DNA in the setting of anti-HBc positive/HBsAg-negative patients with HIV infection. The integration of innovative serological and virological markers can help identifying patients with minimal HBV replication, thus optimizing OBI diagnosis.

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Susceptibility to HIV-1 integrase inhibitors in HIV-1 sub-subtype A6 isolates

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Background: Regimens based on second generation integrase inhibitors (INSTIs) such as dolutegravir (DTG) and bictegravir (BIC) are currently recommended as the preferred first-line HIV-1 therapy. Considering the recent introduction of second generation INSTIs in the Russian Federation, this study aimed to evaluate the natural susceptibility of INSTIs in viral strains belonging to sub-subtype A6, which is the most prevalent genetic variant circulating in Russia and surrounding countries.

Materials and methods The identification of plasma samples harboring viral strains belonging to sub-subtype A6 was carried out through phylogenetic analysis of HIV-1 sequences generated during routine HIV-1 drug resistance testing. Viral sequences assigned to subtype A according to sequence homology were aligned with representative sequences of each subtype including A6 retrieved from the HIV Database of the Los Alamos National Laboratory. Phylogenetic analysis was performed with Mega 7 software. Plasma samples harboring viral strains assigned to sub-subtype A6 were used for the generation of NL4-3 based recombinant viruses carrying patient derived integrase coding region. In vitro susceptibility to the INSTIs raltegravir (RAL), DTG, BIC and cabotegravir (CAB) was determined through a TZM-bl cell line based phenotypic assay and fold-change (FC) values were calculated with respect to the IC50 value obtained with the wild-type NL4-3 strain.

Results: Twenty out of eighty-one (24.7%) viral sequences originally labelled as subtype A were assigned to sub-subtype A6. Residual plasma available in eight cases was successfully used for the construction of recombinant viruses. Seven additional recombinant viruses were created from plasma samples obtained from the Institute of Virology in Cologne, Germany (n=2), and from the Gamaleya National Research Center in Moscow, Russia (n=5). None of the fifteen A6 sequences harbored major INSTIs RAMs while 14/15 (93%) sequences harbored the L74I variant, which is the consensus aminoacid in subtype A and found to be weakly selected in patient under INSTI therapy with no impact on INSTIs susceptibility when alone. Median FC values for RAL, DTG, BIC and CAB were 0.9 (IQR 0.8-1.1), 1.3 (IQR 0.9-1.7), 1.0 (IQR 0.8-1.2), and 0.9 (IQR 0.6-1.4), respectively. According to the available biological or clinical FC cut-offs established by the reference Phenosense Assay, all FC values calculated for RAL and DTG were below the FC threshold associated with reduced susceptibility.

Conclusions: The A6 sub-subtype strains, including the L74I variant, appeared to be naturally susceptible to INSTIs. The A6 consensus L74I variant does not contribute to INSTI resistance.

In addition to analyzing a larger number of samples for phenotypic susceptibility, also the genetic barrier to resistance to INSTIs in sub-subtype A6 isolates should also be investigated in order to give a firm support to a safe use of second generation INSTIs in Russian Federation countries.

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Residual phenotypic susceptibility to second generation NNRTI in multidrug resistant HIV-1 from the PRESTIGIO Registry

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Background: The recently approved NNRTI doravirine (DOR) has a partially distinct resistance profile within the NNRTI class. In order to explore the possible use of second generation NNRTI as salvage therapy, this study evaluated the residual phenotypic susceptibility to DOR, rilpivirine (RPV) and etravirine (ETR) in a panel of multidrug resistant HIV-1 isolates collected from patients enrolled in the PRESTIGIO Registry.

Materials and methods: Recombinant viruses expressing patient derived PR-RT were generated from plasma samples from 22 patients at virological failure (VF) and documented resistance to PIs, NRTI, NNRTI and INSTIs. In vitro susceptibility to DOR, RPV and ETR was assessed through a TZM-bl cell based phenotypic assay measuring fold-change (FC) values with respect to the reference NL4-3 virus. Genotypic susceptibility was computed by the Stanford HIVdb algorithm 8.9-1. Patient demographics and laboratory data were described as median (Q1-Q3) or frequency (%).

Results: Twenty (91%) patients were male, median age 55 years (50-58), time since HIV-1 diagnosis 27 years (23-31), time on ART 23 years (22-26), 12 (55%) with a previous AIDS diagnosis, median viral load (VL) 4.30 log10 copies/mL (3.35-5.14) and median CD4+ cell count 195 cells/µL (80-279); 11 patients (50%) were receiving an NNRTI (ETR=10, RPV=1), while 9 (41%), 5 (23%), 8 (36%) patients had been exposed to 1, 2 and 3 NNRTI, respectively, with a median time of exposure to NNRTI of 1,414 days (298-2158). Median DOR, ETR and RPV FC were 9.8 (2.9-40.4), 42.9 (3.1-100) and 100 (17.9-100), respectively. Median FC values were higher in patients exposed to NNRTI at VF (DOR 17.9 [7.4-80.1] vs. 4.4 [0.9-27.1], p=0.145; ETR 100 [48-100] vs. 4.0 [0.5-26], p=0.004; RPV 100 [100-100] vs. 30.6 [3.9-100], p=0.029). According to clinical or biological FC cut-offs, only 1/22 (5%) viruses was still susceptible to RPV, while 4 (18%), 3 (14%) and 15 (68%) viruses had susceptibility, partial susceptibility and resistance to ETR, respectively. Agreement between phenotypic and genotypic susceptibility was observed in 9 (41%) cases for ETR and 19 (86%) for RPV, with phenotypic ETR activity underestimated by genotype in 9 (41%) cases. Intermediate to high-level resistance to DOR was predicted by genotype in 14 (64%) cases. While DOR FC biological and clinical cut-offs are not available, DOR FC values correlated with predicted susceptibility levels (r=0.746; p=0.0001). Median DOR FC values were significantly higher in viruses harboring major DOR RAMs according to both HIVdb (FC 100 [41.9-100] vs. 6.2 [1.3-18.9], p=0.003) and IAS-USA lists (FC 100 [38.4-100] vs. 6.2 [1.3-22.1], p=0.007).

Conclusions: This panel of multidrug resistant HIV-1 showed limited residual susceptibility to second generation NNRTI. DOR and RPV activity appears to be inferred with fair accuracy by HIVdb algorithm, while ETR activity is underestimated in nearly half of cases.

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Two-drug regimen (2DR) for initial HIV treatment? – Lessons learned from 20 years RESINA cohort

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Background: The classical HIV three-drug regimen (3DR) gives rise to long-term therapy success. The high antiviral potency of integrase inhibitors (INIs), especially the second generation INI Dolutegravir (DTG), was the basis for the idea of two-drug regimens (2DR) in antiretroviral therapy (ART). Since November 2019 the 2DR Dovato® containing the drugs lamivudine (3TC) and DTG is recommended by the European guidelines for first-line treatment in HIV-infected patients without the presence of known or expected drug resistance mutations to the drugs. Thus, we analysed the differences in time to viral suppression in the RESINA cohort depending on used drug classes and screened for baseline drug resistance mutations affecting the use of Dovato® in first-line treatment.

Material and Methods: Overall, 4591 patients from the RESINA cohort, a study initiated in 2000 for the surveillance of transmitted HIV drug resistance, were included in the analysis. The longitudinal analysis of the RESINA cohort includes resistance and clinical data as HIV-RNA, CD4 cell counts and therapies. Baseline drug resistance was analysed for 3TC and INIs [1].

Results: A total of 4591 patients with a majority of men (81.5%) were included. Documented therapies were available in 4076 cases (88.8%) with 3577 corresponding sequence data.

Viral loads of HIV before treatment initiation were stable since 2001 with 60% < 100,000, 30% > 100,000 and 10% > 500,000 copies/ml. In contrast, the number of patients with low CD4+ T-cells < 200 cells/µl decreased from 60% to 35%.

The time to viral suppression after ART initiation significantly differed between 3DR therapies depending on the drug class, INIs < NNRTIs < PIs (median 8w; 14.8w; 17.7w, respectively, p<0.0001). Independent of the drug class, 2DR and 3DR showed no significant differences in time to viral suppression (13.4w and 14.6w, respectively; p=0.688).

The prevalence of baseline drug resistance was rare with 0.42% of the M184V mutation specific for 3TC and 0.8% of transmitted INI mutations.

Conclusion: INI-based therapies showed a significant faster time to viral suppression compared to NNRTI- of PI-based therapies, confirming the high antiretroviral potency of INIs. Although only few 2DR therapies were documented, inferiority to 3DR could not be detected. Baseline resistance to 3TC or INIs was only rarely detected in our cohort. Thus, Dovato® could be an alternative option for initial HIV treatment.

1. Tzou, P.L., et al., Integrase strand transfer inhibitor (INSTI)-resistance mutations for the surveillance of transmitted HIV-1 drug resistance. J Antimicrob Chemother, 2020. 75(1): p. 170-182.

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Dynamics of HIV-1 transmission clusters in North and Central Italy over the years 2012-2019

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Background: To evaluate the dynamics and phylogenetic relationships of HIV-1 strains recently circulating in Northern and Central Italy.

Materials and Methods: HIV-1 pol sequences were obtained from 1770 naïve individuals (1 per patient) diagnosed in different Italian centres in 2012-2019. The phylogenetic tree was built using HIV-TRACE and confirmed by a maximum likelihood approach implemented in FastTree 2.1.4 with automatic selection of the best fit evolutionary model of DNA substitution (GTR+G+I). Sequences linked with at least one other sequence with a bootstrap ≥0.90 and a mean pairwise distance ≤0.015 were considered genetically linked and classified as either pairs (2 members) or transmission-clusters (TCs, ≥3 members). Factors associated with TCs were evaluated by multivariate logistic regression analysis.

Results: Most individuals were men (83.6%) and Italian (69.3%), with a median age of 39 (IQR: 31-48) years. Of them, 83.1% were diagnosed in Central and 16.9% in Northern Italy. Men having sex with men (MSM) represented 44.1%, heterosexuals 32.4%. Individuals were infected mostly by B (63.5%), CRF02_AG (7.3%) or BF recombinant forms (6.9%). Compared to Central Italy, diagnoses in Northern Italy presented a lower proportion of MSM (32.0 vs. 47.3%, p<0.001), a higher proportion of non-Europeans (28.7 vs. 13.8%, p=0.001), higher CD4 cell counts (364 cells/mm3 [141-534] vs. 301 [133-481], p=0.028), and lower viral load (4.86 log10 copies/mL [IQR: 4.14-5.50] vs 5.06 [4.50-5.59], p<0.001). Non-B subtypes, particularly recombinant forms, were more represented in Northern than in Central Italy (39.0 vs. 35.9% and 24.3 vs. 17.2%, respectively, p=0.168 and 0.003). No differences were found in transmitted drug resistance prevalence (13.7% vs. 14.1%, p=0.468).

Regarding TCs, 80 pairs and 43 TCs were observed, corresponding to 15.0% and 21.1% of Northern and Central sequences (45.9% in pairs, and 54.1% in TCs). Northern TCs were characterized by a lower prevalence of Italians (64.4% vs. 81.1%, p=0.003), MSM (37.8% vs. 63.2%, p=0.013), and a higher prevalence of non-B subtypes (55.6% vs. 36.4%, p=0.012). HIV-1 sequences from North and Central Italy rarely intermingled with exception for 31 of them, mixed in 9 pairs and 3 clusters. Two of the 3 mixed TCs involved non-B subtypes (02_AG and 20_BG) and MSM individuals (85.7%), spanning over >5 years.

By multivariate logistic regression analysis, infection by non-B subtype was the only factor significantly associated with being in TCs both in Northern and Central Italy (adjusted odds ratio: 7.87 [2.57-24.06] and 1.67 [1.17-2.40], respectively).

Conclusions: Overall results highlight the existence of different profiles characterizing new HIV-1 diagnoses and transmission groups in Italy, that in absence of an adequate geographical coverage would be underestimated. Compared to Central Italy, Northern Italy is characterized by a higher number of non-B subtypes and non-European infected individuals, actively spreading among TCs, and participating in the epidemiological shift from B to non-B subtypes in Italy.

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Transmitted drug resistance to integrase based first-line HIV antiretroviral treatment in the MeditRes HIV collaboration

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Background and objective: Integrase strand-transfer inhibitors (INSTIs) based regimens are preferred regimens for first-line antiretroviral therapy in Europe. Our objective has been to study the prevalence of transmitted drug resistance to the INSTIs and the NRTI backbone in newly diagnosed patients that are naïve to antiretroviral therapy (ART).

Patients and Methods: MeditRes HIV is a consortium that includes ART naïve people living with HIV that have been newly diagnosed in France, Greece, Italy, Portugal and Spain during the years 2018 and 2019. Reverse transcriptase (RT) and Integrase were sequenced following standard methodologies in use at the participating centres. To evaluate the prevalence of surveillance drug resistance mutations (SDRM) we used the Calibrated Population Resistance (CPR) tools for integrase and RT available at Stanford HIV website. To evaluate clinically relevant transmitted resistance, we used the Stanford v8.9-1 HIVDB Algorithm.

Results: Overall, we included 1844 patients with integrase and RT data available. At diagnosis, 79% were men, 72% of them were men that have sex with men, median age was 40 (IQR, 30-54) years and median viral load was 104.000 (IQR, 22.409-415.000) copies/mL; 47.2% of patients were infected by HIV-1 non-B subtypes. In particular, the most prevalent non-B subtypes were: CRF02_AG (20.0%), A (6.2%), C (4.6%), F (4.6%) and CRF01_AE (1.7%). The prevalence of INSTI SDRMs was 0.22% (T66I, n=1; T66A, n=1; E138T, n=1 and R263K n=1). The prevalence of NRTI SDRMs was 3.6% (M184V, n=16, 0.86%; K65R, n= 2, 0.1%; any STAMs, n=45, 2.44%). Clinically relevant resistance, defined as any resistance level for Stanford interpretation >= 3, was 2.45% for INSTIs (0.05% to Dolutegravir and Bictegravir; 2.4% to Raltegravir; 2.4% to Elvitegravir), and 1.68% to the components of the NRTI backbones (0.76% to TDF/TAF; 1.46% to Abacavir; 0.97% to Lamivudine/Emtricitabine).

Conclusions: Here we describe the most recent data on transmitted drug resistance to integrase based first line regimens in Mediterranean Europe. Given the low prevalence of clinically relevant resistance to second generation INSTIs and to first-line NRTIs, in the years 2018 and 2019 it is very unlikely that a newly diagnosed patient in MeditRes countries would present with baseline resistance to a first-line regimen based on second generation INSTIs.

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Exploring Molecular Characteristics and Evolutionary Dynamics of the HIV-1 Epidemic in Montenegro Through Phylogenetic Analyses

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Introduction: Previous molecular studies of HIV epidemic in Montenegro suggested the predominance of HIV subtype B with low prevalence of non B subtypes, predominantly subtype C. The overall information about the genetic diversity and transmission dynamics of the circulating HIV strains in Montenegro remains insufficient. The aim of this study was to gain insight into molecular diversity and evolutionary characteristics of HIV epidemic in Montenegro.

Materials and Methods: The study included 60 samples from treatment naïve and experienced HIV infected patients monitored at the Clinic for Infectious Diseases, Clinical Center of Montenegro, Podgorica in 2014-2018. For the purpose of transmission cluster analyses additional 26 local sequences, from the NCBI database, were also included. Subtyping was done using the REGA tool and confirmed by phylogenetic analysis. Phylogenetic analyses using maximum likelihood (ML) and Bayesian approach, based on sets of phylogenetic criteria (genetic distance, bootstrap support over 90% and Bayesian posterior probability over 0.9) were used for identification of transmission clusters. Estimation of evolutionary history and dynamics was performed in BEAST and BEAST 2 software packages.

Results: In the studied period, based on the Rega subtyping tool and phylogenetic analyses, the predominance of HIV subtype B was revealed, 42⁄60 (70%). Among non B subtypes, subtype A was identified for the first time in Montenegro in this study, and found with a very high prevalence 14⁄60 (23.3%). Subtype C was found in 3⁄60 (5%) samples. Based on phylogenetic analyses including all 86 local sequences, the presence of 6 transmission clusters, all within subtype B ,that accomplished all predefined criteria sets were identified. Considerable proportion, 39.5% (34/86) of local sequences were identified as a part of 6 local transmission clusters. Sequences within transmission cluster were predominantly isolated from men who have sex with men (MSM), aged from 20 to 35, living in Podgorica, with HIV infection diagnosed within the last 3 years of the study period. Molecular clock analysis suggested the time of the most recent common ancestor (tMRCA) of subtype B sub-epidemic to be in 1985 (HPD interval: 1979–1991) while tMRCA of the most expanded local transmission cluster was dated to 2005 (1999-2011). The subtype A sub-epidemic has a much more recent estimated tMRCA, around 2014 (2010–2018). The estimated birth-death skyline plot for subtype A sub-epidemic, showed initial stationary phase, of Re slight below 1, followed by sharp increasing trend from 2012, reaching maximum value of 2.5.

Conclusion: HIV epidemics in Montenegro continues to marked with the subtype B but with changes in distribution of non B subtypes over time and the emergence of new subtype A in very high prevalence. An important proportion of the local subtype B sequences may be considered to be part of phylogenetically supported transmission clusters. tMRCA for local HIV epidemic in Montenegro, regardless of the transmission risk, dates back to the mid-eighties of the 20st century. HIV subtype A epidemics in Montenegro started to spread later than subtype B, predominantly locally among young MSM with Re suggesting very strong potential for onward spread in the future.

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Prevalence and spatiotemporal dynamics of the HIV-1 circulating recombinant form 03_AB (CRF03_AB) in the former Soviet Union

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Background: The Eastern Europe and Central Asia countries, formerly part of the Soviet Union (FSU), now face the fastest growing HIV/AIDS epidemic in the world. Currently, in this region the steady increase in the prevalence of recombinant forms (CRFs) over time has been noted, though subtype A6 still is responsible for more than half (75.6%) of HIV-1 infections. Among them, CRF03_AB was the third most common recombinant form after AG recombinants. We aimed to investigate the epidemic potential and evolutionary dynamics of HIV-1 CRF03_AB in FSU countries.

Materials and Methods: We studied 145 HIV-1 pol sequences available from twelve FSU countries and the data from fifteen cross-sectional studies describing the genetic diversity of HIV in Russia, Belarus, Lithuania, Latvia, Ukraine and Estonia in 1998-2019 (3,030 HIV cases). Phylogenetic analysis was performed with maximum-likelihood and Bayesian coalescent-based methods using by MEGA v6.0 and BEAST v1.8.2, respectively. Meta-analysis of CRF03_AB prevalence was conducted in Open Meta-analyst with the use of Der Simonian & Laird method and arcsin transformation.

Results: According to our meta-analysis, the total CRF03_AB prevalence was 11.3 % (7.8-14.7%), and was highest in Lithuania 11.6% and lowest in Latvia – 0.3%. In general, a meta-regression has the downward trend which points to stable low circulation of this recombinant form. By analyzing all available sequence, we found that the MRCA for CRF03_AB clade was estimated to be 1992.9; the mean evolutionary rate of the PR-RT genomic region was 2.40x10-3s/s/y. Our estimates suggested that this recombinant virus experienced an initial rapid growth until the beginning of the 2003, soon after that reached a stabilization of the effective population size in 2008, and then it declined after 2010s. The mean growth rate of the CRF03_AB recombinant during the first decades of expansion in FSU was 0.32 year-1. Phylogeographic analysis suggested that the potential origin of CRF03_AB clade was in Kaliningrad, Russia; this city is participated at least in five of the 8 migration pathways which may identify it as a local spread center for CRF03_AB. Apparently, the initial dissemination and subsequent spread of CRF03_AB in the FSU occurred in Russia, thereafter this viral variant spread to Lithuania and Belarus. After expansion in northwestern FSU regions CRF03_AB was introduced to the Central Asian countries (at least in Tajikistan) where apparently has not received epidemic significance.

Conclusions: Previous studies have shown that AG recombinant, which determines the HIV-1 epidemic in Central Asia and Russian Siberian region, had quickly overtaken subtype A6 during the past few years. Our study suggests that the CRF03_AB recombinant likely represents the most widespread genetic form after subtype A6 and reaches a high prevalence in Lithuania, Russia and Belarus. Here we demonstrated that epidemiological history of CRF03_AB recombinant largely repeats the spatiotemporal dynamics of the A6 epidemic in FSU at least in the early stages. These findings are in good agreement with other (sero)epidemiological studies. Along with that, this data represent an excellent opportunity to explore potential CRF-specific differences in the patterns of HIV-1 epidemic growth in FSU.

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Cabotegravir + Rilpivirine Every 2 Months Is Noninferior to Monthly: ATLAS-2M Study

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Background: The 2-drug regimen of long-acting (LA) CAB and RPV dosed i.m. every 4 weeks (Q4W) was noninferior to daily oral 3-drug ART in Phase 3 studies. Long-term Phase 2 data (LATTE-2) provide the rationale for evaluation of a longer and potentially more convenient 8-week dosing interval (Q8W).

Materials and Methods: ATLAS-2M is a multicenter, open-label, Phase 3b noninferiority (NI) study of CAB+RPV LA maintenance therapy administered Q8W or Q4W to treatment-experienced, HIV-infected adults. Virologically suppressed individuals on CAB+RPV LA Q4W (ATLAS study rollover) or oral ART were randomized 1:1 to receive CAB+RPV LA Q8W or Q4W. The primary endpoint at Week 48 was the proportion with plasma HIV-1 RNA ≥50 c/mL (Snapshot, ITT-exposed [ITTe]). The key secondary endpoint was the proportion with HIV-1 RNA <50 c/mL (Snapshot, ITTe).

Results: 1045 participants were randomized to CAB+RPV LA Q8W (n=522) or Q4W (n=523); 27% were female; 63% were naive to CAB+RPV LA. CAB+RPV LA Q8W was noninferior to Q4W dosing in both the primary (1.7% vs 1.0%; adjusted difference [95% CI], 0.8 [−0.6, 2.2]) and secondary analysis (94.3% vs 93.5%; adjusted difference [95% CI], 0.8 [−2.1, 3.7]). There were 8 and 2 confirmed virologic failures (CVFs; 2 sequential measures ≥200 c/mL) on Q8W and Q4W dosing, respectively; 5 and 0 CVFs, respectively, had archived resistance-associated mutations (RAMs) to RPV, either alone (n=3) or with CAB RAMs (n=1) in baseline peripheral blood mononuclear cells (PBMCs). On-treatment RAMs to RPV, CAB, or both not present in baseline PBMCs were found in 6/8 Q8W CVFs and both Q4W CVFs. The safety profile was similar for Q4W and Q8W dosing, and serious adverse events were reported in 5% of participants in the Q8W group (n=26) vs 4% in the Q4W group (n=19). Injection site reactions (ISRs) were mostly mild or moderate (98% overall) with a median duration of 3 days. Discontinuation for an adverse event occurred in 2% of participants (Q8W, n=8; Q4W, n=10), with 6 (1%) and 11 (2%) in each group due to ISRs. There was 1 death (Q8W; sepsis). Of those treated Q8W in ATLAS-2M after ≥48 weeks of Q4W dosing in ATLAS, 94% (180/191) preferred Q8W dosing.

Conclusions: Q8W dosing of CAB+RPV LA was noninferior to Q4W dosing and well tolerated. These results support the therapeutic potential of CAB+RPV LA administered every 2 months.

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Short- and long-term direct medical costs of late and very late HIV presentation

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Background: In Europe, almost 50% of HIV-positive individuals present late to care. Late presentation is associated with higher morbidity and mortality from AIDS-defining illnesses. The costs associated with late presentation have not been comprehensively assessed. This study aims to assess the costs of HIV-care during the first five years after starting antiretroviral therapy (ART) and identify determinants for costs.

Methods: We included clinical data from the ATHENA cohort which prospectively includes nearly all individuals living with HIV in the Netherlands. We included individuals who first initiated ART in 2013 and followed these patients until 2018. Exclusion criteria were enrolment into the cohort six months after starting ART and age below 18 years. Patients were divided in three categories, timely presenters (CD4>350cells/µL), late presenters (CD4 between 200 and 350 cells/µL) and very late presenters (CD4<200cells/µL). Total HIV-care costs included costs of ART, hospitalization, outpatient clinic visits, co-medication and HIV-laboratory tests. Univariate analyses were performed using the chi-square, fisher test and unpaired t-test.

Results: We included 1,296 individuals who initiated ART in 2013, of whom 273(21%) were late presenters and 179(14%) were very late presenters. Patients were predominantly male (88%) and in the age group of 40-60 years. Nearly half (45%) of patients who presented very late were of non-Dutch origin, with 21% originating from Sub-Saharan Africa. The median cost per patient in the first year on ART was €10,477 (Interquartile Range-IQR: 7,466-13,854), of which about two-thirds were costs of antiretroviral drugs (€8,695-IQR:5,656-10,606) and one third non-ART related costs (€1,613-IQR:1,201-3,633). Short- and long-term ART costs were comparable regardless of time of presentation. Non-ART costs were substantially higher among late presenters (€2,206-IQR:1,487-4,816) and very late presenters (€8,399-IQR:5,234-16,539), compared to timely presenters (€1,433-IQR:1106-1957). High short-term non-ART cost is attributable to hospitalization and co-medication. People with lower CD4-cell count, AIDS-defining illness, regimen switching, and malignancies resulted in the highest non-ART cost (all p<0.001). The median total cost of HIV-care per patient in the fifth year was €10,297 for timely presenters, €12,004 for late presenters and €12,095 for very late presenters. Non-ART costs decreased over the years, but remained higher for very late presenters as compared to timely presenters, ascribed to co-medication costs.

Conclusion: Very late presentation is associated with substantial costs, with non-ART costs being nearly seven times higher compared to timely presenters. Importantly, hospitalization and co-medications are key drivers of higher costs for late presenters, indicating that costs of late presentation will remain higher, regardless of future ART regimens. Programs that identify patients earlier will therefore not only improve clinical prognosis of people living with HIV, but likely also provide significant short- and long-term health cost savings.

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Treatment outcome among people living with HIV starting first-line rapid or GRT guided ART from 2015 to 2018

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Introduction: Rapid initiation of antiretroviral therapy (ART) has demonstrated several benefits as compared to ART prescription following baseline genotype resistance test (GRT). However, the role of factors possibly associated with increased risk of virologic failure of rapid ART, including transmitted drug resistance mutations, is still debated.

Within this study, we aimed to examine virologic suppression's (VS) incidence in a cohort of people living with HIV-1 (PLWH) who started first-line ART pending GRT versus those whose ART prescription was GRT-based.

Materials and methods: we included participant in the ARCA cohort who started ART between 2015 and 2018. Mann-Whitney, Pearson $\chi 2$ or Fisher's exact tests were used, as appropriate, to compare the main patients' characteristics between those who started a rapid ART (defined as ART started pending GRT) versus those who started after GRT. Survival analysis (Kaplan-Meier curves with Log rank test and Cox Regression Model) was used to evaluate the probability and predictors of VS stratifying for the two groups.

Results: 521 PLWH were included in this study. Median age was 40 (IQR 31–49) years, 398 (76%) were males. The patients who started a rapid ART were 397 (76.2%), while 124 (23.8%) started a post-GRT ART. CD4 cells at baseline were significantly different [257 (IQR 93-448) cells/µL in pre-GRT vs 383 (IQR 253-564) cells/µL in post-GRT, p<0.001], as well as the median viral load at baseline [4.98 (IQR 4.40 – 5.57)log10 cp/mL in pre-GRT vs 4.61 (IQR 4.01–5.08) log10 cp/mL in post-GRT, p<0.001]. No difference (p=0.765) regarding VS was observed [386 (97.2%) 120 (96.8%) subjects starting pre-GRT ART vs post-GRT, respectively].

By analysing baseline GRTs, at least one resistance associated mutation (RAM) was observed in 15% vs 18% of patients (post-GRT vs pre-GRT respectively, p=0.563). For the Reverse Transcriptase, the most frequent mutation was the 138A/G/K (7.2%) followed by 106I (3.2%), 103N (1.13%) and 41L (1.13%).

About the Protease inhibitor (PI), the 33F was the most frequently observed mutation (2.8%), followed by 6I/L (1.7%). Finally, the only integrase inhibitor mutation was the 66I, identified in one patient.

A NNRTI-based regimen was more frequently prescribed in post-GRT group (27% vs 14%, p<0.001), while a PI-based regimen was preferred in rapid ART group (24% vs 12%, p=0.004). INSTI-based regimens were equally prescribed in both groups.

At a semi-parametric Cox-regression analysis, a more recent calendar year of starting ART was associated with increased probability of VS, whereas a NNRTI-based regimen was associated with increased risk of virologic failure.

Finally, Kaplan Meier curves did not show a significant difference in terms of VS probability between the two groups. Conversely, it was influenced in both groups by calendar year of starting ART and different third drug class (logrank p<0.001 in both cases).

Conclusions: In this cohort, a rapid ART strategy was as effective in achieving VS as a GRT-guided ART. The main predictors of VS were calendar year of starting ART and the anchor drug started.

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Evaluation of HIV-1 tropism in multidrug-resistant cART failing patients

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Background: Heavily treatment-experienced (HTE) patients still represent a fragile population for whom few therapeutic options might be available. According to these considerations, the aim of this study was to evaluate HIV-1 tropism in cART-experienced failing patients to characterize those with exhausted treatment options.

Material and methods: HIV-1 infected patients failing cART with at least one available plasma genotypic resistance test (GRT) for protease/reverse transcriptase (PR/RT), integrase (IN, when available) and gp120-V3 were analysed. For each individual, plasma cumulative PR/RT/IN resistance was evaluated. Multi-drug resistant (MDR) patients were defined as those who accumulated resistance to at least three drug classes among NRTI, NNRTI, PI or INI. Resistance mutations were determined by the Stanford list-2019. HIV-1 tropism was inferred through Geno2Pheno (G2P). The lowest false positive rate (FPR) value in patients' history was considered to determine tropism. X4/dual-mixed strains were defined when a specimen showed a G2P FPR ≤10%. In X4/dual-mixed tropic specimens, FPR was further stratified into two levels (≤5% and 5-10%). Associations between FPR levels and demographic, viro-immunological and resistance parameters were investigated.

Results: Overall, 1382 cART-failing individuals with a median (IQR) time on therapy of 10 (4-16) years were analysed. Most of them were males (68.1%), and infected with HIV-1 B subtype (78.6%), with a median (IQR) age of 46 (39-52) years. Median (IQR) CD4 count nadir and (cells/mm³) at the last GRT available were 98 (33-211) and 312 (155-517), respectively. Median (IQR) viral load (VL) zenith and (log_{10} copies/ml) at the last GRT available were 5.4 (4.9-5.7) and 3.6 (2.5-4.7), respectively. One-hundred and twenty (8.7%), 74 (5.4%) and 30 (2.2%) individuals were previously exposed to maraviroc, enfuvirtide, and both maraviroc/enfuvirtide, respectively.

Four-hundred and twenty-one (30.5%) individuals were infected with X4/dual-mixed strains (FPR ≤5%, n= 307; FPR 5-10%, n=114); 933 (67.5%) accumulated at least one class resistance. In particular, 23.2%, 27.2%, 14.3% and 2.8% of the overall individuals harboured a resistant virus to 1, 2, 3 and 4 drug classes, respectively. Resistance to PI, NRTI, NNRTI and INI (N=780) was 23.5%, 52.7%, 48.2% and 13.2%, respectively.

Among MDR individuals (N=237), 138 (58.2%) harboured R5 strains; while 99 (41.8%) harboured X4/dual-mixed strains, among them 80 (80.8%) showed an FPR ≤5%.

By stratifying according to FPR levels, compared to individuals with FPR 5-10% and FPR >10%, those with FPR ≤5% showed the highest prevalence of resistance to 3 classes (19.2% vs. 16.7% vs. 12.5%), 4 classes (6.8% vs. 0.0% vs. 1.9%), PI (31.6% vs. 19.3% vs. 21.4%), NRTI (59.9% vs. 55.3% vs. 50.1%) and INI (20.6% vs. 11.8% vs. 10.4%) ($p<0.05$). Moreover, by decreasing FPR (from >10% to ≤5%), a decrease of the following variables was found: CD4 cell count nadir (median [IQR] cells/mm³: from 116 [47-225] to 45 [8-128], $p<0.001$); CD4 cell count at last GRT available (median [IQR] cells/mm³: from 334 [179-550] to 256 [87-441], $p=0.003$); proportion of subtype C infected individuals (4.8% to 1.3%, $p=0.017$). By contrast, the proportion of HTE-individuals increased among those who were perinatally infected, experienced >10 previous regimens or were exposed to maraviroc/enfuvirtide ($p<0.05$). No association between FPR levels and VL zenith or plasma VL at the last GRT were found.

Conclusions: Among people failing cART, 17% was infected by an MDR virus. About 60% of these difficult to treat individuals harboured R5-tropic strains, thus maraviroc still represents a valid treatment option.

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DTG+3TC vs DTG+TDF/FTC (GEMINI1&2): Confirmed Virologic Withdrawals Through Week 96

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Background: In GEMINI-1 and -2, the dolutegravir (DTG) + lamivudine (3TC) 2-drug regimen (2DR) is non-inferior to the DTG + tenofovir/emtricitabine (TDF/FTC) 3-drug regimen (3DR) in HIV-1–infected ART-naive participants at Weeks 48 and 96. Eleven participants on 2DR and 7 on 3DR met protocol-defined confirmed virologic withdrawal (CVW) criteria through Week 96. We present a detailed description of these CVWs.

Materials and Methods: Participants were stratified by viral load (VL) ≤ or >100,000 c/mL and CD4+ ≤ or >200 cells/mm3. Participants were not eligible if screening HIV-1 genotype showed major RT/PR resistance mutations. CVW was defined as 2 consecutive VLs meeting virologic non-response (VL ≥200 c/mL after Week 24 or <1.0 log decline in VL by Week 12 unless HIV-1 RNA is <200 c/mL) or virologic rebound criteria (≥200 c/mL after prior suppression to <200 c/mL). Monogram Biosciences performed integrase and RT/PR genotypic and phenotypic resistance testing on Day 1 and virologic withdrawal time point samples. We evaluated CVW participant baseline VL and CD4+ characteristics, adherence, study drug interruption, resistance, and VL progression through the study course.

Results: In GEMINI-1 and -2, 3 participants screen failed due to M184I/V resistance. Overall, 11 participants on DTG + 3TC and 7 on DTG + TDF/FTC met CVW criteria through Week 96. Of these, 5 vs 2 CVWs occurred after Week 48. All CVWs experienced virologic rebound; none had VL blips (VLs between 50 and <200 c/mL with adjacent values <50 c/mL) that preceded CVW. One DTG + 3TC participant never suppressed to <50 c/mL. Among the 11 and 7 participants on DTG + 3TC vs DTG + TDF/FTC, respectively: 9 vs 7 were infected with HIV-1 subtype B; 3 vs 2 had baseline CD4+ <200 cells/mm3; 5 vs 3 had baseline HIV-1 VLs >100,000 c/mL; and HIV-1 VL decreased from CVW time point to the withdrawal visit ≥2 fold for 7 of 9 vs 4 of 5 cases with withdrawal visit VLs. Among the 11 participants with CVW in the DTG + 3TC arm, there was either treatment interruption or evidence of non-adherence in 6 participants, and adherence was unknown in 3 participants. Resistance data were available for all samples except 2 cases on DTG + TDF/FTC where testing failed with HIV-1 VL below the assay cut-off; no treatment-emergent genotypic or phenotypic resistance in IN or RT was observed in any CVWs.

Conclusions: In GEMINI-1 and -2, there were low and comparable numbers of participants meeting CVW through 96 weeks in the DTG + 3TC and DTG + TDF/FTC arms without apparent predisposition by baseline VL or CD4+; no emergent genotypic/phenotypic resistance to INSTI/NRTIs was observed. These data further support the potency and durability of DTG + 3TC.

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Emergence of Resistance in HIV-1 Integrase following Dolutegravir Treatment in Participants Aged 4 Weeks to <18 Years: Results from the IMPAACT P1093 Study

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Background: IMPAACT P1093 (P1093) is a phase I/II, multicenter, open-label pharmacokinetics (PK), safety, and dose-finding study of dolutegravir (DTG) plus optimized background regimen (OBR) in HIV-1 infected children ages 4 weeks (wks)-<18 years (yrs) old and sequentially enrolled by age cohorts (12-<18 yrs, 6-<12 yrs, 2-<6 yrs, 6 months (mo)-<2 yrs and 4 wks-<6 mo). Most participants were highly treatment-experienced. Herein we provide an instream review of virologic failure (VF) across all age cohorts and the emergence of integrase strand transfer inhibitor resistance (INSTI) among children receiving a DTG-containing regimen.

METHODS: VF for P1093 is defined as confirmed decrease in HIV-1 RNA of <1.0 log10 at/after Week 12 (unless <400c/mL); or confirmed >400c/mL at/after Week 24; or confirmed >400c/mL after initial confirmed <400c/mL or confirmed >1 log10 increase above VL nadir (nadir = >400c/mL). At confirmed VF, population resistance testing (RT/PR genotypes, integrase (IN) genotypes, IN phenotypes with IN replication capacity (RC)) were performed. Clonal IN genotypes and phenotypes with IN RC were investigated as available. Adherence was assessed by 3-day recall per visit, and through communication with site investigator.

RESULTS: In this interim analysis of all recruited participants (through 30Sept2018) who completed at least Week 24, 36/142 (25.4%) participants experienced VF. The VF incidence per recruitment age cohort was as follows: 11/23 (48%) for 12-<18 yrs; 7/38 (18%) for 6-<12 yrs; 6/35 (17%) for 2-<6 yrs; 9/29 (31%) for 6 mo-<2 yrs; and 3/17 (18%) for 4 wks-<6 mo. Treatment-emergent INSTI resistance was detected at VF in 6/36 (16.7%) participants. INSTI resistance patterns for the six participants at VF were as follows: R263R/K; L74M, G118R; G118R, E138E/K; E92E/Q, G118G/R; G118R; and T66I, G118R. IN phenotypic results were obtained for 5 of the 6 participants and the presence of G118R was found to have a greater impact on reduced DTG susceptibility as compared to R263K. Corresponding IN RC results for these five participants showed significant decreases as compared to baseline with both R263K and G118R. New resistance to agents in the OBR was detected in 2/6 participants with emergent INSTI substitutions. VF was associated with lack of adherence in most cases. Clonal analyses was conducted for 3/6 participants and confirmed the accumulation of linked IN substitutions that impact DTG susceptibility, however, specific secondary IN substitutions had differing impact on DTG susceptibility, particularly with G118R. An examination of the intasome structure of HIV-1 IN with the resistance patterns detected in P1093 suggests the observed IN substitutions result from an attempt by the virus to preferentially bind substrate over DTG.

CONCLUSION: Among participants aged 4 wks-<18 yrs in P1093 receiving a DTG-containing regimen, the incidence of VF was higher in the 12-<18yrs cohort as compared to other age cohorts. Detection of INSTI resistance in the overall study was low and followed either of two uncommon integrase mutational pathways, R263K and G118R, the latter having greater impact on reduced DTG susceptibility.

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Assessment of Genotypic Patterns Associated with HIV-1 Sensitivity to Ibalizumab

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Background: Ibalizumab (IBA) targets domain-2 of the CD4 receptor and is approved in combination with other antiretroviral(s) for treatment of adults with multi-drug resistant HIV-1 for whom it is otherwise not possible to construct a suppressive antiviral regimen. Decreased susceptibility to IBA has been observed with loss of potential N-linked glycosylation sites (PNGS) within the V5 loop of the envelope protein gp120. We investigated changes in the V5 loop and other regions of gp160 for their contribution to reduced IBA sensitivity in the context of PDTF.

Materials and Methods: Antiviral activity (maximum percent inhibition; MPI) was evaluated against baseline HIV-1 isolates from participants in the phase-2b TMB-202 (n=105) and phase-3 TMB-301 (n=38) trials. In addition, gp160 genotypic and phenotypic analyses were conducted in 45 paired samples, collected at baseline and following protocol-defined treatment failure (PDTF). Viral isolates were assessed using next generation deep sequencing analytics to generate V5 loop nucleotide and derived amino acid (AA) sequences in order to predict V5 PNGS content. Sequence data from gp160 were also evaluated on paired samples to investigate potential contributions to IBA resistance of variations outside the V5 loop.

Results: Baseline IBA susceptibility was consistent (median MPI=97%) for both studies. Nearly all baseline V5 sequences contained 1-2 PNGS motifs. Genotypic analysis of the 45 baseline samples collected from patients with PDTF revealed no pre-treatment correlations between V5 PNGS number or location and IBA susceptibility. PDTF viruses typically had reduced IBA MPI compared to paired baseline isolates but remained partially susceptible to IBA (median MPI=63%). Reduced susceptibility to IBA following PDTF was associated with a reduction in the number of PNGS in V5 compared to baseline but not with PNGS changes observed in other regions of gp160. For baseline viruses with 2 V5 PNGS, reduced IBA MPI at PDTF was associated with loss of PNGS closer to the N-terminus of V5. For baseline viruses with 1 V5 PNGS, reduced IBA MPI at PDTF was associated with its loss. Additionally, 36 AA changes identified at 23 positions scattered throughout gp160 among viruses isolated at PDTF were not associated with reduced IBA MPI.

Conclusions: Baseline HIV-1 susceptibility to IBA indicates rare pre-existing diminished sensitivity. Consistent with published data, loss of V5 PNGS, particularly those closer to the V5 N-terminus, was the primary genetic determinant of reduced susceptibility to IBA following PDTF in two clinical trials. Further, exhibited MPIs indicate persistent residual activities of IBA on viruses following PDTF. No other genotypic changes in gp160 outside the V5 loop were associated with reduced IBA MPI following PDTF. Previous studies have demonstrated that 1) higher IBA MPI is associated with PNGS located closer to the N-terminus of V5 in patients treated with IBA and in other HIV isolates, confirmed by site-directed mutagenesis, and 2) HIV with only 1 V5 PNGS can exhibit complete or partial susceptibility to IBA (Toma, Pace). Previous studies also indicated that higher IBA MPI can be associated with shorter V2 regions, PNGS deletion at position 386, or long side chain AA (H/R/M) at position 375, but only when V5 N-terminal PNGS were absent or deleted. The data described here will inform decision making in the identification of active ARVs to construct suppressive treatment regimens for patients with limited options.

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In vitro activity of Islatravir against HIV-1 mutants harboring multiple NRTI resistance mutations

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Background: Islatravir (ISL, formerly EFdA or MK-8591) is a first-in-class nucleoside reverse transcriptase translocation inhibitor (NRTTI) currently under phase II clinical evaluation. In vitro testing on NRTI resistant viruses revealed that the M184V/I mutation alone or in combination with other NRTI resistance mutations is associated with a variably reduced susceptibility to ISL. This study aimed to evaluate the antiviral activity of ISL on a panel of HIV-1 viruses harboring different NRTI mutational patterns.

Materials and methods: Recombinant viruses, harboring patient-derived PR-RT, were generated from 20 samples of patients enrolled in the Italian PRESTIGIO Registry having different combinations of NRTI mutations. In vitro susceptibility to ISL was determined through a TZM-bl cell-based assay and fold-change (FC) values were calculated with respect to the IC50 value obtained with the wild-type NL4-3 strain. Patients' demographics were described by median (Q1-Q3) or frequency (%); FC data were described by mean±SD and compared by the Mann-Whitney test.

Results: Eighteen (90%) patients were male, median age 54 years (48-58), time since HIV-1 diagnosis 27 years (23-31), time on ART 24 years (22-26), 11 (55%) with a previous AIDS diagnosis, median viral load 4.30 log10 copies/mL (3.32-5.16) and median CD4+ cell count 145 cells/µL (69-280). At sample collection, 13/20 (65%) viruses harbored the M184V mutation. Overall, mean ISL FC value was 6.0±5.1 and higher mean FC values were observed in viruses harboring M184V (7.9±5.2 vs. 2.6±2.6, p=0.006). According to the Stanford HIVdb NRTI mutation list, viruses harboring TAM type 1 (TAM1, n=2) only and TAM1 only plus M184V (n=3) had a mean FC values of 2.3±0.4 and 13.1±4.6, respectively, while the pattern TAM1 only plus M184V and L74V (n=2) appeared to reduce ISL resistance (mean FC 4.0±0.2). Similarly, viruses with TAM2 only (n=2) and TAM2 only plus M184V (n=3) had FC values of 2.1±1.1 and 10.8±6.0, respectively. Viruses with both TAM1 and TAM2 mutations plus either M184V (n=3) or insertion at codon 69 (n=1), or L74V (n=1) had FC values of 4.5±1.9, 8.1 and 0.7, respectively.

Conclusions: This study confirms that M184V and aminoacidic insertions at codon 69 in addition to TAMs are associated with a reduced susceptibility to ISL in vitro. The presence of L74V appears to decrease TAMs+M184V driven resistance. Data from in vivo activity are awaited to define the clinical role of ISL in patients harboring NRTI mutations.

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DETECTION IN HIV PROVIRAL DNA OF DRUG RESISTANCE MUTATIONS IN PATIENTS WITH LOW PERMANENT VIRAL LOADS

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Introduction: There is a clinical need to evaluate HIV patients who after a while on antiretroviral therapy (ART) do not become undetectable (<20 copies / mL) staying with low-level viremias (VBN). In them, adhesion problems are likely to suspect a failure of ART, and the change in therapy is advisable. Currently all genotypic tests available in Chile and the world use RNA and the patient at the time of the test must have a viral load (CV)> 1,000 copies / mL. Therefore, it is recommended to consider proviral DNA (AP) for genotypic resistance test in cases of VBN. It is also important to know the HIV mutations that have been archived for the clinical management of patients naive to ART as well as for those in whom a treatment change will be made.

Objective: Perform a genotypic resistance test in patients under ART with VBN and prepare a report of the resistance profile for HIV antiretroviral drugs.

Material and methods: Between 2017, 2018 and 2019, a total of 545 patients experienced in ART were studied. With viremias that ranged between <20 and <1,000 copies / mL. As a control of amplification, sequencing and genotypic resistance, 30 samples of patients with viral loads> 1,000 copies / mL were included, which were analyzed by RNA. They were studied by AP amplifying by PCR the reverse transcriptase (TR) and protease (PRO). The amplicons obtained were sequenced and analyzed by the RECall bioinformatic software. Only the approved sequences were used to detect mutations of clinical relevance and prepare a report of genotypic resistance to ART in the Standford University database using the HIVdb Program.

Results: Of the 545 patients studied, 309 were Susceptible (56.7%), 153 Resistant (28.1%) and 83 were Not Reportable (15.2%). In the resistant samples the following nucleoside analog mutations were detected: M184V / I (11%), M41L (1.46%), T215C / D / F / Y (3.48%), K70E / G / Q / R (2.2%), A62V (0.9%) and K65R (1.1%). The non-analogous nucleotide mutations detected were: K103N (7.7%), V106A / I / M (3.11%), V108I (2.01%), Y181C / H / I / F (2.93%), M230I / L (1.46%) , H221Y (2.01%). The following mutations were detected in the viral protease gene: M46I / L (0.5%), V82A (0.36%), L90M (0.18%), D30N (0.18%), K43T (0.18%), L76V (0.18%) , G48R / V (0.36%), G73S (0.18%), L33F (0.18%) and Q58E (0.36%). Mutations detected in patients with persistent low-level viremia were associated with resistance to antiretroviral drugs. The non-reportable results were mainly due to the fact that the quality of the sequence obtained is not adequate to make a prediction of resistance. This is due to the high genetic variability of the HIV virus that yields a high level of sequence mixtures that were not approved by the RECall software.

Conclusions: From HIV proviral DNA, a profile of resistance to antiretroviral drugs can be obtained in patients with persistent low-level viremias. Therefore, proviral DNA is a suitable compartment for HIV genotypes because it delivers very useful information that is not available when the viral load is low. In patients with resistance, therapy could be changed more early with the consequent benefit to the patient.

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HIV-DNA DECAY IN ART-NAÏVE PATIENTS STARTING A DTG-BASED DUAL VS TRIPLE THERAPY

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Background: No information is available regarding the HIV-DNA decay in ART-naïve patients starting a dolutegravir(DTG)-based dual regimen (2DR). Our aim was to compare both HIV-DNA and HIV-RNA decay in ART-naïve patients starting a DTG-triple regimen (3DR) or 2DR.

Materials and Methods: This was a retrospective study on patients starting three different regimens: 6 patients with lamivudine (3TC) and DTG (2DR), 17 patients emtricitabine (FTC)/tenofovir alafenamide (TAF) and DTG (3DR-TAF) and 13 patients abacavir/3TC/DTG (3DR-ABC). We quantified the blood-associated total HIV-DNA by droplet digital PCR (detection limit of 32copies/10^6CD4+) using a home-made protocol targeting the HIV-1 LTR region, before starting therapy (baseline, BL) at virological success (VS, HIV-RNA <50 copies/mL) and, for 81% of patients, 6 months after VS (6mVS). Results were expressed as log_{10} HIV-1 DNA copies/10^6CD4+. Non parametric tests were used to compare the medians among the 3 groups and to assess the change of log_{10} HIV-DNA and log_{10} HIV-RNA at the different time points. Linear regression analyses explored predictors of HIV-DNA and HIV-RNA change at VS.

Results: We included 36 ART-naïve patients, mostly males (89%), with homosexual transmission as the main risk factor (53%), a median age of 35 (IQR:28-47) years with an equal distribution of subtype B and non-B (48 and 48%); the main BL characteristics were balanced among the 3 groups, albeit 3DR-TAF group, likely due to AIDS event occurred in 23% of patients, showed a higher BL HIV-RNA and lower CD4+ count. Overall there was a direct correlation between BL HIV-RNA and BL HIV-DNA levels (p=0.001).

At VS among the 3 groups, the proportions of patients reaching the undetectable viremia and the CD4/CD8 ratio improvement was similar. At VS, HIV-RNA significantly decreased in all 3 groups to a comparable level, albeit with a different delta change: both 2DR and 3DR-ABC showed a similar less sharp decay, while, in the 3DR-TAF group the decay was more pronounced, even if the time to reach the VS was longer. BL HIV-DNA levels were similar among groups. At VS there was a significantly reduction with a comparable delta change with all the groups; although this reduction resulted more marked in both triple therapies (3DR-TAF p<0.001 and 3DR-ABC p=0.004) as compared to 2DR (p=0.046). In a sensitivity analysis removing three acutely HIV-infected patients, of whom one in 2DR and two in 3DR-ABC group, in 2DR the HIV-DNA decay resulted no longer statistically significant (p=0.080) while for the other two groups any substantial difference was observed (3DR-TAF p<0.001 and 3DR-ABC p=0.008).

Higher BL HIV-DNA levels predicted a more pronounced decay of HIV-DNA (1 log increase: -0.564; 95%CI -0.994/-0.280, p=0.012). For patients whose samples were available at 6mVS, HIV-RNA levels remained stable as compared to VS (all p values≥0.068), without any difference among the groups (p=0.150), while HIV-DNA levels resulted more reduced in 3DR-ABC.

Conclusion: In ART-naïve patients all three DTG-based regimens determined a significant HIV-DNA decay at VS; however 2DR resulted in a less marked decline when compared to both 3DR DTG-based therapies. A larger sample size is needed to confirm these results.

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ROLE OF ANALITICAL TREATMENT INTERRUPTION ON HIV PERIPHERAL RESERVOIR DIVERSIFICATION

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Background: The impact of analytical ART interruption (ATI) on HIV peripheral reservoir would inform our understanding of viral dynamics and the mechanistic underpinnings of HIV persistence, representing an opportunity to define patients at lower risk to experience viral rebound. The APACHE study shows that ATIs with transient viremia did not alter the size of peripheral HIV-DNA in chronically HIV-1 infected patients with HIV-RNA<50 copies/mL for ≥10 years. Here, we investigate if ATI might impact on the genetic diversity of the HIV peripheral reservoir in this population.

Methods: Six APACHE are analysed for total HIV-DNA (copies/10^6 CD4, ddPCR), residual viremia (copies/ml, ddPCR), and C2-V3 sequences (HXB2 nt: 7029-7448, Illumina MiSeq) at ATI, viral rebound and at achievement of VL <50 copies/mL after ART resumption (post-ATI). These parameters were also analysed at 3 similar time-points for 5 combined-ART treated patients with VL always <50 copies/mL for ≥1 year (control-group: MODAT). To assess similarities between sampled virus populations, pairwise genetic distance (Tamura-Nei 93) is computed at each time point. Maximum likelihood (ML) trees and genealogical sorting indices (GSI, index of monophyly if ≥0.50 with P<0.05) assess compartmentalization of virus populations. Results are described by median [IQR]. Wilcoxon signed-rank test is used to test changes in peripheral reservoir within APACHE and MODAT patients.

Results: APACHE patients experience viral rebound after ATI in 3 [3;5] weeks and, after ART resumption, achieve VL <50 copies/ml in 19 [4;29] weeks. HIV-DNA and residual viremia do not change between pre- and post-ATI (1048 [863;1286] copies/10^6 CD4 and 1 [1;4] copies/ml vs 1333 [553;2183] copies/10^6 CD4 and 3 [1;8] copies/ml, P=0.11 and P=0.60). In MODAT, HIV-DNA and residual viremia are 1037 [730;1750] copies/10^6 CD4 and 3 [2;7] copies/ml at I time-point, and 1104 [527;1598] copies/10^6 CD4 and 2 [0;5] copies/ml at III time-point, with no significant change between time-points (P=0.34 and P=0.10).

C2-V3 genetic distance significantly increases from pre-ATI to post-ATI in 4/6 APACHE (+0.36 [0.11;0.41], P=0.04) and 0/5 MODAT patients (+0.18 [-0.01;0.24], P=0.22). ML trees show that in 5/6 APACHE patients pre-ATI DNA sequences are distinct (bootstrap>70%) from rebound and post-ATI viruses, and that in vivo viral rebound was not predicted by the expansion of pre-ATI viral lineage. By contrary, virus populations at the three time points are highly interspersed in 4/5 MODAT patients.

Statistically significant high GSI values (GSI ≥0.50 with P<0.05) between pre-ATI and post-ATI are found in 5/6 APACHE, confirming a viral evolution of post-ATI viruses with respect to pre-ATI strains. Differently, low and no significant GSI values between I and III time-points are found in 3/5 MODAT patients.

Comparisons of V3 tropism between I and III time-points show a trend of decrease of false-positive-rate (FPR) at third time-point in APACHE patients (delta-FPR, median [IQR]: -27 [-2;-30], P=0.08), but not in MODAT individuals (delta-FPR, median [IQR]: -3.7 [-62;4.14], P=0.50).

Conclusion: This proof of concept study indicates that although transient viremia does not alter size of HIV-DNA and residual viremia from ATI to post-ATI, short treatment interruption might impact on significant genetic diversification of peripheral viral reservoir.

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Similar levels of HIV-DNA and residual viremia are found in virologically suppressed individuals who continue a two-drug regimen with dolutegravir plus one reverse transcriptase inhibitor or switch to elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide enrolled in the Be-OnE Study

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Background: To investigate HIV-DNA and residual viremia levels through 48 weeks (W48) in virologically suppressed HIV-1 infected patients randomized to continue a two-drug regimen (2DR) with dolutegravir (DTG) plus one reverse-transcriptase-inhibitor (RTI, lamivudine or rilpivirine) or to switch to elvitegravir/cobicistat/emtricitabine/tenofovir alafenamide (E/C/F/TAF).

Material and Methods: This is a randomized, single-center, open-label, 96-week superiority Study (NCT03493568; Be-OnE Study). Patients with HIV-RNA <50 copies/mL for at least 6 months while receiving DTG+1RTI for at least 3 months were randomized 1:1 to continue the ongoing treatment or to switch to E/C/F/TAF. Total HIV-DNA (normalized for copies/10^6 CD4+ T-cells) and residual viremia were measured with standardized in-house digital droplet PCR assays. Spearman correlation coefficients (rs) were calculated to assess linear relationship between HIV-DNA, viremia levels and several immunological parameters (including D-Dimer, C-reactive protein [CRP], %CD8+CD38+HLA-DR+, %CD4+CD38+HLA-DR+, CD4+ T-cells, CD8+ T-cells, CD4/CD8) both at baseline and at W48. Differences in HIV-DNA and residual viremia levels were evaluated by using Wilcoxon signed-rank test among patients within the same arm or the Mann-Whitney test between the two arms.

Results: HIV-DNA and residual viremia measurements at baseline and at W48 were available for 40/50 patients enrolled in the Study. Overall, median (IQR) HIV-DNA levels were 2247 (767;4268) copies/10^6 CD4+ T-cells (E/C/F/TAF-arm: 1971 [632;3270]; DTG+1 RTI-arm: 3077 [781;6030], p=0.448) at baseline, and 1587 (556;3543) copies/10^6 CD4+ T-cells (E/C/F/TAF-arm: 1053 [458;3105]; DTG+1RTI-arm: 1922 [982;3804], p=0.330) at W48. Median (IQR) residual viremia levels were 2.9 (1.1;5.3) copies/mL (E/C/F/TAF-arm: 3.0 [2.0;5.9]; DTG+1 RTI-arm: 2.6 [1.3;5.3], p=0.479) at baseline, and 1.2 (0.0;5.5) (E/C/F/TAF-arm: 3.2 [1.2;5.6]; DTG+1RTI-arm: 5.5 [1.3;9.6]; p=0494) copies/mL at W48. No significant correlations were found between HIV-DNA and viremia levels or immunological parameters at either baseline or W48, with the exception of HIV-DNA levels and CD8+ T-cells at W48 (rs=0.411, p=0.008).

No significant changes in HIV-DNA and viremia levels were found from baseline to W48. However, the proportion of patients with target not detected plasma HIV-RNA (TND=0 copies/mL) increased from baseline to W48, overall (from 10% to 40%, p=0.004) and in both arms (E/C/F/TAF: from 4.8% to 38.1%, p=0.008; DTG+1RTI: from 15.8% to 42.1%, p=0.074). Moreover, at W48, a modest decrease in HIV-DNA from baseline was found: -226 (-1189; 890) copies/10^6 CD4+T-cells (p=0.465) in the DTG+1RTI-arm and -137 (-983; 133) copies/10^6 CD4+T-cells (p=0.334) in the E/C/F/TAF-arm, without significant differences between the two arms (p=0.968). In a few participants, HIV-DNA slightly increased from baseline to W48 (DTG+1RTI: 7/19; E/C/F/TAF: 5/21, p=0.495), despite a residual viremia decrease. In a single participant on the DTG+1RTI arm, an increase of both HIV-DNA (from 320 to 1210 copies/10^6 CD4+ T-cells) and residual viremia (from 1.1 to 9.6 copies/mL) was observed.

Conclusions: Changes in HIV-DNA and residual viremia from baseline to W48 in virologically suppressed individuals who switched from a 2DR with DTG+1RTI to E/C/F/TAF were negligible and did not significantly differ from changes in those who continued the 2DR.

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Comprehensive assessment of SARS-CoV-2 key genetic elements single or in clusters underlying geographically-dependent genetic evolutionary adaptation and their impact on drugs binding affinity and immune escape

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Background: To determine key genetic-elements, single or in clusters, underlying SARS-CoV-2 evolutionary diversification across Continents, and their impact on drug binding-affinity, viral antigenicity, and immune response control.

Materials: 12,150 SARS-CoV-2 sequences from a publicly available database (GISAID) cover 69 Countries worldwide are analyzed. Mutational clusters are assessed by hierarchical-clustering. Structure-based virtual screening (SBVS) is used to select the best potential inhibitors acting on the main Protease (3CL-Pr) and RNA-dependent RNA polymerase (RdRp) among the FDA-approved drugs and to evaluate the impact of the identified mutations on binding-affinity for these drugs. Lastly, the impact of mutations on epitope-recognition is assessed in silico through the Immune Epitope Database and Analysis Resource (IEDB), following Grifoni, 2020.

Results: 35 key mutations are identified, all with a prevalence>0.5% and residing in different viral proteins. 16/35 mutations form tight clusters involving multiple SARS-CoV-2 proteins, highlighting inter-genic co-evolution. Some clusters, including D614GSpike+P323LRdRp+R203KN+G204RN, occur in all Continents (bootstrap=1.0). Differently, other clusters show a geographically-restricted circulation: T1198KPL-Pr+P13LN+A97VRdRp in Asia (bootstrap=1.0), L84SORF-8+S197LN in Europe (bootstrap=1.0), Y541CHel+H504CHel+L84SORF-8 in America and in Oceania (bootstrap=1.0 and 0.97, respectively). SBVS identifies 20 best RdRp inhibitors and 21 best 3CL-Pr inhibitors belonging to different drug classes. Notably, mutations in RdRp or 3CL-Pr modulate positively or negatively the binding-affinity of these drugs. Among them, P323LRdRp (prevalence:61.9%) reduces the binding-affinity of specific compounds including remdesivir (G-score: -8.80 vs -9.91 kcal/mol for P323LRdR vs wt). Conversely, P323LRdRp determines an increase of the binding-affinity of the purine analogues penciclovir and tenofovir (G-score: -10.12 vs -8.22 kcal/mol and -8.45 vs -8.25 for P323LRdR vs wt, respectively), suggesting a potential hypersusceptibility to these drug-candidates in presence of this mutation. Finally, specific mutations hamper (up to abrogate as for Y541CHel+H504CHel) Class-I/II epitopes recognition, while D614Gspike profoundly alters the structural-stability of the recently-identified B-cell epitope encompassing amino acids 592-620 of Spike protein.

Conclusion: Key genetic-elements reflect geographically-dependent SARS-CoV-2 genetic adaptation, and can play a role in modulating drug-susceptibility and in hampering viral-antigenicity. Thus, close unremitted monitoring of SARS-CoV-2 mutational patterns is crucial to ensure the effectiveness of treatments and vaccines as well as the accuracy of diagnostic assays worldwide.

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Case report: poor adherence to dolutegravir is associated with selection of resistance mutations T66I, G118R, E138A and L74I

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Background: Dolutegravir (DTG) is recommended as first-line antiretroviral therapy (ART) for HIV-1 infection. DTG is characterized by high antiviral potency and a high genetic barrier to the emergence of resistance. High-level resistance to DTG is rare and clinically significant. DTG resistance has been decribed primarly on raltegravir (RAL) or elvitegravir (EVG) treatment-experienced patients. Here we describe a case of virologic failure to DTG in a ART experienced patient who had not previously received RAL or EVG.

Materials and methods: Clinical data was retrieved from Estonian HIV-positive patients database (E-HIV). Genotypic resistance testing was performed using plasma samples from 2013 to December 2019. Viral RNA was amplified and sequenced in HIV-1 integrase (IN) and protease/revertase (PR/RT) region. PR/RT inhibitors and integrase strand transfer inhibitors (INSTI) drug resistance mutations (DRMs) were detected using Stanford University HIV-1 Drug Resistance Database (Version 8.9-1). Subtyping was conducted using REGA HIV-1 & 2 Automated Subtyping Tool (Version 2.0).

Results: A 38-year old heterosexual man with the history of injecting drug use was diagnosed with HIV-1 in 2013. At the diagnosis, HIV-1 viral load (VL) was 83,576 copies/ml and CD4+ cell count was 367 cells/ul with no baseline PR/RT DRMs. Abacavir/lamivudine and fosamprenavir/ritonavir therapy (ABC/3TC/fAPV/r) was initiated one month after diagnosis. In February 2015 after ART failure (VL 31,310 copies/ml and CD4 cell count 20 cells/ul) the PR mutations V32I, I47V and K20I were detected, but no RT or IN DRMs were found. ART was switched to abacavir/lamivudine/dolutegravir (ABC/3TC/DTG). In March 2015 VL was undetectable for the first time, but CD4+ cell count was 26 cells/ul. In November 2015 VL had increased to 36,145 copies/ml, CD4+ cell count was 21 cells/ul and resistance testing showed high level resistance to all INSTIs (T66I, G118R, E138A and L74I) and no DRMs in PR and RT region. The patient confirmed poor adherence during the last two months prior to the DRMs testing. In February 2016 patient started ABC/3TC with LPV/r and was lost to follow up in October 2017. In February 2019 on re-engagement in the care his VL was 2,865,214 copies/ml and CD4+ cell count was 2 cells/ul and he restarted ART with ABC/3TC and double-dose DTG. As VL (42,864copies/ml) was still detectable and CD4+ count remained low (4 cells/ul), a new resistance test was performed in December 2019 revealing M184V and previously detected INSTI mutations (T66I, G118R, E138A and L74I).

Conclusions: Although resistance to DTG is generally rare, in this case a poor adherence to DTG-containing regimen was likely associated with the selection of resistance mutations T66I, G118R, E138A and L74I determines high level resistance to all INSTIs. We emphasize that resistance to DTG can occur in treatment experienced patients with poor adherence and should be considered in an inadequate response to ART.

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Integrase Resistance development after long-term suppressive treatment with Abacavir, Lamivudine and Dolutegravir

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Introduction: Integrase inhibitors (INI) are one of the main components in modern anti-retroviral therapy against HIV. Especially the 2nd generation Inhibitors like Dolutegravir or Bictegravir have overcome the problem of the low genetic resistance barrier of the first generation inhibitors. A low rate of adverse effects and low risk of pharmacokinetic drug-drug interactions make this group to ideal candidates for long term successful anti-retroviral treatment.

Objectives: We here report a case of development of clinical relevant resistance after long term treatment with a combination of Abacavir (ABC), Lamivudine (3TC) and Dolutegravir (DTG).

Patient & methods: The 47 year old male patient was diagnosed with HIV in 2009 (CDC C3), having a CD4 Nadir of 20/µl and as clinical representation of his immunodeficiency a CMV retinitis. Baseline genotypic resistance testing from viral RNA was performed 04/2009 showing no clinical relevant mutations. Treatment was initiated with TDF/FTC and LPV/r and switched to TDF/FTC and EFV in 2013. After genotypic resistance testing from proviral DNA in 11/2014, showing also no clinical relevant mutations, treatment was changed to ABC/3TC/DTG as a single tablet regimen. Treatment response was stable, despite some detectable HIV-RNA measurements below 50 cop./ml, until 05/2019 where a viral load below 200 cop./ml could be detected. In the follow-up sample from 12/2019 a viral load of 1090 cop./ml could be detected and a genotypic resistance test using NGS was performed.

Results: Following mutations could be detected: reverse Transcriptase M184V (99.5%), V189I (99.7%); Protease I62V (99.6%), L63P (99.5%), A71T (98.6%); Integrase R263K (64.6%). This mutational pattern leads to a resistance against 3TC/FTC, low level resistance against ABC and resistance against Dolutegravir.

Discussion: While few reports of treatment failure with resistance against DTG and mutation R263K exist, this is to our knowledge the first report of treatment failure after 5 years of successful treatment with a DTG containing regimen. Based on insufficient prescription refill of the drugs we suspect an adherence problem with temporarily insufficient drug levels as reason for resistance development. Despite the high resistance barrier of 2nd generation INIs, a high level of adherence remains a crucial factor in successful treatment.

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Difficulty in reaching virological suppression in a long-term treated HIV-1 patient: rather the role of the reservoir than drug resistance?

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The size of HIV-1 reservoir highly impacts virological outcomes. We here present the well-documented story of a long-term treated HIV-1 patient with a high reservoir, who failed to reach undetectable plasma viral load, despite the use of potent antiretroviral molecules and the absence of significant resistance associated mutations investigated by both Sanger and NGS methods.

A French MSM patient was diagnosed in October 2003 with symptomatic HIV-1 primary infection at the age of 21 years. At diagnosis, the medical history was unremarkable. The CD4 count was 992 cells/mm3 and the plasma HIV-1 viral load (VL) was 5.1 log copies/mL. The patient was followed by his GP with no antiretroviral treatment (ART) from October 2003 to November 2010. Anal abscess and anogenital warts have been recorded on July 2005 with a progressive decrease of CD4 count to 233 cells/mm3, and a VL fluctuating between 4 and 5 log copies/mL. ART was initiated on December 2010 with abacavir (HLA B57 negative and no HBV co-infection), lamivudine, atazanavir, and ritonavir. The subtype B virus was fully susceptible and the Sanger resistance genotyping test found only minor L63P and A71T protease mutations. The next-generation sequencing (NGS) resistance test (Sentosa® HIV assay, Vela Diagnostics) performed later on this sample additionally detected only the reverse transcriptase D67N mutation at a very low prevalence (around 2%). Adherence to treatment was not optimal. CD4 count increased to 500 cells/mm3 while VL remained around 3.5 log copies/mL at 6 months post-treatment. A new resistance test was performed and found the same profile both with Sanger and NGS methods. The ART was switched to tenofovir, emtricitabine, atazanavir, and ritonavir. Acceptable drug monitoring results confirmed the adherence but the VL remained detectable 8 months later. The patient stopped the treatment for 3 months until he felt ready to restart it. Then the VL increased with no additional resistance associated mutations (RAMs) detected. The new ART regimen included tenofovir, emtricitabine, darunavir and ritonavir and failed to clear the virus after 12 months. To estimate the reservoir, total HIV-1 DNA quantification was performed (Generic HIV DNA cell, Biocentric) and found a high level around 4 log copies/106 PBMCs. Thereafter, CD4 count reached 1000 cells/mm3 but VL fluctuated between 1.7 and 2.5 log copies/mL under multiple subsequent ART regimens (tenofovir, etravirine, darunavir and ritonavir; tenofovir, dolutegravir, darunavir and ritonavir; tenofovir, darunavir and ritonavir). Several resistance tests were performed during the follow-up with both Sanger and NGS methods. In plasma, Sanger sequencing did not detect RAMs explaining the persistent viremia. NGS found the reverse transcriptase E138A mutation (RT) at 9% after the initiation of etravirine-containing regimen. In PBMCs, some RAMs usually associated with defective variants (M184I, E138K, M230I, G73S) were also detected by NGS at prevalence between 3 and 7%. In addition, the integrase G140S mutation was found in PBMCs around 20% before and after dolutegravir initiation.

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HIV Seronegativity After Rapid ART: A Case Demonstrating Future Challenges in HIV Confirmation Testing in the Era of iART

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Current guidelines recommend initiation of antiretroviral therapy (ART) as soon as possible after diagnosis of human immunodeficiency virus (HIV) infection [1]. This can result in the failure to develop HIV antibodies or a subsequent waning of antibodies from early treatment [2, 3, 4]. One small study involving 15 children started on early ART before age 6 months found undetectable HIV-specific immune responses in the majority of patients [5]. We present a case of an adult started on ART nine days after diagnosis of HIV who experienced a loss of HIV-1 antibody (Ab) reactivity, or seroreversion.

The patient underwent 4th generation HIV-1/2 antigen (Ag) and antibody testing on March 31st, 2015, resulting in a reactive Ag/Ab screening but indeterminate HIV-1 Ab and negative HIV-2 Ab. Two weeks later, repeat 4th generation testing was positive for HIV-1 Ab with HIV-1 RNA PCR detecting 230 copies/mL. Nine days later, the patient was started on Complera (FTC/RPV/TDF) resulting in sustained viral suppression. The patient then switched to a different clinic for 2 years before re-establishing care on January 31st, 2020, at which time HIV confirmation testing was performed. While 4th generation HIV Ag/Ab screening was positive, HIV-1/2 Abs were not detected. This result was reproduced on repeat testing, and both HIV-1 RNA and proviral DNA were also not detected. Presence of HIV infection was then confirmed by DNA Archive resistance testing.

The push for early or even rapid “same-day” initiation of ART, or “iART”, may lead to providers encountering HIV seroreversion with increasing frequency, which raises several concerns regarding current practice.

First, 4th generation testing was insufficient in confirming HIV infection in this patient. Formal recommendations are needed to help guide confirmation testing in a cost effective and readily accessible manner in patients who had been started on iART.

Second, because HIV seroreversion is not widely recognized it carries the risk of causing diagnostic confusion for providers. This could result in misclassification of a patient's HIV status, especially when switching between clinical settings. Educating providers will facilitate communicating these results to patients to reinforce ART adherence and retention in care.

Finally, there lacks an evidence-based approach for ongoing treatment in HIV seroreversion. There are no studies comparing the risks of continuing ART versus adopting a monitoring approach off ART. More data is needed to help guide this discussion, especially as the increasing age of patients living with HIV calls for greater focus upon potential long term adverse effects of ART and polypharmacy.

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Assessment of quality of life among patients with hepatitis C in Georgia

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Background: Georgia is the country with high prevalence of viral hepatitis C. Results from a serosurvey conducted in 2015 among adults found that the estimated seroprevalence of HCV is 7.70% with 5.45 being chronically infected. Hepatitis C elimination program has been launched in Georgia since 2015. One of the major goals of treatment among HCV infected people, particularly those with advanced liver disease, is to improve the quality of life after cure. According to studies conducted mainly among patients treated with interferon/ribavirin regimen, hepatitis C treatment has a positive effect on the general health and improves quality of life. There are no published data about the impact of HCV treatment on the quality of life among patients treated within HCV elimination program in Georgia. The objective of this study was to evaluate baseline, pre-treatment quality of life (QOL) among patients enrolled in HCV elimination program and study the associations of QOL with different clinical and laboratory parameters to collect the data for further comparison to the follow-up quality of life data after the cure from HCV.

Methods: HCV RNA positive patients with advanced liver fibrosis were selected through random sampling from the list of patients from medical clinic NeoLab (one of the National HCV Elimination Program providers). Degree of liver fibrosis was assessed by FIB4 scores (ALT, AST, Platelet count and patients age) and among patients with FIB4 score between 1.45-3.25 by liver elastography was performed. A special questionnaire was specifically developed to study pre-treatment quality of life and piloted prior to administration. The questionnaire was used to obtain detailed demographic and general health information. The social-demographic characteristics included information about age, sex and place of residence. Participants were recruited from January 2019 to January 2020.

Results: Out of 285 patients approached, 267 agreed to participate (refusal rate 6.4%). Among them 49 (18.4%) were females and 218 (81.6%) - males. The mean age was 52.8 (range 26-83 years). 74 (27.7%) patients had liver fibrosis level F3 with 193 (72.3%) having F4 (or ≥3.25 by FIB4 score). 85 (31.8%) study subjects reported often feeling weakness in the last two weeks, 53 (19.9%) often suffered from insomnia. One-third of respondents (28.8%) reported that they often feel a lack of energy and 19.4% have the problem of lifting or carrying heavy objects. Almost 30% of respondents feel abdominal discomfort and pain. 15% of interviewed individuals described their health as poor and according to 16.1% of respondents their health condition has deteriorated, as compared with the one a year ago.

Conclusion: About one third of patients with HCV infection and advanced liver fibrosis reported poor quality of life before DAA treatment.

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Multicentric Castleman Disease HHV-8 related in a HIV patient

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Objective: To describe clinical, diagnostic and therapeutic features of CD related with HIV.

Methods: This is a case report of a patient diagnosed and treated in ID Service at UHCT, 2017.

Results: Male 26 years old, known as intravenous drug user was diagnosed with HIV infection 4 years ago, in AIDS stage-C3, CD4 baseline 2%-21 cell/mm3. Opportunistic infections at admission were: wasting syndrome and HCV coinfection. Since the moment of HIV diagnosis the patient started first line of antiretroviral therapy, Zidovudin/Emtricitabine and Efavirenz. Because of his problematic lifestyle, the adherence to treatment was poor. In February 2017 the patient was diagnosed with pleuropneumonia and the CD4 was reduced 2,1%-19cell/mm3. He switched on second line of ARV with Truvada, Efavirenz, Raltegravir. One month later the patient presented with fever, oral thrush, malaise, odinophagia and evident tonsil hypertrophy, lymphandenopathy in cervical and axillar regions and in abdominal CT scan lymphonodes. Laboratoric studies showed: pancytopenia, hypoalbuminemia, hypergammaglobulinemia. The patient underwent to biopsy of cervical lymphonode and the histopathological distinct concluded for Castleman disease HHV-8 associated. Based on treatment protocols the patient started the chemotherapy with CHOP (Ciclophosphamide, doxorubicin, vincristin and prednisolone) and also supportive treatment with allopurinol, antiemetic and blood transfusions. The cervical lymphnodes reduced the diameter after one week and the patient was without fever and had a good clinical performance. The second cycle with CHOP after 3 weeks was complicated with ARDS due to Chemotherapy. After two weeks the patient presented again with high fever, lymphadenopathy, malaise, anemia, thrombocytopenia, hypertransaminasemia, hepatosplenomegalia. Despite intensive care the patient couldn't survive because of multiorgan failure after the second cycle of CHOP. The patient wasn't treated for HCV coinfection due to poor resources of our country for DAA regimen.

Conclusions: Castleman's disease also known as angiofollicular or giant lymph node hyperplasia, is a rare B-cell lymphoproliferative disorder, etiologically is linked to HHV-8, and manifesting clinically as a unicentric or multicentric disease that occurs especially in men with HIV infection. It is an aggressive disorder, with diffuse lymphadenopathy, constitutional symptoms and a usually fatal course. Gold-standard approach to clinical management has yet to be formally established, although there is increasing support for the use of rituximab-based approaches.

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Variability in HIV Proviral DNA Genotyping Results from a Single Blood Draw

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Background: The HIV latent reservoir represents all viral strains that have replicated during the course of HIV infection, including those with drug resistance. DNA genotyping of the proviral archive is increasingly utilized to obtain resistance data in virologically suppressed patients. Proviral DNA genotyping is limited by the small amount of target HIV DNA available in a patient's blood sample and only detects high frequency mutations circulating in the periphery. In addition, circulating antigen-specific CD4+ T cells expand and contract upon cognate antigen recognition and further influence which strains are available for amplification. To assess the reproducibility of DNA genotyping, the GenoSure Archive® assay was performed on multiple aliquots from the same blood draw for five individuals from Study 4030 (NCT03110380), a study switching virologically suppressed participants with no known integrase (IN) inhibitor resistance from a dolutegravir (DTG)-based regimen to bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF).

Methods: Proviral DNA genotyping of whole blood samples was conducted in duplicate or triplicate using the GenoSure Archive® assay, Monogram Biosciences. This assay requires 4 mL of whole blood and tests 1 mL per test. It can detect resistance of HIV-1 to protease, reverse transcriptase (RT), and IN inhibitors in cell-associated viral DNA.

Results: Separate aliquots of one blood draw from five participants were tested. For two participants who each had two results, concordance between the tests was high. One participant had the D67N, K70R, M184V, and K219Q mutations in RT in both reports and had similar polymorphisms in both RT and IN. The other participant had M184V in RT and M50I in IN in both reports and had similar polymorphisms. For the other three participants with multiple sample aliquots tested, results varied. One participant had two results, with three-class resistance in one (M184V, K101P/Q/T, Y181C, and H221Y in RT; Q148H and G140S in IN) and no resistance in the other. The other two participants each had three results, with M184V/I detected in only one of the three. For one of these participants, M184I was reported once and other mutations in RT and IN were seen consistently in all three reports. For the other participant, M184V and other mutations were reported in one of three results.

Conclusions: In this study looking at the reproducibility of HIV proviral DNA genotyping, test results of separate aliquots of the same blood draw varied. These results provide only a small snapshot of the total viral reservoir, and results are limited by sample size and can only report the HIV resistance mutants that are currently circulating at high frequency. The implication of these findings is that absence of resistance by a single test cannot convincingly be interpreted as lack of resistance in the archived reservoir.

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Virological efficacy and resistance evaluation in people living with HIV starting an STR regimen

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Background: Despite the increasing use of single tablet regimens (STRs), few data from real life are available regarding potential impact of pre-existent drug resistance to each single drug. The study aimed to evaluate the impact of resistance in people living with HIV who start an STR regimen and the emergence of resistance at virological failure (VF).

Methods: We selected from the ARCA database treatment-naïve (G1), treatment-experienced aviremic (G2) and viremic (G3) HIV-1 infected individuals with at least one resistance profile available before the start of the STR regimen. Cumulative resistance associated mutations (RAMs) before STR start and cumulative STR genotypic susceptibility score (cGSS=3: full susceptibility; <3: reduced susceptibility to at least one drug) were evaluated according to the Stanford algorithm (HIVdb version 8.9-1). The impact on VF of pre-treatment resistance to at least one component included in the following STRs was evaluated: EFV/FTC/TDF, FTC/RPV/TDF, FTC/RPV/TAF, EVG/COBI/FTC/TDF, EVG/COBI/FTC/TAF, ABC/DTG/3TC. Survival analysis (Kaplan-Meier curves and Cox regression) was used to assess the probability and predictors of VF. In patients who failed STR and for whom a genotypic resistance test at VF was available, the emergence of RAMs was also evaluated. McNemar's test was used to compare RAMs frequency detected before and after STR failure.

Results: Overall, 3916 individuals (73.1% males; median [IQR] age: 44 [36-52] years) were included, of whom 678 (17.3%) in G1, 2309 (59.0%) in G2, and 929 (23.7%) in G3. Viral subtype was reported B in 2926 (74.7%) patients. Median (IQR) zenith viral load (VL) was 5.0 (4.3-5.5) log10 copies/mL. Before STR start, RAMs were present in 82 (12.1%) individuals in G1, 580 (25.1%) individuals in G2 and 279 (30.0%) individuals in G3. Median cGSS was 3 (IQR 3-3) in G1, 3 (IQR 2.5-3) in G2, 3 (IQR 2.5-3) in G3. Considering virological efficacy, overall, by one year of STR treatment, VF probability was 3.1% in G1, 5.2% in G2 and 12.8% in G3. Stratifying according to cGSS, VF probability was higher in patients with cGSS<3 than in those with cGSS=3 both in G2 (respectively 6.4% and 4.7%, p=0.031) and in G3 (respectively 20.3% and 11%, p<0.001), without significant differences in G1. Adjusting for confounder factors, at multivariable analyses a higher risk of VF was predicted by higher zenith VL (per 1 log increase; aHR [95%CI] 1.47 [1.01-1.50], p=0.047) in G1; by female gender (vs. male; 1.58 [1.14-2.20], p=0.007), higher zenith VL (1.30 [1.12-1.50], p<0.001), previous use of PI (per 1 drug increase; 1.70 [1.18-2.45], p=0.004) and a longer ART (per 1 year increase; 1.04 [1.01-1.08], p=0.014) in G2; by previous use of PI (2.31 [1.26-4.22], p=0.007) in G3. Conversely, a lower risk of VF was predicted by higher CD4 count nadir (per 100 cells increase; 0.84 [0.73-0.97], p=0.015) and previous use of NNRTI (0.61 [0.38-0.96] p=0.034) in G3. Among individuals who failed STR, only in those in G3 exposed to EFV/FTC/TDF a significant selection of RAMs was observed: K103N (pre-STR=15.7%, post-STR=56.9%, p<0.001) and M184V (pre-STR=19.6%, post-STR=47.1%, p<0.001).

Conclusions: Despite high virological efficacy of STR regimens, higher VL and the reduced predicted susceptibility score to at least 1 drug seems to influence virological efficacy of STR. In treatment-experienced viremic individuals who started EFV/FTC/TDF, an increment of K103N and M184V mutations was observed. Conversely, no significant increment of mutations was observed in naïve and treatment experienced aviremic individuals.

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Resistant minority variants observed in failing patients at week 48 in ANRS 170 QUATUOR trial (a 4/7 days maintenance strategy vs a 7/7 days regimen in patients with controlled viral load)

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Background: ANRS 170 QUATUOR study demonstrated the non-inferiority of a 4/7 days maintenance strategy vs a 7/7 days regimen in patients with controlled viral load (VL) under triple therapy with either PI, NNRTI, or InSTI based regimen at week 48 (W48). As the traditional Sanger sequencing may underestimate the prevalence of ARV-resistant variants, this study therefore aims to determine minority and majority drug resistant variants in failing patients.

Methods: Resistance was retrospectively evaluated in virological failures which were 6 versus 4 in the 4/7 and the 7/7 days arm respectively, at failure in RNA samples and at baseline in DNA samples. Sanger and ultra-deep sequencing (UDS) were performed in reverse transcriptase (RT), protease and integrase (INT) regions according to the Agence Nationale de recherche sur le SIDA et les hepatites virales (ANRS) consensus and using Illumina technology (Illumina, San Diego, CA, USA). The sequence reads were analyzed with IDNSVR software v3_8_0r4 (VCSmartGene), and resistance was interpreted using the latest ANRS resistance algorithm. For UDS, the minimum coverage was set at 50 and the ambiguity filter at 2%.

Results: At baseline, no drug resistance mutation (DRM) was detected in DNA with Sanger sequencing; but with UDS, 2/6 patients in the 4/7 days arm harbored DRM (one T215S (98.5%) implicated in resistance to TDF/TAF, and one Y188L (22.5%) responsible of resistance to RPV); moreover, 3 patients presented viruses with Apobec mutations. At failure in the 4/7 days arm, 3/6 patients presented resistance to at least one drug of their current treatment by RNA Sanger sequencing 2 patients with TDF/FTC and RPV DRM (RT:M184I, E138K, Y188L and M184V, E138K, V179I, H221Y) and 1 patient with ABC/3TC and RAL DRM (RT: M184I; INT: N155H); UDS revealed 2 more patients with resistant variants: one resistant to TAF/FTC and EVG/c (RT: K65R(98,1%), M184V(10%), INT: Q148K (2%)) and one resistant to TAF (RT: M41L (3.7%), D67N (5.7%), T69N (5.6%), T215S (99.4%)). At failure in the 7/7 days arm, only 1/4 patient presented a virus resistant to RPV (K101E) using Sanger sequencing, and UDS revealed 3 more patients presenting resistant variants to at least one drug of their current treatment: one resistant to TDF/FTC and RPV (RT:M184I (3.9%), L210W(3.2%), T215Y (3.1%), F227C (3%)), one resistant to RPV (RT: K101E (4.5%)), and one resistant to DTG-QD (INT: G140A (9.9%)).

Conclusions: Resistance mutations related to current antiretroviral treatment were detected more frequently by UDS. Among these 10 patients with virological failure, 2 presented resistant minority variants at baseline which could have favored virological failure. At failure, UDS method revealed 5 more patients presenting resistant variants to their current treatment, especially for drugs with low genetic barrier to resistance. Emerging resistance at failure was equivalent in the 2 arms, detected by Sanger in 3/6 in the 4/7 days arm versus 1/4 in the 7/7 days arm, and 5/6 versus 4/4 respectively with UDS.

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Molecular characterization in HCV late relapser patients treated with direct-acting antiviral (DAA) drugs

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Background: Sustained virologic response (SVR) is defined as aviremia 24 weeks after end of treatment (EOT) with direct-acting antiviral drugs (DAAs) for HCV infection. The introduction of DAA therapy increased the possibility of HCV elimination. The success of this treatment overcome 95%, however in some patients HCVRNA rebound has been observed after 24 week (late relapse or recurrence phenomenon). This issue underlines the relevance to assess the optimal follow-up after EOT to establish the true treatment efficacy.

In this study, we present preliminary results of Sanger and NGS analysis used to distinguish between late relapser from reinfection in patients showing HCVRNA rebound after > 24 weeks following EOT.

Materials and Methods: NS5B region was amplified by using nested PCR. Sanger analysis was performed on HCVRNA samples collected before the beginning of treatment (T0) and after HCVRNA rebound (T1) of 5 late relapsers (Pt1-Pt5) and 9 relapsers (Pt6-Pt14).

Pangenotypic primers were specifically designed to amplify a NS5B fragment of suitable length for NGS. Libraries were prepared using the Ion 520 & Ion 530 ExT Kit (Life Technologies). The chemistry of this new kit allows to obtain sequences up to 600 bp long. NGS was performed using Ion-Torrent S5 platform. Pollux software was adopted to correct for erroneous substitutions, while insertion and deletions were manually adjusted after global alignment. Within samples, genetic diversity were calculated by p-distance and Wilcoxon signed-rank test was adopted to determine whether two samples at T0 and T1 from the same patient belonged to the same viral population.

Results: No RAS was observed at T0 and T1 in the late relapsers.

Sanger phylogenetic tree showed that viral sequences present at T0 and at T1 segregate in different clusters with significant bootstrap values in two patients: Pt5 and Pt3. Pt5 presented a HCV GT1a at T0. He was treated with Glecaprevir/Pibrentasvir. He achieved SVR, but he relapsed after about one year with a strain of GT1b. Since Gt1b was not found at T0, HCV viremia rebound was considered as a highly suggestive of a reinfection.

Pt3 was an HCV genotype (GT)1a/HIV coinfected. He was treated with Sofosbuvir/Lepidasvir. During the follow-up, Pt3 showed a rebound with a different strain of HCV GT1a (T1) and a second rebound with a similar strain of HCV genotype 1a (T2), in fact sequences corresponding to T1 and T2 doesn't segregate in phylogenetic tree. However, p-distance between T0 and T1, calculated on Sanger sequences, was 0.05458 and did not markedly differ from p-distance observed in two early recurrents Pt11 (0.01965) and Pt12 (0.01747). NGS analysis was thus performed on Pt3 at T0 and T1 in order to corroborate the hypothesis of a putative reinfection. Mean genetic distance between T0 and T1 was 0.06437, and it was found significantly greater (p-value<0.001) than intra-sample diversities, suggesting a reinfections.

Conclusions: These results show that late relapse phenomenon could be linked both reinfection and reactivation of host HCV strain after a "transient SVR". Moreover, NGS provides a sensitive and cost-effective platform for discovery/confirmation of reinfection in late relapser cases.

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Clinical and virological characteristics and retreatment of patients with chronic hepatitis C virus infection and failure to sofosbuvir, velapatasvir, and voxilaprevir in real-world

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Background & Aims: Data on the characteristics of patients with chronic HCV infection who failed VOX/VEL/SOF rescue treatment and possible re-treatment options for these patients are limited.

Methods: Samples of 40 patients with HCV genotypes (GT) 1-4 who failed to VOX/VEL/as retreatment were collected within the European Resistance study group. Population-based NS3, NS5A and NS5B resistance analyses were conducted and clinical parameters and retreatment efficacies were evaluated retrospectively.

Results: The majority of VOX/VEL/SOF failure patients were infected with HCV GT3 (n=19, 45%) or GT1a (n=11, 28%) and had a cirrhosis (n=28, 70%). Previous treatments included NS3-inhibitor (30%), NS5A-inhibitor (100%) and SOF (85%). Baseline RASs available in a subgroup of patients before VOX/VEL/SOF (>70%) included rarely NS3 RAS with exception of Q80K in GT1a (40%), typical NS5A RASs pattern in the majority of patients (80%) and no S282T in NS5B. Sequencing after VOX/VEL/SOF failure available in >95% of patients showed only minor changes for NS3 and NS5A RASs. In 16 patients rescue treatment was initiated with glecaprevir, pibrentasvir alone (n=1) or with sofosbuvir +/- ribavirin (n=11), VOX/VEL/SOF +/- ribavirin (n=3) or VEL/SOF and ribavirin (n=1) for 12 to 24 weeks. Sustained virologic response was achieved in 6/9 (67%) patients with final treatment outcome. Two patients with GT3a had virologic failure after VEL/SOF and G/P+SOF+R, respectively, and one patient with decompensated cirrhosis died while on therapy.

Conclusion: VOX/VEL/SOF failure was mainly observed in GT3 and GT1a infected patients with cirrhosis and was not associated with specific RASs pattern within NS3, NS5A or NS5B target regions. Rescue treatment with multiple targeted therapies was effective in the majority of patients.

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Pharmaco-virological algorithm to target risk of resistance among a population of HIV-infected female sex workers in Togo

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Background: The first epidemiologic survey among the key population of female sex workers (FSW) in Togo was led in 2017 showing an HIV prevalence of 13.1%. No data about ARV treatment and virological control were collected due to recruitment in hot-spots. The aim of this study was to explore therapeutic and virological status using an algorithm combining pharmacology and HIV viral load to identify FSW at risk to harbour drug-resistant HIV.

Patients and Methods: This cross-sectional study was conducted in 10 sites in Togo. Viral load (VL) was determined using COBAS®HIV-1 (Roche). Plasma ARV concentrations (Cp1) were determined by UPLC-MS/MS (Waters Xevo). Genotypic resistance tests of protease and RT were performed according to the procedures described at www.hivfrenchresistance.org and interpreted using the ANRS algorithm.

Results: A total of 123 HIV FSW with median age 32 years (IQR=28-38) were included and their median number of clients within the 7 days preceding recruitment was 5 (IQR=3-10). Prevalence of HBV and HCV co-infections was 9.2% and 3.4%, respectively. Plasma VL was <100 c/mL in 44 (36%). In case of VL >100 c/mL (n=79), median VL was 4.7 log10 c/mL (IQR=4.4-4.9). Plasma ARV concentrations measurement enabled to show that 60 FSW (48.8%) were receiving ARV including 58 with TDF/3TC/EFV, one with TDF/3TC/NVP and one with TDF/3TC/LPV/r. Among them, 51 FSW showed adequate Cp1, all but one receiving EFV-based regimen (median=3075 ng/mL, IQR=1638-5385). Fifteen (29%) had a VL >100 c/mL (median=836 c/mL, IQR=326-3450) suggesting resistance. Regarding the 9 FSW with suboptimal Cp1, 4 had positive VL also suggesting resistance. Sixty of the 63 FSW (95%) with Cp1 below limit of quantification displayed VL >100 c/mL (median=46200 c/mL, IQR=10515-142500). Out of the 19 samples eligible to resistance testing, 12 genotypes were successfully carried out exhibiting at least one drug resistance mutation in 7. Resistance interpretation brought out one profile still susceptible to all three ARV of the regimen, one resistant to EFV, two resistant to both 3TC and EFV, and two resistant to all regimen drugs. NRTI M184V (n=3) and NNRTI K103N (n=4) mutations were the most frequently present. Hypothesizing that only the seven resistant samples would have been brought out by resistance tests, eligibility to resistance test defined only on VL would have led to 9% tests revealing ARV resistance while the additional data of plasma ARV concentration enabled to enhance resistance testing efficacy to 37% of tests revealing ARV resistance.

Conclusion: In limited-resource countries, HIV biological monitoring is a crucial issue while treatment access is increasing. In these settings, VL availability is still very limited and has to be generalized while genotyping resistance tests are currently not feasible. To get around this hindrance, pharmacology may be a decision tool to sort out patients harboring resistant virus in need to ARV switch. It also enables to narrow down the number of samples eligible to resistance testing. In addition, pharmacological tests are cheap and can be carried out on dried blood spots which also enable resistance tests. In the present study this algorithm allowed to target resistance testing from 79 to 19 FSW at risk of HIV drug resistance.

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Characterization of the NS5A gene of HCV genotype 4 isolates from HIV-1 co-infected patients in a third-level hospital in Mexico

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Background: Other than access to treatment, the success of direct antiviral agents (DAA's) against HCV infection has been limited by low adherence, HCV re-infection and rare HCV genotypes for the most part. As for today, it is known that HCV genotype 1b is responsible for around 700,000 infections in Mexico. However, the data might not be comprehensive and the information for HCV genotype 4 is very limited. The aim of this study was to identify HCV genotype 4 strains from Mexican isolates and characterize the NS5A gene.

Material and Methods: We identified 12 viral isolates from HIV-1 positive patients with diagnosis of HCV genotype 4 co-infection from our samples repository. The HCV viral load and genotype were performed by a commercial kit (Abbott RealTime HCV viral load and genotype). NS5A gene characterization was performed with an in-house RT-PCR assay with a second nested-PCR amplification step and Sanger sequencing. The analysis was performed with SeqScape v2.5 and MEGA X software, and the antiviral susceptibility analysis with the GLUE HCV algorithm 1.1.50.

Results: The mean plasma HCV viral load was 1,625,793 UI/mL (n=11, range 31,926-6,782,489 UI/mL). Interestingly, the GLUE analysis shown that all 12 strains were related to the 4d subtype, and all closely related to two strains (EU392172 and DQ418786). The occurrence of resistance-associated substitutions (RAS) in the NS5A gene was practically absent in all samples. Nine isolates harbored the P/T58T substitution, a probable signature of circulating strains, which has been associated with low resistance to elbasvir and three samples show P/T58P associated with probable resistance to elbasvir and ombitasvir. Given that all samples had HIV-1 undetectable viral load, we were not able to compare the relation between HIV strains and their association with the HCV strains.

Conclusions: This is the first report of the characterization of NS5A gene from HCV genotype 4 isolates in Mexico. We found a close genetic relationship between all our HCV isolates according to GLUE HCV and MEGA algorithm and these were all practically susceptible to all DAA's. The characterization of HCV clusters could provide valuable epidemiological information for treatment and prevention strategies in our country. It will be interesting to further investigate the association with the HIV-1 strains using sensitive assays or/and proviral DNA.

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Impact of Sexually Transmitted Infections on seminal HIV levels among undetectable patients

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Background: Sexually transmitted infections (STIs) are known to increase the HIV shedding in ART naïve patients' seminal fluid. Their role in influencing the seminal compartment HIV-RNA levels despite peripheral undetectability is still unclear.

Materials and Methods: 30 HIV-1+ undetectable (HIV-RNA<20 cps/mL) patients (pts) were included in this ongoing study. Among these, 15 were STI-positive (cases: 10 syphilis, 1 M.genitalium, 1 U.urealyticum, 1 N. gonorrhoeae, 1 C. trachomatis/M.genitalium urethritis, 1 syphilis/C. trachomatis/U.urealyticum co-infection), while 15 were STI-negative (controls).

Total HIV-DNA and residual viremia (detection limit 32 cps/10^6CD4+cells and 2 cps/mL, respectively) were quantified in both blood and seminal compartments by home-made protocols using droplet digital PCR (ddPCR). Blood and semen specimens from cases were collected at enrolment and after STI treatment.

Results: Pts were mainly Men having sex with men (MSM, 83.4%) and infected by HIV-1 B subtype (73.3%), with a median (Interquartile-range, IQR) age of 35 (30-45) years. At enrolment, median (IQR) CD4+ and CD8+ counts were 730 (578-992) and 770 (618-941) cells/mm^3, respectively.

24 pts were on successful combined triple NRTI-based regimen (3rd drug: 14 INSTI; 6 NNRTI; 4 PI), while 6 pts were on a dual regimen: 3DRV/c+3TC, 1DRV/r+RAL, 1ETR+RAL, 1DTG+3TC.

At enrolment, total peripheral HIV-DNA was detectable in 24/30 pts (80%), with a median (IQR) value of 614 (163-1851) cps/10^6CD4+cells. Instead, seminal total HIV-DNA was detectable only in 3/30 pts (10%) 1 case and 2 controls, always with values lower than 32cps/10^6CD4+cells.

Peripheral and seminal residual HIV-RNA levels were detectable in 19/30 (63%) and 18/30 (60%) pts, with median (IQR) values of 2.8 (<2.0-4.4) and 5.4 (2.3-9.3) cps/mL, respectively.

In both compartments, residual HIV-RNA never exceeded 20 cps/mL with the exception of 2 patients. The former was a STI-free control (congenital HIV infection) who had 39.0 cps/mL in the seminal compartment and 10.0 cps/mL in plasma. The latter had a M.genitalium/C. trachomatis urethritis and his residual HIV-RNA levels were 55.8 cps/mL (semen) and 9.0 cps/mL (plasma).

No differences were found when HIV-DNA and -RNA values in both compartments were compared between cases and controls (P>0.30).

However, 7 out of 30 pts (23.3%) showed a seminal HIV-RNA positivity despite the peripheral HIV-RNA undetectability. This discordance was more frequently observed in cases (33.3%) respect to controls (13.3%) (P=0.195).

Finally, for 9 STI cases a follow-up after antibiotic treatment was available. Among these, 4 (44.4%) maintained undetectable seminal HIV-RNA, 4 (44.4%) showed a reduction (-3.4, -2.3, -12.2, and 55.8) and only one (11.1%) experienced a seminal HIV-RNA increase to 12.1cps/ml.

On note, when we compared residual HIV-RNA levels from one of the STI-positive cases (N.gonorrhoeae infection) with previous collected samples of plasma and semen (both undetectable), a slight increase of 3.6 and 9.0 cps/mL respectively was observed in the contest of the urethritis.

Conclusions: These preliminary data show that successful combined antiretroviral treatment (3DR or 2DR) avoids the presence of HIV-DNA in the seminal cells in the majority of pts, maintaining HIV-RNA in seminal compartment at non-relevant levels, despite STI and independently by drug regimen.

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Bone mineral density in patients with HIV infection and chronic hepatitis C

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Background: Today the HIV infection is considered chronic disease which is under effective control. In focus are acquired coinfections, non AIDS related conditions, prolonged antiretroviral therapy and its complications. Osteoporosis is a systemic skeletal disorder characterized by low bone mass. Osteopenia (BMD <1 standard deviations of the mean BMD of a sex-matched, young healthy population below normal BMD, i.e. T-score –1 to –2.5) often precedes osteoporosis (i.e. T-score <–2.5), predisposing affected patients to fracture. Low BMD is a recognized complication of HIV infection, use of ART or HIV/HCV co-infection.

Due to shared transmission routes, co-infections with HIV and HCV are very common. Multiple cross-sectional studies have reported an association between chronic HCV infections, reduced BMD, and increased risk of fracture. HCV co-infection, compared to HIV mono-infected patients, significantly increases the risk of fractures at many sites. Thus, the risk of fracture in co-infected patients may be greater than that of HIV or HCV mono-infected patients. It is suspected synergistic effects of co-infection, including increased inflammation.

Therefore, the objective of this study is to estimate the association of HIV/HCV co-infection on adverse skeletal outcomes (low BMD). We have compared HIV+HCV co-infected and HIV+ patients on ART. The causes of low BMD in HIV-infected patients are multifactorial and constitute a complex of interactions. Current points of interest are the persistent immune activation, chronic inflammation and adverse effects of ART. Renal tubular toxicity has also been reported with ART and may increase the risk of bone loss.

Materials and Methods: In the current study we included 26 HIV+HCV co-infected and 42 HIV+ monoinfected patients currently on treatment in the outpatient department for treating HIV infection in Hospital for Infectious and Parasitic Diseases „Prof. Ivan Kirov“. Description of baseline parameters: age, sex, CD4 count, HIV1+ RNA viral load. We analysed deviations in laboratory tests, total protein, albumin, erythrocyte sedimentation rate (ESR), cystatin C, and bone mineral density in all patients. HCV RNA and genotype were assessed in HIV+HCV co-infected patients. Bone mineral density (BMD) was measured by dual-energy X-ray

Results: HIV/HCV-co-infected patients had lower BMI and BMD hip T- and Z-scores ($p = 0.036$ and $p = 0.025$) compared to HIV+ monoinfected patients. Also we found that HIV/HCV-co-infected patients had higher ESR, TP and cystatin C compared to HIV+ monoinfected patients ($p<0.05$).

Conclusions: HIV/HCV co-infection is associated with significantly reduced BMD and increased fracture risk. We suppose the potential contribution of chronic inflammation to bone deficits as well as associated metabolic and body composition abnormalities among HIV/HCV co-infected patients.

Keywords: HIV infection, HIV+HCV co-infection, antiretroviral therapy, persistent immune activation, Cystatin C, bone mineral density

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The burden of serious non-AIDS-defining events among admitted cART-naive HIV/AIDS patients in China: An observational cohort study

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Objective. The objective for this study was to elucidate the burden, risk factors and prognosis of serious non-AIDS-defining events (NADEs) among admitted cART-naive HIV/AIDS patients in China.

Methods. The evaluation of the burden, risk factors and prognosis of serious NADEs was carried out among 1309 cART-naive HIV/AIDS patients admitted in Beijing Ditan Hospital between January 2009 to December 2018.

Results. Among 1309 patients, 143 patients (10.9%) had at least one serious NADEs, including 49 (3.8%) with cerebrovascular diseases, 37 (2.8%) with non-AIDS-defining cancers, 28(2.1%) with chronic kidney diseases, 26 (2.0%) with cardiovascular diseases and 18(1.4%) wth liver cirrhosis. Serious NADEs distributed in different age and CD4 levels, especially with age≥50 years and CD4≤350cells/ul. Other traditional risk factors, including exposure to cigarette smoking(OR=1.872, CI=1.271-2.757, p=0.002), hypertension (OR=2.473, CI=1.659-3.684, p<0.001), chronic HCV infection (OR=2.765, CI=1.375-5.562, p=0.004) and hypercholesterolemia (OR=4.084, CI=1.184-14.084, p=0.026), were also associated with serious NADEs, 17 cases (1.3%) with serious NADEs were died among cART-naive HIV/AIDS patients when hospitalized, severe pneumonia (HR=6.322, CI=2.186-18.280, p=0.001) and AIDS-defining cancers (HR=5.562, CI=1.549-19.975, p=0.009) as risk factors were associated with an increased hazard of mortality among these patients with serious NADEs.

Conclusions. Serious NADEs also occurred in cART-naive HIV/AIDS patients in China with low prevalence. Our results reminded physicians that earlier screening serious NADEs, timely intervention of their risk factors and management of severe AIDS-defining events, multi-disciplinary cooperation and earlier initiation of cART was essential to reduce the burden of serious NADEs.

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The prevalence of hepatitis B virus seromarkers in patients with HIV/HCV coinfection. An Egyptian cross-sectional study.

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Background: Because of their overlap in transmission routes, individuals with predisposing risk behaviours are at risk of co-occurrence of HIV, HBV and HCV. This modifies the outcome of each infection alone and leads to significant morbidity. The aim of our study was to estimate the prevalence of HBV serological markers, HBsAg and HBcAb total, among HIV/HCV coinfected patients in Egypt.

Methods: This is a pilot cross-sectional study conducted in Imbaba Fever hospital, Cairo, over the period between November 2018 and May 2019. It included 131 patients with confirmed HIV/HCV co-infection presenting to the HIV/HCV co-infection clinic to start HCV treatment with direct-acting antivirals (DAAs). Demographic, clinical and laboratory data were collected and all patients signed an informed consent to use their data anonymously. Enrolled patients were screened for HBc Ab total and HBs Ag using Enzyme Linked Immunosorbent Assay (ELISA).Chi-square test and Student t- test were used to compare categorical and continuous variables.

Results: A total of 131 samples were analyzed, 89 (67.9 %) were found to have isolated anti-HBc and five (3.8%) with both anti-HBc and HBs Ag. The mean age of the patients was 35 years,95% were males. 86.3%(n=113) gave history of intravenous drug use amongst whom 78(69%) were positive for HBcAb total and four (3.5%)positive for both HBc Ab and HBs Ag. Only 15 individuals reported risky sexual behavior(11.5%), one of them was positive for both tests(6.7%) and 11 had isolated HBcAb seropositivity (73.3%). The mean HCV viral load 4,038,433 copies/ml.The mean CD4 T-cell count was 467.38/mm , the mean HIV viral load 94,560.46 copies/ml, the mean time since HIV diagnosis was 1.5 years and 49.6%(n=65) were receiving antiretroviral therapy. There was no significant association between any of these clinical , virological/immunolgical varibles and the studied HBV serological markers.

Conclusions and Recommendations: The prevalence of Isolated HBcAb among this subgroup is high. The presence of which may denote occult HBV infection and risk of HBV reactivation. HBV infection is particularly relevant in the HIV/HCV co infection group and we recommend screening for HbcAb total alongside other HBV markers as a routine.

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Seroprevalence and co-infections HIV, HBV and HCV among clinic attenders at Laquintinie Hospital in Douala, Cameroon

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Background: In Cameroon, the new national AIDS-control-strategy ''test and treat" apart from lymphocytes TCD4 rate to achieve 90-90-90'' target prompt the systematic screening and the early management of HIV. However, HIV, HBV, and HBC share the same routes of transmissions increasing the risk of co-infection and of severe liver damages. This study was undertaken to evaluate the prevalence of the co-infection HIV/HBV/HCV among subjects at Laquintinie Hospital in Douala, Cameroon.

Methodology: A cross-sectional study was held from October, 2017 to March 2018 at Laquintinie Hospital in Douala. Rapid diagnosis tests (RDTs) for the detection of HIV antibodies 1,2,0 (immunochrotography) and ELISA for HBs Antigen and HCV antibodies were performed on each ignorant participant and HIV positives cases were confirmed with oral Quick as recommended by the National AIDS-control-Committee in 2016. Data's analysis were performed using Epi info 7.0. P value <0.05 was considered as statistically significant.

Results : Out of 247 patients enrolled, there were 51.5% of women and the mean age among participants was 42.3(2 years old. The seroprevalences were 10.1%, 7.7%, and 4% respectively for HIV, HBV and HCV. The co-infection rates were 1.2%, 2% and 1.6% respectively for dual HIV/HBV, HIV/HCV and HBV/HCV. Women seemed to be more HIV infected (12.5%) than men (7.5%, p=0.28). Most co-infections were encountered in women. Subjects aged between 45 and 60 years old were more likely to be positives either for HIV (23.6%), HBV (12.1%), and HCV (7.8%) or for co-infection HIV/HBV (7.8%) and HIV/HCV (7.8%).

Conclusion: Regarding the prevalence of HIV and viral hepatitis infections, prevention measures should be intensified in Douala.

Key words : Seroprevalence, co-infection, HIV, viral hepatitis, Douala.

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HIV-DNA from rectal GALT is a valid marker to explore the viral latency

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Background: The extension of HIV reservoir in the gut associated lymphoid tissue (GALT) and the ART influence on this compartment are still poorly studied. Aim of our study is to evaluate the extent of HIV-DNA in undetectable pts' rectal GALT.

Methods: 26 HIV1+ pts on ART were included in this ongoing study. Among these, 25 were undetectable (HIV-RNA <50 cp/ml), 1 had 54 cp/ml. Blood and GALT HIV-DNA levels and residual viremia were quantified by using digital droplet-PCR (detection limit: 32cp/106CD4+ for HIV-DNA and 2 cp/ml for residual RNA). Rectal biopsies were collected during High Resolution Anoscopy, subjected to collagenase digestion, mechanical disruption and filtering through cell strainer. Washed cells were analysed to characterize the lymphocyte subsets and activation markers by flow cytometry.

Results: Pts median age (IQR) was 37 (32-47), 24 were males, 1 female, 1 MtoF transexual. 61.6% had B subtype. Median (IQR) CD4 count and viremia at diagnosis were 463 (310-625) cells/µL and 124527 (28606-522298) cp/ml, respectively. Instead, at the enrolment, median (IQR) CD4 count was 679 (552-1070) cells/µL. All pts were under NRTI-based regimen (3rd drug: 14 INSTI, 8 NNRTI, 4 PI). Median (IQR) time of undetectability was 36 (30-57) months.

Median (IQR) residual viremia was 5 (2-11) cp/ml. Although without statistical significance, HIV-DNA levels in GALT were higher than peripheral ones (median [IQR]: 736 [237-1620] vs 539 [137-927] cp/106CD4. p=0.527) (table 1). Moreover, lower median (IQR) levels of HIV-DNA were observed in GALT among those on INSTI-based regimen when compared with non-INSTI (522 [0-1590] vs 930[468-1538] cp/106CD4. p=0.462) as well as in peripheral compartment (163[29-2409] vs 688[444-840] cp/106CD4. p=0.297).

Focusing on GALT HIV-DNA, a positive correlation emerged with peripheral HIV-DNA (Rho= 0.685; p <0.001), residual viremia (Rho=0.425; p=0.030), pre-ART viremia (Rho=0.563; p=0.023), pre-ART peripheral HIV-DNA (9 samples available; Rho=0.783; p=0.013), time (weeks) necessary to achieve the undetectability (Rho 0.439; p=0.068). Moreover, the immunophenotype analysis showed that the percentage of CD19+CD38+ subpopulation from GALT negatively correlated with both GALT HIV-DNA (although not significantly, Rho= -0.448; p =0.108) and peripheral HIV-DNA (Rho= -0.587; p <0.027). 2 pts had undetectable HIV-DNA levels in both blood and GALT compartments. These were on INSTI-based treatment and interestingly, the HIV-DNA in their seminal fluid was undetectable too. The former had started ART during acute infection and his residual viremia was 5cp/ml. The latter had undergone allogeneic bone marrow transplant and his residual viremia was completely undetectable.

Conclusions: These preliminary results show that HIV-DNA from rectal GALT is a valid marker to characterize the viral reservoir. Further details on a larger cohort are needed to assess possible correlations with drugs regimens, viral latency and mucosal immunoactivation status

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The efficacy of first-line therapy based on RPV in HIV-infected patients with pre-existing E138A mutation in reverse transcriptase

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Background. The variant of HIV-1, sub-subtype A6, that caused the first epidemic in Russia and accounts for more than 70% of HIV-infection cases in Russia now, has a typical polymorphic mutation E138A in reverse transcriptase. This mutation in A6 viruses is present in 4-8% of ART-naive patients, depending on the geographic region, and associated with the resistance to rilpivirine (RPV) and etravirine (ETR). RPV, a second-generation non-nucleoside inhibitor (NNRTI), with confirmed efficacy, safety and tolerability, is currently approved for the treatment of HIV-infection in first-line antiretroviral therapy and included in perspective dual therapy scheme (DTG/RPV) in European guidelines. At present, the HIV treatment coverage in Russia is increasing. In 2018 the Russian generic form of RPV (lakonivir) was registered in Russia (№LP-004807 of April 19, 2018). These give us the reasons to predict possible widespread use of RPV in the near future in Russia. The HIV genotyping at baseline is not recommended in Russia, and that is why the clinical significance of the singleton pre-existing E138A mutation in ART-naive patients should be clarified.

Materials and methods. We asked the international EuResist network (https://www.euresist.org/) for data that met the following criteria: a) age over 18 years, b) confirmed as HIV-infected, c) received RPV as part of the first-line ART regimen, d) maintained it for more than 40 weeks, e) had E138A mutation at baseline, and f) did not have major mutations to NRTI. There were 11 participants fulfilling these criteria. Patients were observed in Italian and Swedish centers, being EuResist partners. The follow-up periods and frequency of viral load (VL) measuring varied from 78 to 209 weeks and from 5 to 14 times, respectively. All patients were infected with non-A6 HIV-1 subtypes viruses. The virologic outcome was analyzed according to Russian guidelines, and the criteria of virologic failure were based on European and DHHS clinical guidelines.

Results. Virologic efficacy of first-line ART regimen based on RPV in HIV-infected patients with pre-existed E138A mutation in reverse transcriptase was demonstrated for all patients, according to both Russian and European and DHHS guidelines.

Conclusion. The study has a number of limitations: very small number of patients was observed, with different observation periods; the measuring of VL was performed with different equipment and test-systems; the measuring of VL and patient examination were irregular; the patients were infected with different non-A6 HIV-1 subtypes viruses. Nevertheless, the data obtained give reason to believe that the effect of a pre-existing E138A mutation on the RPV-based regimens virologic efficacy is negligible.

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A Joinpoint Regression Analysis of Trends in HIV Incidence in Balkan Countries in 15 Years Period

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Background: In Europe, 141.552 new cases of Human immunodeficiency virus (HIV) infection was registered in 2018, with an incidence rate of 16.2/100.000 inhabitants. The Central European surveillance region, in which the majority of Balkan countries are located, is considered as low incidence region of 3.3/100.000 inhabitants. So far, several molecular studies and surveillance reports in the region concluded that the epidemic is compartmentalized into several key populations such as men who have sex with men (MSM) or people who inject drugs (PWID).

The Aim: Our study aimed to analyze trends of HIV incidence in the adult population and key populations in Balkan countries.

Materials and Methods: Epidemiological data and basic demographic data for the period 2004 to 2018 from Serbia, Croatia, Slovenia, Romania, Greece and Bulgaria were obtained from the ECDC HIV/AIDS surveillance reports as well as annual statistical yearbooks. The population of interest was adult population age 15-65 for which age-specific incidence rates were calculated. Also, transmission specific incidences were calculated based on key population size estimates, provided in the literature and UNAIDS reports. Time trends of HIV incidence in the adult population and key populations were assessed using annual percentage change (APC), estimated by Joinpoint Trend Analysis Software.

Results: HIV incidences in Serbia and Croatia show a constant increase with APCs of 5.74 and 5.53 respectively. In Serbia, a modest reduction in HIV incidence was seen in PWID with APC of -1.95. The APCs for MSM and heterosexual key populations were 9.13 and 1.79 respectively, showing that in Serbia MSM population is the most affected.

In Croatia the highest APC of 9.28 is seen in MSM as well, but with a significant decrease of incidence in heterosexuals and PWID with APCs of around -5.5.

In Slovenia, in the last two years, a trend of reduction of HIV incidence is observed in the general population and three main key populations (MSM, heterosexuals and PWID) with APCs of -21, probably reflecting effective harm reduction programs, implementation of PrEP and compliance to ECDC testing guidelines as well as prompt therapy initiation and viral suppression.

In Romania, there is a reduction in HIV incidence since 2012 with APC of -6.16 mainly due to a reduction of incidence in PWID after the outbreak detected in the mid-'00s. In MSM and heterosexuals, there is a reduction in APC since 2010 from 23 to 5.14 and 52 to 7.4 respectively.

In Bulgaria, there is an increase in HIV incidence (APC 9) mainly due to MSM and heterosexual transmission with APCs of 21.84 and 5 respectively. In PWID since 2009 APC has been -9.

In Greece, little change was observed in heterosexuals with APC of -0.05. In the last three years there is a reduction in MSM transmissions with APC of -14 and in PWID transmissions since the year 2012 (APC -28.29) mirroring successful fight against HIV outbreak among PWID in Greece.

Conclusion: The results of this study imply very dynamic and heterogeneous epidemic trends in the studied populations. Preventive measures tailored to the specific needs of an HIV epidemic, aimed at dominant key populations such as PrEP for MSM, harm reduction programs for PWID and good linkage to care as well as good HIV test coverage, safe sex promotion and prompt therapy initiation are needed to successfully reduce HIV incidence.

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Using next-generation sequencing to understand the phylogenetics and networks of generalised HIV epidemics in Africa

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HIV transmission prevention methods, such as antiretroviral (ART) therapies are becoming increasingly available worldwide. ART is a particularly effective way to prevent the spread of HIV amongst populations, however, there are issues with determining how best to deploy them and how to evaluate their impact. Previous targeted interventions strategies such as the Treatment as Prevention (TasP) trial, were inconclusive in determining what contribution to the HIV epidemic was had. The role of acute and early infection is still disputed, particularly for heterosexual epidemics, and there is an increasing realisation that movement and migration of people between areas, may be an important epidemic driver.

The HIV epidemic is described as a model of connected sources, sinks, and hubs, whereby sources are groups in a population who disproportionately infections; sinks disproportionately receive infections and; hubs act as both sources and sinks. These populations are often defined according to age, gender, riskiness of sexual behaviour, geography, occupation, culture and migratory behaviour. The PANGEA (Phylogenetics and Networks of Generalised HIV Epidemics in Africa) consortium seeks to use phylogenetic methods in association with next-generation sequencing, to determine transmission pathways and assess potentially transmitted and acquired ART resistance.

The incidence of HIV in many parts of Africa is not falling as fast as expected, despite a dramatic increase of ART coverage with new prevention approaches. One theory to explain this is that with the rapidly expanding use of ART, there has been rapid emergence of HIV drug resistance. Phylogenetic approaches may allow for the discovery groups at high-risk of HIV infection and target them in prevention approaches. Viral phylogenies reveal which patients (of HIV-infected people) are closely related within transmission network. By using phylogenetics as well as epidemiological data, mathematical modelling approaches can be used to identify likely sources of new infections. Phyloscanner is a tool which is used for source attribution of NGS data. Once sources have been identified with quantified uncertainty, epidemiological questions about the characteristics of transmitters can be addressed.

PANGEA-HIV 2 (Phylogenetics And Networks for Generalised Epidemics in Africa) is a collaboration between scientists from the Africa Health Research Institute (South Africa), Rakai Health Sciences Program (Uganda), Johns Hopkins University (USA), Medical Research Council/Uganda Virus Research Institute (Uganda), Zambart Project (Zambia), London School of Hygiene and Tropical Medicine (UK), Imperial College London (UK), Partners in Prevention Project at the University of Washington (USA), Botswana Harvard AIDS Institute Partnership (Botswana/USA), University of Edinburgh (UK), and the University of Oxford (UK).

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Number, type and cost of microbiological assays during HIV Pre-Exposure Prophylaxis: the experience of a French hospital

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Aim: regular microbiological tests are required for patients on HIV Pre-Exposure Prophylaxis (PrEP), but their number, type, and cost in the real-life are poorly described.

Methods: number, type, and results of microbiological tests performed in Besançon Hospital-associated laboratory, France, from 2016 to 2019, in the setting of PrEP consultations were retrospectively collected. Costs were estimated by the current reimbursement rate set by the French national protection system.

Results: 756 consultations for PrEP initiation or follow-up in 135 patients were performed over 4 years. The global cost of microbiological tests was 45983.2 euros, corresponding to a mean cost of 60.8 euros per consultation. Virological assays accounted for 31.5% (n=1083) of the 3434 microbiological tests and for 37.7% of their total cost (17334.8 euros). Serology was predominant in virology (98% of virological tests), with HIV, HCV, and HBV screening as the 3 more frequent assays, whereas molecular biology was predominant in bacteriology (63.1% of bacteriological tests) with N. gonorrhoeae and C. trachomatis screening as leader assays. Before PrEP initiation, 48.5% (n=32/66) and 14.3% (n=10/70) of patients had appropriate immunization against HBV and HAV, respectively. No seroconversion was observed for HIV, HCV, and HBV, but one patient was diagnosed with an acute hepatitis A (genotype 1a). By contrast, a bacterial infection due to N. gonorrhoeae, C. trachomatis or T. pallidum was observed in 50.5% (n=54/107) of patients tested for the 3 pathogens. Among 17 HIV RNA testing performed, 8 were carried out in emergency due to symptoms compatible with a primary infection.

Conclusions: since numerous virological tests are required for patients on PrEP, the availability of adapted specific technical platforms should not be neglected by centers wishing to set up PrEP consultations.

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BREAKING BAD: CHEMS, SEX AND SEXUAL HEALTH

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Background: Recurrent use of Chems during sex raises interest and concerns from both a sexual health cares perspective and HIV and STIs prevention. Nowadays there's no epidemiological register of Chems and sex usage from health authorities. Adhara/SevillaCheckpoint started the program Breaking Bad to implement primary prevention strategies complementary to the ones already implemented in Seville and Andalusia by health responsibles.

Materials: After the pilot we set up from 2016-17 in Adhara/SevillaCheckpoint with 496 Chem sex users – mefedrone and tina-, we started in 2018 with the help of a multidisciplinary team a program based in reach out and rescue strategies. During the interviews with the clients we collect data that allows us to identify those in need of an active follow up due to sexual and life habits that put them at higher risk of contracting HIV and other STIs, and act as transmission vectors as they do not know their HIV status. After screening, those clients with addictive behaviors are attended by the psychological team of our centre.

Results: From January 2016 and June 2019, 1738 interventions with Chem Sex users have been done (16% are currently taking PrEP). 9.7% showed problematic behavior with the use of toxics. This collective of Chem Sex users presented 33.1% of new HIV diagnosis (41 of 124), 38.3% of Syphilis reactive tests (46 of 120) 44.8% of Chlamydia infections (43 of 96) and 40.5% of Gonorrheas (32 of 79). We also detected 3 acute HIV infections by PCR in this collective the last year.

Conclusions: The Breaking Bad program implemented by Adhara is consolidated as a source of information that allows to obtain hard conclusions to lead prevention strategies more effectively from public administrations. Furthermore, by applying the algorithms generated with this information, the really early detection of HIV / STIs is facilitated, improving the quality of life of PLHIV and helping to control the transmission chains of HIV and other STIs.

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Using next-generation sequencing to assess the differences in HIV drug resistance pre- and post-treatment in a Treatment as Prevention Trial

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The proportion of people on HIV antiretroviral therapy (ART) in South Africa has doubled since 2010, reducing AIDS-related deaths by 36%. ART has numerous benefits and South Africa are implementing universal test-and-treat strategies, increasing the number of people eligible for ART. However, high ART usage may lead to higher levels of acquired and transmitted drug resistance, compromising ART efficacy. Previous NNRTI-based regimens were highly effective, but the low genetic barrier to resistance has resulted in increasing prevalence of pretreatment drug resistance (PDR). Within the Treatment as Prevention (TasP) trial, the prevalence of PDR was determined, using next-generation sequencing of HIV+ patients who reported no use of ART at the start of the trial. Patients often carry several different HIV quasispecies with different inherent resistance patterns; by investigating the evolutionary change and carriage of DRMs using haplotyping methods, policymakers can be informed on appropriate treatment regimens and target specific groups for interventions.

Plasma samples with viral loads ≥100 copies/mL were sequenced using Illumina MiSeq. NGS reads were used to reconstruct haplotypes by mapping reads to the closest matching reference genome. Haplotypes were then constructed using HaROLD: "Haplotype RecOnstruction using Longitudinal Data". Haplotypes were interrogated using a custom script to detect DRMs using the Stanford HIV database. Phylogenies were constructed across longitudinal timepoints and these were overlayed on maximum-likelihood trees. Coreceptor usage was measured with both geno2pheno and webPSSM and annotate on trees where there was concordance between the two methods.

Haplotype reconstruction provides insights into viral population shifts at whole genome level of 17 patients. Results indicated that selective sweeps during PI failure occurred even in the absence of major PI mutations. In two patients who experienced >1 log fall with high concentrations of PI drug there was a viral population shift consistent with preferential suppression of susceptible viral strains or residual clonal virus coming from proliferating T cells. The Tenofovir mutation K65R was not observed on the same NGS reads as the thymidine analogue mutation (TAM) D67N in a patient with both variants in the quasispecies, which is consistent with antagonism. Coreceptor usage appeared to switch from CCR5 to CXCR4 at consensus level during treatment failure but needs to be further explored.

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Knowledge, attitude and practice survey about blood borne infections among Georgian health care workers

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Background: The prevalence of viral hepatitis B and C in Georgia is among the highest in the region. US Centers for Disease Control and Prevention (CDC) has selected Georgia as a pilot country for hepatitis C elimination program. Since 2015, Georgia launched a multi-year program of HCV elimination, including treatment of infected individuals with modern Direct Acting Antivirals (DAAs) and implementation of prevention programs, including infection control in health care facilities. The objective of this study was to evaluate the attitude and knowledge of blood borne infections (HIV, HCV, HBV) among Georgian Health Care Workers(HCWs).

Methods: HCWs were recruited from ten participating multi-profile hospitals and thirteen dental care institutions in three large cities of Georgia (Batumi (Western Georgia), Rustavi (Eastern Georgia) and the capital city, Tbilisi). A self - administered questionnaire included sections regarding sociodemographic and professional characteristics; awareness of blood-borne infections; practice for transmission risk reduction and perceived educational interventions acceptable among HCWs. The selection of HCWs was done through simple random sampling from the list of staff as a sampling frame. HCW's survey results were compared to the one from Dental health care workers (DHCWs).

Results: The total number of surveyed individuals was 442. Among them, 246 (55.6%) were HCWs (physicians, nurses, physician assistants and residents) from different departments, including family medicine (38.6 %), surgery (21.7%), gynecology (23.4%) and intensive care (13.9%) and 196 DHCWs (44.6%). Only few respondents (15.6%) correctly identified the prevalence of HIV infection in Georgia. HCWs have better understanding about the prevalence of viral hepatitis compared to DHCWs (Prevalence of HBsAg was correctly identified by 33.2 % vs 22.3%; prevalence of HCV- by 18.9 % vs 17.3%). Knowledge regarding transmission risks of blood-borne infections among HCWs is higher compared to DHCWS (correctly identified transmission of HIV - 73.0% vs 45.3 , HCV - 49.2 % vs 37.9%, HBV - 54.8% - 33.7%) ($p<0.005$). Vast majority of DHCWs as well as HCWs believed that probability of transmission of blood-borne infections after contaminated needle stick is 50 - 70% ($p<0.05$). There was a to poor knowledge on availability of post exposure prophylaxis (42.9% of HCWs compare to 36.1% DHCWs believed that HCV post-exposure prophylaxis is available ($p<0.005$). The practice of using facemasks (81% vs 74.4% always use, respectively), protective clothes (96.8% vs 83.3% always use) and eyewear's (46.9% vs 27.4% always use) was reported by DHCWs and HCWs. Some nosocomial risk events were reported by higher proportion of DHCWs, compared to HCWs and included accidental needle stick injuries (65.1% vs 45.5%) and blood splashes (48.3% vs 28.2%). Cuts with contaminated instruments was more common among HCWs compared to DCHWs (41.4% vs 35.1%) during medical procedures.

Conclusion: The study suggests that level of knowledge on blood borne infections among both HCWs and DHCWs is not adequate. Data from this study can be utilized to design educational programs for Georgian HCWs/DHCWs to improve knowledge and practice about blood borne diseases.

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Determination of drug resistance of hepatitis C virus to NS5A protein inhibitors as a new approach to personalized medicine

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Introduction. The use of direct-acting antiviral drugs (DAAs), that block specific proteins and enzymes of the virus, became a breakthrough in the treatment of HCV infection. At the same time during the process of virus replication nucleotide substitutions accumulate affecting both treatment outcomes and disease progress. The decrease in drug sensitivity is mainly associated with the occurrence of mutations in the region of the HCV genome encoding the NS5A RAS protein (resistance-associated substitutions), which differ in different genetic variants of HCV. In order to select effective treatment regimens, it is necessary to conduct a test to identify RAS (resistance-associated substitutions).

Materials and methods. The study included 64 blood plasma samples obtained from patients infected with 3a and 1b HCV subgenotypes. Amplification of the NS5A region of the genome was performed by “nesting” in house PCR. Fragment sequencing was carried out on an automated genetic analyzer ABI PRISM 3100-AVANT (Applied Biosystems, USA). Bioinformational sequence analysis was performed using the programs "SeqScape® Software v.3.0", "BioEdit v.7.2.5", "SeqA6". Clustal W program was used to align genetic sequences. Resistance mutations were analyzed using the on-line program https://hcv.geno2pheno.org/.

Results. Among 34 patients (50.0%) infected with HCV 3a subtype, RAS NS5A were found in 19 (55.9%): Y93H (41.2%), A/E62S/L/T/+ Y93H (44, 1%) and A30K (11.8%). In single cases, amino acid substitutions such as A30K/S/Q +A62L/W, A30K+A62S+Y93H and A30K +A60S were detected. Among 34 patients infected with HCV 1b subgenotype RAS NS5a were found in 27 (79.4%): Y93H +L31M/I (26.5%), Y93H (20.6%), L31M/V (17.6%). The least significant amino acid substitutions were found in isolated cases: A92T, P58A+L31M, Y93H+P58S, Y93H+L31M+R30Q, Y93H+L31M+P58A, Y93H+P32A+P58S.

Conclusion. Thus we have developed a method for detecting RAS NS5A and the results of this study demonstrated a high frequency of occurrence of such mutations among HCV-infected patients receiving treatment with direct antiviral drugs. If such mutations are widely detected among “naive” patients and among patients who have not achieved a successful virological response, it will be necessary to screen all patients for RAS NS5A before treatment.

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Next generation sequencing for routine HIV-1 resistance genotyping in a clinical laboratory: unsolved issues

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Several studies have evaluated the use next generation sequencing (NGS) for HIV-1 resistance genotyping and its impact in clinical routine. The availability of a commercial automated system has prompted some centers to switch from Sanger sequencing to NGS for their routine activity; however, clear answers are still needed for several questions.

We here present our experience, one year after the implementation of the Sentosa HIV NGS platform (Vela Diagnostics) in routine in our laboratory and our attempts to address issues raised by the facts.

A total of 411 consecutive plasma samples were run using the NGS-based Vela Diagnostics Sentosa SQ HIV-1 Genotyping Assay. A non-optimal result (sequencing failure or insufficient coverage) for at least one target region was observed for 103 sampes (25 %). For most of these samples, the viral load (VL) was below the treshold reported by the manufacturer (1000 copies/mL). Ultracentrigation from a larger volume could be a solution for samples with a VL below 1000 copies/mL.

The analysis of resistance associated mutations (RAMs) was routinely done at the cut-off of 20%. Then data were retrospectively analyzed with a cut-off of 5% (subject to sufficient coverage). A RAM at prevalence between 5 and 20%, yielding a significant change in resistance interpretation (S to R) for at least one drug, was found for only 12 samples.

HIV-1 proviral DNA genotyping was also performed in 52 DNA extracts from whole blood. An optimal result was observed for 50% of samples. DNA extraction from a larger volume of whole blood could help to reach better results.

In conclusion, the use of a commercial NGS solution for routine HIV-1 resistance genotyping can be interesting regarding the advantage of the automation, but optimization is still required in order to process all the samples, formerly tested with the Sanger solution. Consensus interpretation rules are also needed, especially regarding the cut-off.

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Association of viral reservoir with HIV infection duration, viral load and CD4 count

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Background: Viral reservoir refers to a pool of cells infected with replicatively competent forms of HIV that do not produce new viral particles because of latency. Measuring the size of a viral reservoir has become an increasingly relevant topic, not only due to the development of different curing strategies but also because it has been shown that the reservoir is an important clinical marker. It has been shown that in naive patients the size of the viral reservoir is associated with the rate of disease progression and the risk of developing HIV-associated diseases. In patients receiving antiretroviral therapy (ART), the size of the reservoir affects the speed of achievement of undetectable viral load, the risk of virologic failure, and time to viral rebound following ART interruption. The aim of our work was to study the HIV reservoir in patients without experience of ART and its association with the duration of infection, as well as with viral load (VL) and the CD4-lymphocytes.

Materials and methods: In PBMC samples from 97 HIV patients, total HIV DNA (integrated and non-integrated forms) was measured using quantitative real-time PCR. All patients at the time of the study had no experience with antiretroviral drugs. The duration of infection ranged from 55 to 3405 days and was determined on the basis of the last negative and first positive ELISA test (the time difference between the tests did not exceed 6 months). At the time of sampling to measure the viral reservoir, the concentration of HIV RNA and the CD4 count were determined.

Results: In the studied cohort, the reservoir size ranged from 129 (1st quartile (Q1)) to 1103 (3rd quartile (Q3)) copies of HIV DNA per million PBMC, the median was 376 copies of HIV DNA per million PBMC. When analyzing the association between the reservoir and the duration of infection, it was found that Q1, median, and Q3 patients with infection duration up to 3 years were 130, 387, 1095 copies of HIV DNA per million PBMC, and in patients with an infection duration of more than 3 years - 829, 1450, 2616 copies of HIV DNA per million PBMC, respectively.

In patients with VL<1000 copies/ml, Q1, median, Q3 were 55, 70, 106; in patients with VL from 1.000 to10.000 copies/ml - 75, 122, 618; in patients with VL from 10.001 to100.000 copies/ml - 203, 400, 956; in patients with VL from 100.001 to 1.000.000 copies/ml - 798, 1365, 2776; VL>1.000.000 - 2363, 2737, 2854, respectively.

In patients with CD4<250 cells/µl, Q1, median, Q3 were 585, 1313, 2534; in patients with CD4 from 250 to 500 cells/µl - 158, 554, 1065; in patients with CD4>500 cells/µl - 117, 203, 651, respectively.

Conclusion.

In the studied cohort, the size of the HIV viral reservoir was larger in patients with long-term infection (more than 3 years), high levels of HIV RNA in blood plasma and low CD4 counts. Despite the wide range in the size of the reservoir within the stratified groups, significant differences in the medians and interquartile ranges between them allow the use of this marker to assess the stage of the infectious process and to refine the prognosis of the development of the disease in the patient.

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Comparison of the Vela Dx Sentosa and in-house MiSeq Next-Generation Sequencing Systems for HIV-1 DNA Genotypic Resistance

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Background: Standard genotypic tests performed on HIV DNA from patients on suppressive ART, with previous drug-resistance mutations (DRM) detected in their plasma, underestimate resistance. In this context, the sensitivity of Next Generation Sequencing (NGS) genotyping techniques and the detection of minority variants can improve the characterization of resistance reservoir. The goal of this study was to compare performance of two NGS platform, Vela Sentosa and MiSeq for detection of DRM on HIV proviral DNA.

Materials and Methods: Fifty-eight highly treatment experienced patients with suppressed viraemia (ANRS 138-EASIER trial) were included in this study. HIV DNA was extracted from whole blood with the DSP DNA Mini Kit on a QiaSymphony instrument (Qiagen, Courtaboeuf, France). Reverse transcriptase (RT) and protease (PR) genes were sequenced using NGS techniques (Sentosa SQ HIV Genotyping Assay (Vela-Dx) and in-house NGS (ANRS) on MiSeq platform (Illumina). DRM analysis was performed with SmartGene software for both techniques using 2018 ANRS algorithm v9.

Results: MiSeq was successful for all patients and Vela assay failed for 3 samples which were excluded from the analysis. Median depth sequencing of MiSeq and Vela was 20388 and 4323 reads on the RT gene, and 23870 and 1984 reads on the PR gene, respectively. On the RT gene, 56 and 53 DRM positions were detected with MiSeq and Vela, respectively. On the PR gene, 45 and 46 DRM positions were detected with MiSeq and Vela, respectively. A mean of 6.6 DRM per patient was identified using both MiSeq and Vela, 8.1 with MiSeq alone and 9.8 with Vela alone. Both assays showed a good agreement for DRM detection in RT and PR genes at a threshold of 1 % (kappa index respectively 0.67 and 0.72), at a threshold of 5 % (kappa index 0.68 and 0.73) and at a threshold of 20% (kappa index 0.71 and 0.75). Analysis of frequency of DRM at the threshold of 1% showed a coefficient of correlation of 0.38 for RT gene and 0.40 for PR gene between Vela and MiSeq. DRM detected with both assays had a significantly higher frequency (median 72%) than DRM found only by MiSeq (median 20%) or Vela (median 15%), ($p < 0.0001$). For interpretation of ART drug resistance at the threshold of 1%, MiSeq estimated higher levels of resistance than Vela for PI in 24% of patients, for NNRTI in 18% and for NRTI in 16%. Vela estimated higher levels of resistance than MiSeq for PI in 27% of patients, for NNRTI in 36% and for NRTI in 12%.

Conclusion: The comparison of the two NGS genotyping techniques at the threshold of 1% showed differences in the detection and quantification of DRM on HIV DNA. Identification of DRM at a low frequency using NGS techniques must be interpreted carefully.

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Creation of an Italian HIV DNA Network for the validation and clinical use of HIV-1 DNA quantification assays

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Background: Although total cell-associated HIV-1 DNA (CAD) has gained attention as a rough estimate of the HIV-1 reservoir, certified systems for CAD quantification are not available and many different quantitative PCR-based methods are being used to determine CAD levels in blood of HIV-1 infected patients. Assays include commercial kits not yet marked for in vitro diagnostic use and homebrew quantitative PCR protocols based on digital (ddPCR) or real time (rtPCR) formats. The "Italian HIV DNA network" was launched to create a collaborative network to test and validate CAD quantification methods in use at University and Hospital laboratories.

Material and methods: In the stage 1 of Network program, detailed information was collected from all participating centers concerning sensitivity, specificity, accuracy, precision and linear range of the individual methods, generated by self-assessment. Each parameter was assigned a score based on the type and number of standards used and on results obtained. A minimum threshold score of 39/65 points had to be reached to access the stage 2 of the Network program, consisting in analyzing blindly a composite panel of reference material and reconstructed samples as an extensive quality control and assay validation. We report data of stage 1 with mean±SD or median (IQR) values.

Results: Of the 12 selected centers, 9 perform rtPCR, 2 ddPCR and 1 both methodologies. Quantification standards used included certified international standards (n=5), home-made (n=3) or commercial (n=1) plasmids, DNA from cell lines harboring known numbers of HIV-1 proviruses, quantified either by user (n=2) or by provider (n=1). The lower limit of detection (CAD copies/reaction) was calculated through single-reaction limiting dilution by 1 center (6.0), replicate limiting dilution by 4 centers (8.5 [6.0-10.0]) or complete Probit analysis by 7 centers (8.7 [3.0-13.0], 95% Hit rate). To assess precision, the centers used 3-5 different standard input copies and a median of 5 (3-5) replicates and obtained a coefficient of variation (CV) value 10.3±7.7%. A median of 8 (4-20) blood/PBMC clinical samples were used to evaluate the precision yielding 14.0±9.1% CV values, calculated on copies per million of cells. For linear range assessment, the centers tested 4 (3.8-4.0) standard input copies and CAD quantification was linear over the tested range. To assess accuracy, 5, 4 and 3 centers tested 4, 3 and 5 standard input copies, respectively. The accuracy (log expected values – log measured values) was 0.1±0.2. Only 6 centers verified the alignment of their probe/primers on publicly available HIV-1 sequences, however 8 declared to be able to quantify CAD across different subtypes. Few centers had performed extra analysis, including testing commercial kit, evaluating different extraction systems. All the centers reached a passing score to proceed to stage 2.

Conclusions: The centers had broadly but variably investigated the performance of their own assay and data based on self-assessment were reassuring. However, a blind and comprehensive analysis planned in stage 2 is required for external validation of the systems.

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Exploratory evaluation for the optimization of next generation sequencing HIV-1 drug resistance outcomes based on an in-house RNA extraction protocol

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Background: One of the major challenges for next generation sequencing (NGS) is the standardization of protocols and analysis of the data, especially for clinical use in HIV-1 drug resistance test. Different laboratory strategies and pipelines have been designed to accomplish this objective. The aim of this study was to test an in-house strategy and compare different parameters of the available algorithms for the analysis of HIV-1 drug resistance by NGS.

Materials and methods: We evaluated a panel of 4 samples with viral loads between 1.81-5.37 log of copies/mL, testing them by duplicate using two different RNA extraction methods: an in-house protocol and a commercial protocol (QIAGEN). Amplification and sequencing of the protease (PR) and reverse transcriptase (RT) genes of HIV-1 were performed with the MiSeq system (Illumina) using a commercial kit (ABL). Next, we analyzed and compared the data using four different pipelines for HIV drug resistance (HyDRA, PASeq, DeepChek and Stanford HIVdb-NGS beta version). In parallel, Sanger sequencing was performed as standard control. The susceptibility analysis was performed with the Stanford HIVdb algorithm 8.9-1.

Results: The samples were successfully extracted, amplified and sequenced by NGS and Sanger. Only 1 sample (with 65 copies/mL of viral load) couldn't be sequenced by Sanger. Two of the samples perfectly matched the resistance profile in all conditions: extraction method, sensitivity threshold (down to 5%) and the specific analysis pipeline. In the other two samples, we identified punctual resistance differences associated with the quality of the sequences, number of reads, the type and parameters of the analysis pipeline (including the sensitivity threshold) and the extraction method. The bigger differences were associated to the extraction method, favoring the in-house protocol compared to the commercial protocol.

Conclusions: Harmonizing criteria is essential to obtain comparable results and for the outcome of patients. Differences in each step during the workflow for sequencing can likely influence outcomes. At a threshold of 20%, results with our in-house RNA extraction protocol were most comparable to the control Sanger sequences and between pipelines. Nevertheless, our in-house RNA extraction protocol produced sequences with higher quality than those from the commercial protocol. Optimization of steps like RNA extraction could provide higher quality sequences, specially for samples with very low viral load. An evaluation with a larger number of samples is required to confirm our results.

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ASSOCIATIONS BETWEEN VITAMIN D AND FIBROSIS, GENOTYPE, VIRAL LOAD IN PATIENTS HAVING CHRONIC HEPATITIS C

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To date, evidence has been increasing in various scientific studies that vitamin D levels are inversely correlated with the stage of liver fibrosis in patients with HCV, viral load and genotype of the virus. However, data from other studies do not confirm the relation between serum vitamin D levels and the degree of morphological changes in liver tissue in patients with HCV, viral load, genotype.

The aim of our study was to evaluate the levels of vitamin D in the blood serum of patients with HCV depending on fibrotic changes in the liver, genotype of the virus, viral load.

Materials and methods – 100 patients having chronic viral hepatitis C in Dnipro were examined. Patients were divided into 2 groups, depending on the level of vitamin D: group I - with normal level of vitamin D, group II - decrease in vitamin D (lack and deficiency). A liver-synthesized vitamin D metabolite was defined in the blood serum – 25-hydroxycalciferol, which today is an indicator of vitamin D level adequacy in the human body. To verify the diagnosis of vitamin D deficiency, a classification (M.F.Holick, 2011), adopted by the International Institute of Medicine and the Committee of Endocrinologists, was used. According to this classification, the standard level of 25(OH)D in blood serum is 30-85 ng/ml; the 25-hydroxycalciferol blood level of 29-20 ng/ml is considered to be the vitamin insufficiency, and the value less than 20 ng/ml corresponds to vitamin D deficiency. All patients were examined for determination of the virus genotype, the stage of fibrosis and the viral load. Data processing as well as analysis was performed using Libre Office and R. Comparison of qualitative data was performed using Pearson`s criterion $\chi 2$. Correlations between variables were analyzed by using Spearman's correlation analysis (ρ).

Results. During the Spearman`s correlation analysis, no significant correlation was found between vitamin D level and fibrosis stage (Spearman`s correlation coefficient in group I $\rho = 0.16$, $p = 0.51$, in group II $\rho = -0.012$, $p = 0.90$), however, the study found out that in patients with normal level of vitamin D, fibrosis stage F1-F2 was recorded 2.5 times more frequently than liver fibrosis stage F3-F4, whereas in patients with the insufficiency and deficiency of vitamin D, this ratio was 1:1, the stage of severe liver fibrosis F3-F4 was observed in almost half of the patients in this group. This brings us to the assumption that the stage of fibrosis has some relation with the level of vitamin D. The percentage of deficiency and lack of vitamin D increased along with rising stage of fibrosis ($p= 0.51$). The results of the conducted study found out that abnormal level of vitamin D associated with HCV had no relation to the genotype of hepatitis C virus. Vitamin D levels between the groups were not statistically different ($p= 0.14$). Performing a linear regression revealed a statistically unreliable weak positive relation between viral load and vitamin D levels ($p= 0.32$).

Conclusion. The stage of fibrosis F3-F4 in patients was associated with the frequency of vitamin D deficiency.The results of the conducted study found out that abnormal level of vitamin D associated with HCV had no relation to the genotype of hepatitis C virus. Performing a linear regression revealed a statistically unreliable weak positive relation between viral load and vitamin D levels.

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OVERVIEW OF THE NATIONAL HEPATITIS B VIRAL LOAD TESTING PROGRAME IN UGANDA

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Background: Uganda is a high endemic country for Hepatitis B with an estimated prevalence of 4.1% [UPHIA, 2016]. The testing program started in 2014 with highly endemic regions of Uganda. MOH testing and treatment guidelines, training materials and laboratory tools were developed. The Hepatitis B viral load testing program started in 2017, leveraging on the already existing HIV systems. A viral load is necessary in informing treatment legibility (>20,000 IU/ML) and as well as a monitoring tool for those who are on treatment (WHO, 2017). Against this background, this presentation is aimed at showing trends in the Hepatitis B viral load testing at the National Health Laboratory and Diagnostic Services.

Methods: Samples were collected from facilities and transported to NHLDS through the Hub sample transport network.11, 296 samples were analyzed in a period between 2017 November and 2019 February. All the samples were tested for viral load using the HBV DNA PCR test. All analyzed samples were subjected to Quality control checks to ensure release of quality results. The viral load of 20,000 IU/ml was used as a threshold for clinical interpretation as guided by the WHO guidelines for managing CHB. Data analysis was done using SPSS software.

Results: A total of 11,296 samples have been tested for hepatitis B viral load of which 5316 were female and 5256 were male. The overall rejection rate was at 2.24% and the main reason for sample rejection being sample hemolysis at 0.3%. In 2017, 1978 samples were tested, 4731 in 2018 and 4587 have been tested from January to February 2019. 31.5% samples were from Mid-eastern region, central 2 region at 11%, Kampala district at 10.9%, North East at 5.3%, West Nile at 4%. Of the patients tested 16% had non suppressed viral load (above 20,000IU/ML). Pregnant women were a total of 88 patients out of whom 8% were non suppressed. The age category of above 30 years had relatively higher viral load of above 20,000 IU/ML at 7.7% of patients followed by patients aged 17-30 years that were at 5.5% and almost insignificant cases in patients below 17 years at 1.7% and there was a statistically significant relationship between age categories and viral load levels at a P value of <0.001 at 95% CI.

Conclusion: Attention should be given to the female patients that had un suppressed viral load in a way to reduce mother to child transmission. Age is a predisposing factor to liver disease and delayed suppression so the need for early diagnosis and timely management is necessary.

KEY WORDS: HBV DNA, CHB, WHO.

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Comparison of the new Alinity m system with the established Abbott RealTime and Hologic Aptima Quant Assay in HIV-1, HCV and HBV viral load measurement including performance and turn-around times

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Background: Molecular diagnostics is an essential part of diagnosis and treatment monitoring in infectious diseases. Short turn-around-times are a requirement for rapid initiation of treatment and fast identification of treatment failures. New molecular diagnostic platforms allow to perform multiple parallel tests like detection of HIV, Hepatitis C Virus (HCV), Hepatitis B Virus (HBV) without the restriction of batching samples. We compare the assay performance of the new Abbott Alinity m system with the Abbott m2000 and the Hologic Panther platforms.

Materials/methods: We tested 1841 clinical samples on the Alinity m system and compared the results to either the m2000 or the Panther. 1089 samples were tested for HIV viral load, 307 for HBV viral load and 445 for HCV viral load. Regression and Bland-Altman analysis was performed for samples with quantitative results. Turn-around-times (TAT) were analysed as time difference between sample arrival in the laboratory and availability of results in the laboratory information system with the same samples tested in parallel.

Results: 198 samples showed quantitative results for HIV (HBV: 151, HCV: 225). Comparison between Alinity m and m2000 showed high coefficients of correlation (HIV: R^2=0.96, HCV: R^2=0.96, HBV: R^2= 0.95) with similar results between Alinity m and Panther (HIV: R^2=0.94, HCV: R^2=0.99, HBV: R^2= 0.90). TAT analysis showed in 1125 samples with one Alinity m 90% of results were available after 11:09 hours compared to 71:22 hours with three m2000. A second set of 1033 samples was compared between one Alinity m and two Panther showing 90% of results were available after 5:46 hours (Alinity m) and 5:13 hours (Panther).

Conclusions: Our analysis shows excellent performance of the Abbott Alinity m system compared to Abbott m2000 and Hologic Panther with high levels of correlation. The analysis of turn-around-time shows a relevant reduction of duration until results can be reported to the clinicians compared to the m2000 system, while both random access systems with an optimized pre-analytic workflow showed similar turn-around-times. The Abbott Alinity m allows to run 20 different assays in parallel as compared to 4 on Panther. This increase in automation leads to faster reportable results with less hands-on-time.

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The Significance of M2BPGi Marker and CCC DNA in Unknown Etiology Hepatitis

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Background: M2BPGi is Mac-2-binding protein glycosylation isomer, a novel serum marker for liver fibrosis. Determining the level of M2BPGi does not require a surgery operation, as with a liver biopsy, and its accuracy is very high. This non-invasive method has a high correlation with the development in patients of complications in the form of liver fibrosis and hepatocellular carcinoma, and also allows the doctor to see the prognosis of the course of the disease. Laboratory diagnosis of viral hepatitis is an actively developing branch of medical science. Over the 50 years since the opening of the "Australian antigen", many viruses have been discovered that are responsible for the onset of hepatitis. The variety of serological markers of infection, methods for their detection using highly sensitive and specific methods have opened the possibility of not only the etiological decoding of acute or chronic viral hepatitis, but also monitoring of treatment.

Materials & Methods: For the study, blood plasma and liver biopsies from 26 patients were investigated. All patients were admitted to the intensive care unit of the Research Institute of Virology in serious condition. All subjects were given written consent to conduct this analysis. CCC DNA HBV was detected according to the Pollicino T. method. Using TaqMan probes for real-time PCR. DNA was pretreated with MungBean endonuclease at the rate of 1 unit. enzyme per 1 µg of DNA to remove single-stranded DNA and RNA and cleavage of genomic DNA of HBV (partially circulated). For each sample, the reaction was carried out three times; then, the Ct value for them was averaged. Analysis of the results was carried out by the method of relative calculation with normalization by the endogenous reference gene GAPDH. In order to confirm the presence of HBV, untreated endonuclease DNA was used as a template for PCR with further sequencing. The M2BPGi values were measured using HISCL M2BPGi kit (Sysmex, Kobe, Japan) on fully-auto¬mated immunoanalyzer, HISCL-800 (Sysmex). M2BP levels were indexed by the following equation: Threshold index (COI)=([WFA+M2BP]sample-[WFA+M2BP]NC)/([WFA+M2BP]PC-[WFA+M2BP]NC), if [WFA+M2BP] sample - level of WFA+M2BP in sample, [WFA+M2BP]PC - positive control, [WFA+M2BP]NC - negative control. The positive control was supplied in the form of a calibration solution standardized to obtain a COI value of 1.0.

Results: A total of 25 patients with a diagnosis of hepatitis of unknown etiology were examined. For all patients, ELISA tests for HBsAg and AntiHCV were preliminarily performed. All samples were negative for the presence of HBV surface antigen and antibodies to HCV. Further, testing was carried out for the presence of CCV of HBV DNA in liver biopsies. The calculation of the number of copies of CCV of HBV DNA per 1 hepatocyte was also performed. All patients tested for a serum M2BPGi marker level.

Of the 25 patients examined, CCC was detected in 13, which indicates an occult form of hepatitis B. The average number of copies of CCC per 1 hepatocyte was 0.2.

Conclusions: There was no correlation between the detection of an occult form of hepatitis B by CCC DNA HBV testing and serum marker M2BPGi level in our study. However, there is good potential for using serum marker M2BPGi to assess liver fibrosis and treatment success in patients with hepatitis of unknown etiology.

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A pan-genotypic nearly whole genome amplification approach of HCV by a simple two-step RT-PCR combined with parallel Oxford Nanopore and Illumina sequencing produce highly accurate long-range sequences

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Background: Phylogenetic studies of HCV evolution and transmission are often limited to the analyses of partial viral genome segments, mainly due to technical obstacles to amplify the full-length HCV genome. Despite some improvements in full genome amplification and sequencing methodologies, these approaches are either genotype-restricted or expensive.

Methods: Using pimers in the 'core'- and NS5B-region in a two-step RT-PCR with Superscript IV (Invitrogen) and Phusion hot start II hifi enzyme (Thermo Scientific), respectively, we were able to generate an approximately 8.2 kb long fragment. Sequencing was done by Illumina MiSeq (Nextera XT protocol) and Oxford Nanopore (ONT) sequencing on MinION with a Flongle flow cell. The protocol was applied to 15 clinical samples with different genotypes [1a (1x), 1b (2x), 2a (1x), 2b (2x), 2c (1x), 3a (4x), 4a (1x), 4d (3x)].

Results: A pan-genotypic protocol for nearly whole genome amplification of four major HCV genotypes was established. The limit of detection/amplification was determined to be between 15.000 and 20.000 IU/ml.

Amplicons were verified with both, Illumina MiSeq and Oxford Nanopore (ONT) sequencing technologies. Illumina sequencing resulted in a high coverage and enabled for detection of minority variants. ONT sequencing resulted in sequence reads close to 8 kb, covering the entire HCV amplicon in a single read. Combining the data from both approaches resulted in highly accurate long-range sequences, enabling for determination of mutation linkages between distantly separated positions.

Conclusion: This easy and fast protocol for amplification and sequencing of the nearly whole HCV genome enables the analysis of the nearly full-length HCV quasispecies, e.g. in the context of analyzing transmission chains.

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A hybrid sequencing approach for accurate minority detection and resolving haplotype information

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Background: HIV-1 drug resistance testing is routinely performed prior to initiation of antiretroviral treatment or resistance-related therapy changes.

While next generation sequencing approaches using Illumina's technology have been applied in clinical routine to detect minor variants, they are not capable of detecting linkage information between distantly separated mutations or regions.

In contrast, Oxford Nanopore's technology (ONT) is capable of sequencing large amplicons within one read, but has the drawback of having a relatively high error rate rendering it less suitable for HIV drug resistance testing.

In this work, we combined the strengths of both technologies to accurately assess the frequencies of mutations, thereby enabling a better insight into the linkages within the viral population.

Materials and Methods: The complete genes of HIV-1 gag, pol and env were amplified from 12 plasma samples. Additionally, env only was amplified from 14 proviral DNA samples.

All amplicons were sequenced with both Illumina's Miseq (fragmented) and Oxford Nanopore's MinION (whole amplicons, plasma samples on regular flow cell, proviral on Flongle flow cell).

Analysis was performed using SeqIT's drug resistance testing pipeline for MiSeq-data and an in-house developed analysis pipeline for ONT-data and combined/hybrid analysis.

The most prevalent highly variable loops of gp120 were determined from MiSeq-data and used to construct virtual genotypes for hybrid analysis. ONT-data was mapped onto these and further processed. Covariation information of both correlated mutations as well as hypervariable regions was calculated.

ONT-data was used to establish linkage information between mutations and hypervariable loops determined by MiSeq. Covariation matrices and dendrograms of linked regions were generated.

To assess the quality and sensitivity of the approach, sequencing data from two samples was mixed with different quantities (1-99%, 2-98%, 5-95%, ...).

Results: MiSeq generated sequencing data of around 5.000x coverage on average for all genes and samples tested. The regular ONT flow cell was stopped after around 2.5 hours with approximately 766.000 reads. The Flongle flow cell generated approximately 158.000 reads.

High quality data was extracted from MiSeq data. Mutations above 2% and the highly variable loops of gp120 were extracted and used for further analysis with the ONT-data while mutations below 0.5% were used to polish ONT-reads.

Polishing increased accuracy of ONT-reads by about 4%. ONT-reads readily linked variable loops and mutations.

In the mixing experiments, linkage analysis could correctly separate mutations from the two samples for all mixes where the minor population had a prevalence of at least 5%. Even for mixes 2-98% and 98-2%, more than 50% of the mutations of the minor sample could still be linked to each other.

Conclusions: In this study we demonstrate that a hybrid sequencing approach is capable of detecting mutations with high accuracy as well as determining correlations between mutations and regions over long ranges.

The method can be used for better analyzing the haplotype structure of the viral quasispecies within a patient. A possible application for the method is its use in proviral drug resistance testing. There it can be used to better define if resistance mutations are on replication competent or deficient strains with Apobec mutations further apart from them.

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The quantification of HBeAg levels differ among the different phases of HBV infection, and can predict therapeutic outcome in the setting of immunosuppression driven HBV reactivation.

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Background: Qualitative HBeAg is a marker of active HBV replication and HBeAg loss is an important clinical and therapeutic end-point. Here, we quantify HBeAg level in different phases of HBV infection, its correlation with virological and biochemical markers and its role in predicting virological response to anti-HBV therapy.

Material and Methods: This study includes 86 HBeAg-positive patients: 20 with acute infection (HBcIgM+, median[IQR] HBV-DNA: 8.3[7.9-8.7]logIU/mL, ALT: 1556[142-2027]U/L), 14 with HBeAg-positive chronic infection (median[IQR] HBV-DNA:8.1[4.7-8.5]logIU/mL, ALT <40U/L), 23 with HBeAg-positive chronic hepatitis (median[IQR] HBV-DNA: 8[6.8-8.5]logIU/mL, ALT: 85[62-179]U/L) and 29 patients with immunosuppression-driven HBV-reactivation (median[IQR] HBV-DNA: 6.8 [5.5-8]logIU/mL, ALT: 143[40-528]U/L, pre-reactivation status HBcAb-positive/HBsAg-negative). 15/29 patients with HBV-reactivation were monitored for >12months after starting TDF or ETV therapy (median[min-max] months of follow-up: 21[12-54]). Quantitative HBeAg (qHBeAg) is assessed by DiaSorin LIAISON assay. Association of qHBeAg at HBV-R with the achievement of HBeAg loss after starting anti-HBV therapy is assessed by Fisher exact test.

Results: qHBeAg is higher in patients with HBV-reactivation and acute infection (median[IQR] 930[206-1945] and 754[210-3379]PEIU/mL) and decreases in patients with chronic infection and chronic hepatitis (median[IQR] 655[0.9-1773] and 412[17-1850]).

qHBeAg positively correlates with qHBsAg in all the subsets of patients (Rho=0.61, P=0.003 for HBV-reactivation, Rho=0.78, P<0.001 for acute infection, Rho=0.71, P=0.023 for chronic infection and Rho=0.75, P<0.001 for chronic hepatitis) and with HBV-DNA only in chronic hepatitis (Rho=0.59, P=0.005). Moreover, qHBeAg negatively correlates with ALT in acute infection and chronic hepatitis (Rho= -0.59, P=0.035; Rho= -0.42, P=0.044), reflecting a modulation in HBeAg production by immune response.

Focusing on 15 pts with HBV-Reactivation starting anti-HBV therapy for >12 months, ALT normalization is achieved in 93% of pts while virological suppression and HBeAg loss in 60% and 53.3%, respectively. The combination of qHBeAg >2000PEIU/mL + qHBsAg >52000IU/mL at HBV reactivation is the only factor predicting no HBeAg loss during anti-HBV therapy (HBeAg loss in 0% pts with qHBeAg >2000PEIU/mL+qHBsAg >52000IU/mL vs 72.7% pts without this combination, P=0.01, result not significant considering qHBeAg and qHBsAg separately).

Conclusion: HBeAg levels differ during the natural history of HBV infection and according to the extent of immunological pressure. In the setting of HBV reactivation, HBeAg levels can be useful in predicting virological response to anti-HBV therapy under iatrogenic immunosuppression.

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The effectiveness of the application of serological tests and laboratory indicators as part of RITA for the detection of recent HIV infection

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Background: Estimation of HIV incidence and detecting recent HIV infections in a population are important for monitoring, rapid initiation of treatment, plan and implement prevention activities. The accurate identification of recent HIV infection continues to be an important research area. Recent infection is defined as the period during the first 6-12 months after infection. Today there is no gold standard method to accurately define the time passed since infection, but already there are a number of kits that allow determining the approximate time of HIV infection. However, concerns about the accuracy of incidence estimates derived from using single tests have arisen and can be addressed by combining multiple assays in a recent infection testing algorithm (RITA). RITA is an approach that allows for differentiating recent and established HIV infection. Laboratory tests include detection of antibody titer, avidity index (AI), viral load (VL), and CD4-count. The aim of this study was to assess the sensitivity (correct detection of recent infection) and accuracy (correct detection of the duration of all samples in accordance with the epidemiological data) of this algorithm for the determination of HIV infection duration.

Materials & Methods: Plasma samples (n=311) was obtained from ARV-naïve HIV patients: 185 samples from patients with infection duration up to 12 months (recent infection samples) and 126 samples from patients with duration more than 12 months (established infection samples). The duration of infection was determined on the last negative and first positive ELISA and immunoblot tests (indicators of seroconversion), by epidemiological and clinical data. Determination of antibody titers was carried out with DS-EIA-HIV-Ab-TERM kit (Diagnostic Systems, Russia) and antibody avidity was estimated by Architect HIV Ag/Ab Combo kit (Abbott, USA).

Results: In the first step, all samples were analyzed by the antibody avidity and sensitive-less sensitive assays. The concordance of DS-EIA-HIV-Ab-TERM results and epidemiological data were obtained for 290/311 (93.2%) of all samples. The concurrence data for Architect HIV Ag/Ab Combo were 241/308 (78.2%) respectively (three samples had an invalid result). For using RITA in addition to serologic assays the following criteria have been defined for recent infection: CD4-count> 200 cells / mm3; viral load> 75 copies / ml; the absence AIDS-defining illness. Further, we had to establish a threshold for determining recent HIV-infection. According to WHO recommendations, this rate can range from 6 to 12 months, and it should also be calibrated and determined for each country (study or cohort) individually. In our case, the maximum values of sensitivity and accuracy of the algorithm were achieved at a threshold of 9 months: sensitivity was 67.4%, accuracy 79.7%. Moreover, these indicators were equal to 70.0% and 81.1% for subtype A, and 47.6% and 69.4% for subtype B respectively.

Conclusions: Study results showed that serological tests (DS and Abbott) correctly identified the duration of HIV infection in 93.2% and 78.2% respectively. If we consider RITA as a whole, then the results for subtypes A and B are extremely different, which is an important fact because subtype A predominates in the Russian Federation. Therefore, the algorithm requires further refinement and calibration and validation as recommended by WHO.

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Combined use of high-sensitivity serological and virological HBV markers can make better in predicting the occurrence of HBV reactivation and to optimize prophylaxis duration in HBsAg-negative/anti-HBc-positive patients in the context of oncohematological diseases

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Introduction: Prevention of HBV-reactivation (HBV-R) in patients undergoing immunosuppressive therapy is still challenging. This study is aimed at investigating the role of HBV markers in predicting HBV-R in HBsAg-negative/anti-HBc-positive patients with oncohematological diseases.

Methods: HBV-R rate is estimated in 107 HBsAg-negative/anti-HBc-positive patients (42 receiving rituximab [RTX], 40 hematopoietic stem cell transplantation [HSCT], 25 other chemotherapies). All patients received lamivudine-prophylaxis for >18 months after stopping immunosuppression (EASL guidelines) and were prospectively monitored every 3 months. 58/107 patients have completed lamivudine-prophylaxis and were monitored for a median time of 25.4 (9-32) months after prophylaxis completion. The role of HBV markers in predicting HBV-R is evaluated by testing 831 serum samples for highly-sensitive HBsAg (Fujirebio, HS-HBs; lower limit of quantification [LLOQ]: 5 vs 50 mIU/ml of routinely-used assays), HBV-DNA (Roche, LLOQ:20IU/ml), quantitative anti-HBs and anti-HBc (Fujirebio, LLOQ:1.0COI). HBV-R is defined as serum HBV-DNA >20IU/ml (Seto, 2016).

Results: At baseline-screening, all patients have undetectable HBV-DNA and 67.3% is anti-HBs positive (median [IQR]:152[47-976] mIU/ml]. HBV-R occurs in 14/107 patients with the highest 5-year cumulative reactivation rate in HSCT (63.4% vs 15.8% for RTX and 9.6% for other chemotherapies, P=0.026).

At HBV-R, median (IQR) HBV-DNA is 42(23-682) IU/ml and ALT>ULN for 46% (median [IQR]:88[60-763] U/L). Among HBV-R cases, 6 develops HBV-R during and 8 after completing prophylaxis (median [min-max] months after prophylaxis completion:3[1-27]).

The analysis of serological markers during the entire monitoring reveals that the combination of anti-HBc>3COI and anti-HBs persistently or declining to <50mIU/ml correlates with a higher risk to develop HBV-R (53.8% of patients with anti-HBc>3COI+anti-HBs<50mIU/ml vs 14% without this combination experiences HBV-R, P=0.004, OR [95%CI]: 7.1[1.9-26.1]). Results confirmed in the subset of 58 patients completing lamivudine-prophylaxis (63% of patients with anti-HBc>3COI+anti-HBs<50mIU/ml vs 26% without this combination experiences HBV-R, P=0.038, OR [95%CI]: 4.7[1-22.7]).

Furthermore, by monitoring virological markers, the positivity, confirmed in at least 2 time-points, to HS-HBs (detection failed by routinely used HBsAg-assays) and/or to HBV-DNA (detected below LLOQ) is another factor predicting HBV-R (44.4% of patients positive to HS-HBs and/or HBV-DNA vs 7.4% never positive to these markers experiences HBV-R, P=0.007, OR [95%CI]: 10.1[2.2-46.1]).

Conclusions: In the setting of oncohematological diseases, HBV-R frequently occurs in anti-HBc-positive/HBsAg-negative patients, particularly after completing antiviral prophylaxis. The combined usage of accurate HBV-markers can guide to identify patients at higher HBV-R risk who need an extended prophylaxis.

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Ibalizumab shows in vitro activity against group A and group B HIV-2 clinical isolates

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Background: In addition to being naturally resistant to NNRTI and enfuvirtide, HIV-2 easily selects drug-resistance associated mutations to protease, NRTI and integrase inhibitors at time of virological failure, leading to multi-drug resistant (MDR) viruses. Ibalizumab (IBA) is a long-acting humanized monoclonal antibody that blocks entry of virus into the host cell. It is approved by the FDA and the EMA for treatment-experienced persons infected with MDR HIV-1. No data were available on the activity of IBA against HIV-2 isolates.

Methods: Isolates from 6 HIV-2-infected persons (4 group B, 2 group A), the ROD HIV-2 group A reference strain and the BRU HIV-1 reference strain were assessed for IBA phenotypic susceptibility. We adapted a PBMC phenotypic assay. Briefly, PHA-activated PBMC were incubated with increasing concentrations of IBA for 1h, prior to infection. Two hours post-infection, cells were washed and then resuspended in complete RPMI media containing IBA. At day 4 post-infection, HIV-2 replication was assessed on cell supernatant using a qRT-PCR (Biocentric-HIV-2). Phenotypic susceptibility was assessed through 50% inhibitory concentrations (IC50) and Maximum-Percent-Inhibition (MPI). All HIV-2 isolates were previously obtained by co-cultivation of PHA-activated PBMC pool obtained from healthy blood donors.

Results: IBA inhibited viral replication for all seven HIV-2 isolates, with IC50 ranging from 0.002 to 0.18µg/mL, and for the HIV-1 reference strain (IC50=0.01µg/mL). MPI was below 80%, between 80 and 90%, and >90% for 2, 1 and 4 strains, respectively. The 2 isolates with the lowest MPI (74 and 77%) also had the highest IC50 (0.18 and 0.09µg/mL, respectively).

Conclusions: These data demonstrate for the first time that IBA is active in vitro against both HIV-2 epidemic groups, with similar IC50 and MPI to those observed for HIV-1. IBA could be included in therapies for HIV-2-infected-persons displaying MDR viruses, a more frequently observed situation in HIV-2 than in HIV-1. Clinical studies of IBA-based regimens in HIV-2-infected-patients are warranted.

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TDR SURVEILLANCE AND CLINICALLY RELEVANT RESISTANCE IN SPAIN: 2019 UPDATE

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Background: Treatment guidelines currently recommend resistance testing in the RT and protease of HIV as a part of the initial evaluation of HIV infected patients. Thus, it is of interest to evaluate the prevalence of drug resistance mutations (DRM) and, also of clinically relevant resistance. Here, we present data on trends in DRM and clinically relevant transmitted drug resistance to ARVs recommended for first-line treatment in Spain

Methods: We analysed 6849 RT & Pro Fasta sequences from CoRIS (2007-2019). As Integrase resistance is not part of routine testing in naïve patients in Spain, we run a surveillance programme (2012-2019) and tested 1687 patients. We evaluated the prevalence of RT/Pro and INI SDRMs using the CPR tools from Stanford. Clinically relevant resistance was evaluated using the Stanford v8.9-1 Algorithm. First line regimens for each study period were those recommended by the Spanish treatment guidelines (GESIDA).

Results: Overall, we found that 9.1% of the patients were infected by viruses carrying at least one SDRM (3.5% NRTI, 4.3% NNRTI, 2.0% PIs, 2.7% INIs). Our results indicated a similar trend in NNRTIs and NRTIs SDRM prevalence with values ranging from 2.5-5.9% through the study period. However, we observed a decrease in PIs TDR, going down to prevalence below 1% for years 2018 and 2019. In regard to INsTIs, TDR was low with no significant changes over years. Clinically Relevant resistance to recommended GESIDA's first line regimens showed no trend from 2007-2012 (always close to 9%), peaked in 2013-2014 due to the inclusion of Rilpivirine for 1st line in the Spanish recommendations, and went down to levels always below 3% for the years 2015-2019. For year 2019, clinically relevant resistance to first line antiretrovirals in Spain was 0% to Dolutegravir, Bictegravir and Darunavir, 1,0% for 3TC/FTC; 1,2% for TDF/TAF, 1.9% for Abacavir, and 2.6% for Raltegravir.

Conclusions: While NNRTIs and NRTIs SDRM prevalence remained stable in Spain through 2007-2019, we observed low levels of PIs and INIs SDRMs. Clinically relevant TDR to approved first line regimens is at low levels from 2016 to 2019. These findings support GESIDA's recommendations on baseline resistance testing and test and treat strategies.

Key words: CoRIS, TDR, first-line regimens, resistance.

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PREVALENCE OF DRUG RESISTANCE MUTATIONS TO RILPIVIRINE AND DORAVIRINE AMONG NNRTI-EXPERIENCED PATIENTS IN ITALY

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Background: NNRTI class still represents a cornerstone of combination HAART. Despite new generation drugs with lower drug-drug interaction, side effects and higher genetic barrier were recently developed, drug resistance mutations (DRM) surveillance is warmly recommended by WHO.

Materials and methods: This was a cross-sectional analysis from the Antiviral Response Cohort Analysis (ARCA) database. To assess pre-treatment DRM prevalence to rilpivirine (RPV), we included only genotypic resistance tests (GRT) from NNRTIs-experienced patients who were naïve to RPV; while for DRM prevalence to doravirine (DOR) all GRT from NNRTIs-experienced patients were included in the analysis. All GRT performed at least three months after NNRTI containing regimen start since 1998 onwards were collected. Resistance interpretation was made according to the Stanford algorithm.

Results: Overall, 3,214 GRTs were included from 1,773 subjects of RPV group, and 3,530 from 1,911 patients of DOR group: mostly men, Caucasians and carrying B subtype in both groups.

Prevalence of susceptible strands was 71.5% in RPV group and 73.5% in DOR group.

Similar overall prevalence of DRMs was observed for the RPV (16.9%) and the DOR (16.5%) groups. Low resistance was mainly attributable to E138A (3.4%) in RPV group, while in DOR group it was principally due to V108I (3.1%) and K101E (2.3%); the latter showed the same percentage in RPV group. L100I in combination with K103N, conferring intermediate resistance to DOR and high resistance to RPV, was present in 1.7% and 1.5% of cases respectively, being this pattern second only to Y188L among all high DRM considered in our analyses (1.8% vs. 1.9%).

Regarding the V106A mutation was significantly more frequent among Caucasians compared to non Caucasians (1.8% vs. 0%, p=.002) and in subjects with B subtype (27.4% vs. 20.6%, p=.001).

In both RPV and DOR groups, statistically significant association has been found between B subtype and the presence of at least one DRM (p=.003 and p=.001, respectively), and between male The multivariable logistic regression indicated that: male sex and number of previous cART regimens were associated with detection of DRM, while a low CD4+ count resulted associated with a reduced risk of DRM, in both groups. By contrast, calendar year of GRT and controlled viremia were inversely associated with DRM in the RPV group only, and subtype B decreases DRM odds in DOR group only.

Conclusion: High level DRM to both RPV and DOR are uncommon in our samples. Relative higher prevalence of Y188L and K103N+L100I arises concern about use of both drugs among efavirenz and nevirapine experienced patients.

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HCV resistance associated variants following directly acting agent therapy failure- real life data from Poland

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Introduction: Directly-acting antiviral (DAA) HCV treatment failure remains the issue even in the era of the widespread access to the therapy, as unsuccessfully treated patients may not only progress to the more advanced liver disease but also potentially limit the efficacy of the HCV elimination efforts. This study aimed to analyse the NS3 and NS5A mutation frequency in the cohort of the real-life patients failing HCV therapy following DAA treatment.

Methods: In total, NS3/NS5A Sanger sequences from 105 DAA failing patients infected with genotype (G) 1a (6, 5.7%), G1b (94, 89.5%), G3a (4, 3.8%) or G4 (1, 1.0%) were analysed. This study included samples from patients with HCV-RNA >1000 copies after at least 3 months of DAA therapy termination, collected at a discretion of the treating physician at variable timepoints after virologic failure. Data on the age, gender, HCV transmission route, DAA exposure history, HBV coinfection status, HCV-RNA level at the date of failure and liver fibrosis stage were collected. NS3 and NS5A RAVs were identified using geno2pheno algorithm HCV v.0.92.

Results: Majority of patients in the analysed cohort were male (n=61, 58.1%), with median age of the group 54 (IQR: 42-61) years. Of these, 49 (55.7) had either advanced liver fibrosis or cirrhosis. Pegylated interferon/ribavirin + boceprevir, simeprevir or telaprevir treatment failures were observed in 38 (36.2%) cases, 27 (25.7) individuals were failing NS3 + NS5A inhibitor therapy with the most common being asunaprevir/dalclatasvir (19, 18.2%) or less frequently grazoprevir/elbasvir combinations (6, 5.7%), NS3+NS5A+NS5B inhibitor (ombitasvir/pibrentasvir+dasabuvir) combinations were found in 28 (26.7%) cases while 11 (10.5%) participants were exposed to NS5A inhibitor+NS5B±RBV, most commonly ledipasvir/sofosbuvir (9, 8.6%). NS5A resistance associated substitutions (RAS) were found in 87.9% of sequences derived from patients exposed to this class of agents, while NS3 RAS in 59.1% protease exposed subjects. If analysed by the codon positions the NS5A mutation frequencies were as follows: 7.6% for 28A/V/M, 10.6% for 30K/Q/R, 42.4% for 31I/F/M/V and 75.8% for 93H; for NS3 the most common RAVs were: 56F-23.7%, 168A/E/I/Y/T/V-14.0%, and 117H-5.4%. Frequency of the NS3 drug resistance variants was increasing with the fibrosis stage, from 47.8% among F0/F1 individuals to 81.8% among patients with liver cirrhosis (F4). Also NS3 RAVs were notably more frequent among patients with advanced liver fibrosis (F3) or cirrhosis (n=29, 72.5%) compared to F0-F2 cases (n=18, 48.7%), p=0.03 and for cirrhotic patients (F4, n=18, 81.8%) compared to non-cirrhotics (F0-F3, n=29, 52.7%). Similarly, there was an increase in the observed NS3+NS5A RAVs frequency with progression of liver fibrosis from 33% in F0/F1 to 80% in F4, with resistance associated variants being notably more common among F3 and F4 patients (n=17, 70.8%) compared to lower fibrosis stages (F0-F2, n=8, 40%), p=0.025 and cases with cirrhosis (n=12, 80%) versus non-cirrhotic ones (n=12, 44.8%), p=0.039. No similar associations were observed for any of the fibrosis stages and NS5A RAVs analysed separately. Among currently recommended NS3/NS5A combination full susceptibility to the Glecaprevir/Pibrentasvir was observed in 92.9% of patients, with 25.3% susceptibility for Elbasvir/Grazoprevir and 43.0% for Ombitasvir/paritaprevir/ritonavir.

Conclusions: Following DAA treatment failure the NS5A associated are common with increasing frequency in more advanced liver disease. In most of the observed cases, despite presence of RAVs susceptibility to the DAA combinations with higher genetic barrier is retained.

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Absence of GS-6207 Phenotypic Resistance in HIV Gag Cleavage Site Mutants, Patient Isolates With Gag Polymorphisms, and Isolates With Resistance To Existing Drug Classes

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Background: GS-6207 is a potent, first in class, multistage inhibitor of HIV-1 capsid function with the potential to be used as a subcutaneous (SC) long-acting agent with dosing every 3 months or longer. In a clinical trial, a single SC injection of GS-6207 (50 mg to 750 mg) in people living with HIV (PLWH) showed a rapid and strong antiviral effect, with a ≥1.8 mean log10 decrease in HIV-1 RNA through day 10. Pre-existing mutations in the capsid polyprotein precursor gag, such as protease inhibitor (PI)-induced resistance mutations at gag cleavage sites or naturally occurring gag polymorphisms, are known to affect the antiviral activity of PIs or maturation inhibitors (MIs). Additionally, resistance mutations to currently approved drug classes may also affect the activity of GS-6207. Here we have characterized the activity of GS-6207 in mutants with HIV-1 gag cleavage site mutations, patient isolates with gag polymorphisms, as well as mutants with resistance to existing drug classes.

Methods: HIV mutations were inserted into the pXXLAI infectious clone either by site-directed mutagenesis (n=19) or by cloning of plasma samples (n=51). The resulting infectious clones with HIV gag cleavage site mutations, or HIV gag-PR fragments from treatment-naïve or experienced PLWH were evaluated using a standard 5-day antiviral assay (MT-2-cells). Isolates with resistance mutations against one or more of the 4 major antiretroviral (ARV) drug classes (NRTI, NNRTI, PI, INSTI) were tested phenotypically using a single-cycle assay (Monogram Biosciences).

Results: In all, 19 HIV gag cleavage site mutants (single and double mutants with L363F/M, A364V, Q430R, A431V, K436E, I437T/V, L449H/V/F, P453L) with or without PR mutations V82A or I84V, as well as 51 patient derived isolates, 24 of which contained gag cleavage site mutations, were analyzed phenotypically. The GS-6207 EC50 fold-change compared to wild-type (WT) ranged from 0.4 to 1.9 for these mutants. In contrast, high levels of reduced susceptibility to PIs (>100-fold) and MIs (>64 fold) were noted in some mutants. Isolates with resistance mutations against the 4 main classes of ARV drugs (NRTI, NNRTI, PI, INSTI; n=40) remained fully susceptible to GS-6207 (0.3- to 1.1-fold change), while highly reduced susceptibility was observed for control drugs from each class.

Conclusions: HIV gag cleavage site mutations did not affect the activity of GS-6207, while some conferred resistance to PIs and MIs. Similarly, GS-6207 activity was not affected by naturally occurring variations in HIV gag, in contrast to the loss of activity observed for MIs against nearly half of the mutants. Finally, the activity of GS-6207 was unchanged in the presence of resistance mutations to the 4 main ARV drug classes. These data indicate that the activity of GS-6207 is not altered by the presence of gag cleavage site mutations, gag polymorphisms, or resistance mutations to the main approved drug classes, supporting the evaluation of GS-6207 in all PLWH regardless of treatment history, including those with multi-class resistance.

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Prevalence of transmitted resistance mutations to rilpivirine and doravirine in treatment-naive patients in a large clinical and resistance database

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Background: Non-nucleoside reverse transcriptase inhibitors (NNRTIs), first approved in 1996, have historically been a cornerstone of antiretroviral therapy. However, their low genetic barrier to resistance may affect the HIV transmitted drug resistance (TDR). TDR, therefore, can lead to virological failure as a consequence of pre-existing resistance to one or more drugs in the first-line antiretroviral regimen. The aim of this study was to investigate the prevalence of TDR to both rilpivirine (RPV) and doravirine (DOR) among naive patients from a large Italian cohort, in the 1996-2019 period.

Materials and methods: In this retrospective study, we selected all ART-naïve HIV-1 infected patients with at least one plasma RT/PR genotypic resistance test (GRT) available, performed from 1996 to 2019 from the ARCA database. We considered major resistance mutations paneled by the Stanford HIV Drug-Resistance Database 2019. We evaluated the overall and 5-year prevalence of TDR to RPV and DOR in all isolates.

Results: We retrieved 2,441 isolates from 1,667 ART-naive patients: mostly males(69%), Caucasian (82%) with subtype B (71%) and with a median age of 51 (IQR 42-58) years. The overall prevalence of DOR and RPV resistance-associated mutations were 3.6% and 10.6% respectively, while 3.6% was the prevalence of TDR affecting both DOR and RPV. The DOR-TDR frequency decreased from 9% in 1996-2000 to 2% in 2006-2010 (p=0.001), remaining stable until 2016-2019 (p=0.500). On the contrary, the frequency of RPV-TDR increased from 3% in 1996-2000 to 15% in 2001-2005 (p<0.001) and thereafter decreased, but not significantly, to 12% in 2006-2010 (p=0.257), staying stable until 2016-2019 (p=0.158). Resistance to both DOR and RPV was comparable to RPV-TDR trend. The percentage of GRTs susceptible to both DOR and RPV remained stable around 85% during the study period except for a minimum percentage (70%) reached in 2001-2005. The most frequent DOR mutation was V106I (68 isolates), followed by Y188L (26 isolates); the most frequent RPV substitutions were E138A and Y181C (96 and 71 isolates, respectively). Similar TDR frequency and prevalence were observed when classifying the isolates according to B and non-B subtype over time.

Based on Stanford algorithm, the prevalence of GRTs considered with Potential Low-level, Low-level, Intermediate and High-level resistance to DOR and RPV were 3, 6, 5 and 4% and 3, 8, 3 and 7%, respectively.

After stratifying by viral load, we observed RPV-TDRs being more frequent in patients with HIV-RNA under 25,000 copies/mL(p<0.001) while DOR-TDRs were evenly distributed over the whole viral load range (p=0.138). Oppositely when we tested the distribution of TDRs based on CD4 levels, it emerged that the highest frequency of DOR-affecting mutations was detected in patients with CD4 levels lower than 350 cell/µL (p<0.001) while RPV-TDRs showed a constant distribution over the whole CD4 levels range (p=0.274).

Conclusion: Although a substantial increase of an overall resistance emerges in the period 2001-2005 we also observed a decrease and stabilization of TDR percent fluctuation in the last decade. Our hypothesis is that this stabilization is due to the availability of new NNRTIs which result in a selective pressure spanning along more residues. This highlight the necessity of having large therapeutic spectra as well as the necessity to execute genotype test on naive patient in order to drive the clinician's decision toward the selection of the most effective drug.

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Genotyping and treatment issues with unusual 1e and 1g HCV subtypes from African subjects: report of two cases.

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Background: Unusual HCV genotype 1 (HCV1) subtypes are rare in Europe and more prevalent in Africa. The majority of unusual HCV1 subtypes have natural resistance associate substitutions (RASs) in NS5A region and treatment with first generation DAAs has shown a lower sustained virological response (SVR) rate compared with common HCV1 a/b subtypes.

Material and Methods: We analysed the HCV sequence database at the HIV and Hepatitis unit of the University Hospital of Siena, for unusual non a/b HCV1 subtypes. Genotyping by Sanger sequencing of the NS3/NS5A/NS5B and 5' UTR/core regions was compared with commercially available 2nd generation rapid genotyping assays results. The Geno2pheno[HCV] version 0.92 web system was used to compute genotype/subtype and predict drug resistance based on sequence data.

Results: Of 136 HCV genotype 1 infected patients with complete sequencing of the NS3/NS5A/NS5B and 5'UTR/core regions, 44 harboured subtype 1b (32%), 90 subtype 1a (66%) and 2 unusual non 1a/1b subtypes (1.5%), namely one 1e (ptE) and one 1g (ptG). Both patients were from sub-Saharan Africa (SSA). By commercially available 2nd generation HCV genotyping assays, the 1e virus was classified as 1b (GEN-C 2.0, Nuclear Laser Medicine) or indeterminate (Abbott RealTime HCV Genotype II) and the 1g virus was classified as 1a (ABBOTT RealTime HCV Genotype II). At baseline, the subtype 1e sequence had two NS3 (positions 36, 54) and multiple NS5A (positions 24, 28, 30, 31, 93) polymorphisms and the subtype 1g sequence had two NS5A polymorphisms (positions 24, 30) labelled as substitutions at scored positions by Geno2pheno[HCV]. PtE (75 years old, fibrosis stage F3) failed DAA treatment with the pangenotypic regimen sofosbuvir/velpatasvir for 12 weeks, while SVR 12 was achieved in ptG (62 years old, fibrosis stage F1) after treatment with the same regimen.

Conclusions: Unusual non a/b HCV1 harbour resistance associated natural polymorphisms which may reduce SVR rates compared with more common subtypes. Rapid genotyping assays fail to detect these sequences and treatment with a triple DAA regimen may be a preferred option.

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SUSCEPTIBILITY TO BNABS OF TRANSMITTED HIV VARIANTS AMONG RECENT INFECTIONS IN FRANCE

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Background: Pre-existing resistance to broadly neutralizing antibodies (bNAbs) restrains their use for prevention and treatment of HIV infection. In addition, an increasing resistance of HIV to neutralization over time has been observed arguing for a prospective monitoring of the sensitivity to bnAbs of all prevalent HIV subtypes. Here, we analyzed the susceptibility to bNAbs of HIV transmitted variants among recently infected individuals in France with a focus on evolution over time.

Methods: We assessed the sensitivity to seven bnAbs against a panel of 73 early-transmitted subtype B and CRF02_AG viruses (the most prevalent subtypes in Europe) over a 25-year period of the French epidemic (1987-2012). Samples were obtained during acute/recent infection from individuals included in the ANRS PRIMO cohort. Env pseudoviruses were constructed and neutralization assays on TZM-bl cells were performed using bnAbs targeting the CD4-binding site (CD4bs; VRC01, 3BNC117), the V1/V2-glycan region (PG9, PGT145), the V3-glycan region (PGT121, 10-1074), and the gp41 membrane proximal external region (MPER; 10E8).

Results: Participants' median CD4 count was 506 cells/mm3, median viral load was 5.1 log10 copies/mL and the estimated time from infection was 41 days. bNAbs targeting the CD4bs and 108E were the most potent and broadly neutralizing. VRC01 neutralized 92.5% of all variants at the target concentration of 10 µg/mL. 3BNC117 IC50s were the lowest of all bNAbs (respectively 0.01 et 0.25 µg/mL for B and CRF02_AG variants; Mann-Whitney P<0.05). CRF02_AG were more resistant than B viruses regarding bNAbs targeting V3 (64-67% of the strains neutralized at 10 µg/mL vs 78-88%, respectively). This resistance was associated with the absence of the glycosylation site N332 (p<0.01). Both subtypes were more resistant to bNAbs targeting V2 (55-65% of the strains neutralized at 10 µg/mL). Finally, we observed an increased resistance to several bNAbs over the course of the epidemic - especially those targeting the CD4bs – which correlated with the continuous diversication of Env sequences over time (Spearman P<0.05).

Conclusion: Of the bNAbs in clinical development tested here, none neutralized 100% of T/F variants, indicating that combinations will be required to achieve a full coverage for prevention and treatment. As in other countries, we conrmed the natural drift of HIV towards higher resistance to bNAbs for the most prevalent subtypes spreading in France, arguing for a continuous surveillance of HIV transmitted variants around the globe.

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Baseline Hepatitis C Virus NS5A Resistance-Associated Polymorphisms in Patients with and without HIV coinfection in Mexico

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Background: The rate of treatment success of hepatitis C virus (HCV) infection with direct-acting antivirals (DAAs) has improved amazingly. However, The DAAs efficacy is diminished by resistance-associated substitutions (RASs); among these, the NS5A RASs, which might be found at baseline and virological treatment failure, are clinically significant because of its persistence and association with elevated fold changes to several NS5A inhibitors. Therefore, we aimed to evaluate the frequency and predisposing factors of baseline NS5A RASs in patients coinfected with HIV and HCV monoinfected patients with genotype 1b (GT1b) and genotype 1a (GT1a), which are the most prevalent genotype in Mexico, and a comparison between them was performed. Moreover, we evaluated evolutive viral relationships between both groups of patients by phylogenetic analysis

Materials and Methods: We retrieved blood samples of HCV-infected patients stored in the molecular virology laboratory of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran within the period 2011-2018. Samples of 49 patients with HCV/HIV co-infection and 30 HCV monoinfected were included. Viral load quantification, genotypification, and NS5A Sanger sequencing were performed. RASs known to generate elevated fold changes to NS5A inhibitors in vitro or related to treatment failures in clinical trials were sought. We performed multivariate analysis for seeking factors associated with RAAs presence. The relationship among sequences with RASs was evaluated by a maximum likelihood phylogenetic tree.

Results: Fifty-five patients were infected with G1a, of whom 44 (80%) were HIV-infected patients. The presence of RASs was of 14% (6/44), and were distributed as follows; 5(11%) harbored M28V RAS and 1(2%) A92T RAS. Twenty-four patients were infected with HCV GT1b, of which only 5 (21%) were HIV-infected and the presence of RAS was found in 17/24 (71%), of which were as follows; Y93H+F37L+Q54H (1/24), Y93H+F37L (1/24), P58S (1/24), L31F+F37L (1/24), F37L+H/Q54H 3/24), F37L (10/24). We did not find significant differences in the RASs presence between coinfected and monoinfected patients. In the multivariate analysis of factors associated with RASs presence, only GT1B was significant (aOR,16.37; 95% CI, 2.74-97.48; P=0.002). A cluster of sequences from HIV/HCV GT1a patients was found; however, we did not find phylogenetic relationships among sequences with NS5A RASs.

Conclusions: In our population of HCV-infected patients, the frequency of NS5A RASs at baseline was somewhat similar to the previously reported worldwide. HCV GT1B showed the most significant predisposition to harboring NS5A RASs. Of note, despite there were clusters of transmission among HIV-infected patients, NS5A RASs were not transmitted. More studies are needed about if GT1b harboring a higher prevalence of RASs might have clinical implications as well as the risk of NS5A RASs transmission with the forthcoming extended use of DAAs.

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Substantial clustered transmission of the acute hepatitis C among HIV co-infected men-who-have-sex-with-men (MSM)

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Introduction: The World Health Organization (WHO) has declared elimination of hepatitis C virus (HCV) transmission as a global health threat. To achieve this ambitious goal, WHO recommends expanding direct-acting antivirals (DAAs), which has high cure rates and thereby prevents onward transmission. Widespread use of DAAs has reduced the number of new HCV infections by 50% in the Netherlands. Unfortunately, virological failure can occur and is associated with emergence of resistance associated substitutions (RAS). Transmission of RAS can hamper HCV elimination efforts. In Western Europe, HCV is predominantly transmitted between HIV-positive men-who-have-sex-with-men (MSM). We investigated the transmission dynamics of HCV and RAS among MSM, before and after the widespread use of DAAs.

Methods: We included 90 plasma samples from 101 acute HCV genotype 1a infected HIV-positive MSM that were diagnosed in one Belgian and ten Dutch HIV-treatment centres between 2013 and 2018. Samples were subjected to Sanger sequencing or Illumina Sequencing (15% cut-off for variant calling). RAS were defined based on the EASL guidelines. Phylogenetic analysis was based on concatenated NS5A and NS5B sequences from the included plasma samples and from 425 publicly available sequences. A cluster was defined using a bootstrap support of 100% and a genetic distance threshold of 1.5% (maximum likelihood analysis and GTR+G4+I).

Results: We found strong clustering of HCV sequences and distinguished five major clusters including 84% of individuals. Four clusters included ≥10 individuals that were sampled in different treatment centres. One-third of all new HCV infections (28 individuals) clustered in one large cluster, of which 96% harboured the NS5A RAS M28V. The number of clusters and the proportion of individuals belonging to a cluster remained stable in the period before and after introduction of DAAs in 2015.

Conclusion: Large clusters of acute HCV infections were detected in the years preceding as well as after introduction of DAAs, suggesting active transmission of HCV among HIV-infected MSM. A stable transmission of the RAS M28V was found, which is known to influence susceptibility to NS5A inhibitors. The continuing transmission of M28V illustrates the need for resistance surveillance in populations with ongoing HCV transmission. Despite elimination efforts, most clusters persisted, highlighting the need for targeted monitoring and risk reduction strategies to achieve the WHO elimination goal.

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Ibalizumab shows in vitro activity against group A and group B HIV-2 clinical isolates

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Background: In addition to being naturally resistant to NNRTI and enfuvirtide, HIV-2 easily selects drug-resistance associated mutations to protease, NRTI and integrase inhibitors at time of virological failure. This has led to a worrying rise in the number of patients with multi-drug resistant (MDR) viruses. Ibalizumab (IBA) is a long-acting humanized monoclonal antibody that blocks entry of virus into the host cell. It is approved by the FDA and the EMA for treatment-experienced persons infected with MDR HIV-1. No data were available on the activity of IBA against HIV-2 isolates.

Methods: Isolates from 6 HIV-2-infected persons (4 group B, 2 group A), the ROD HIV-2 group A reference strain and the BRU HIV-1 reference strain were assessed for IBA phenotypic susceptibility. We adapted the ANRS peripheral blood mononuclear cells (PBMC) phenotypic assay. Briefly, PHA-activated PBMC were incubated with increasing concentrations of IBA for 1h, prior to infection. Two hours post-infection, cells were washed and then resuspended in complete RPMI media containing IBA. At day 4 post-infection, HIV-2 replication was assessed on cell supernatant using a qRT-PCR (Biocentric-HIV-2). Phenotypic susceptibility was assessed through 50% inhibitory concentrations (IC50) and Maximum-Percent-Inhibition (MPI). All HIV-2 isolates had previously been obtained by co-cultivation of PHA-activated PBMC pool obtained from healthy blood donors.

Results: IBA inhibited viral replication for all seven HIV-2 isolates, with IC50 ranging from 0.002 to 0.18µg/mL, and for the HIV-1 reference strain (IC50=0.01µg/mL). MPI was below 80%, between 80 and 90%, and >90% for 2, 1 and 4 strains, respectively. The 2 isolates with the lowest MPI (74 and 77%) also had the highest IC50 (0.18 and 0.09µg/mL, respectively).

Conclusions: These data demonstrate for the first time that IBA is active in vitro against both HIV-2 epidemic groups, with IC50 and MPI similar to those observed for HIV-1. IBA could be included in therapies for HIV-2-infected-persons displaying MDR viruses, a situation more frequently observed in HIV-2 than in HIV-1. Clinical studies of IBA-based regimens in HIV-2-infected-patients are warranted.

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Antiretroviral resistance in patients with multiple treatment failure in a low-middle income setting

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Background: Failure to suppress viral replication during the antiretroviral therapy drives to the selection of resistant virus, which has an impact on treatment sequencing as well as in morbidity and mortality. In low-income settings availability of resistance tests is very limited and ARV changes are made frequently after prolonged virological failure. Our aim was to establish the prevalence of ARV resistance in patients with multiple failures (at least 2 failing ARV regimens).

Material and methods: In this cross-sectional retrospective study we included 77 patients with a resistance test after a history of multiple regimen failure (>2) from 2009 to 2019. For the purpose of this analysis we determined the level of resistance using Stanford HIV database program having 2 groups based of the score: Susceptible <30 and resistant >30.

Results: Resistance to DRV was present in 6.89% (6/87), for other PIs in 22.98% (20/87), for ETV in 27.58% (24/87), DOR in 27.58% (24/87), TDF in 17.24% (15/87) and for RAL/EVG and BIC/DTG was 6.89% (6/87) and 1.14% (1/87) respectively. Tropism test was performed only in 6 cases, one of which had a Non-R5 tropism.

Conclusions: Most ARV combinations for multiple failure cases include DRV, ETV/DOR TDF and/or an INSTI. While with our results we can create a salvage combination in most cases, it is worrisome the high resistance to ETV and DOR despite most cases were not using NNRTIs in the current failing regimen. Due to its recent introduction, INSTIs could still be a good option for salvage combinations.

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Prevalence and Correlates of Pre-Treatment HIV Drug Resistance among HIV-Infected in the Republic of Belarus

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Human immunodeficiency virus, subtypes, drug resistance mutations, antiretroviral drugs.

Introduction: In the Republic of Belarus 28748 cases of HIV infection were recorded over the entire observation period. HIV prevalence rate among people living with this infection on November 1, 2019 was 230.48 per 100,000 population. In some regions, this indicator varies from 88.56 (Grodno region) to 574.97 (Gomel region). In 2018, for the first time a pilot study was conducted to analyze the genetic diversity of HIV and the prevalence of PDR mutations in our country.

Objective: to establish the genotypic diversity and prevalence of drug resistance mutations to ART in the Pre-Treatment HIV Drug Resistance (PDR) group in the Republic of Belarus.

Materials and methods: Serum/ plasma obtained in 2018 under the project RESEDA from 199 patients residing in the territory of the Republic of Belarus. Drug resistance mutations were detected by sequencing the gag-pol region using the "Bel HIV-1 resistance genotype" test systems produced by The Republican Research and Practical Center for Epidemiology and Microbiology (RRPCEM) (Belarus) and AmpliSense HIV-Resist-Seq produced by the Central Research Institute of Epidemiology (Russia). Multiple nucleotide sequence alignment was performed using the ClustelW algorithm and MAFFT. The phylogenetic tree was constructed using the ML maximum likelihood algorithm in the MEGA 6.0 Phylogenetic maximum likelihood program (PHML) with the GTR + I + G nucleotide replacement model. Tree topology optimization was conducted with Best of NNIs and SPRs. The trees were visualized in the FigTree v.1.4.2 program. Nucleotide sequences were analyzed for mutations significant for epidemiological surveillance according to SDRM sheet (2009) and for major mutations using the Stanford database (https://hivdb.stanford.edu/hivdb/by-sequences/).

Results: Among 199 samples 186 (93.5%) belonged to the HIV-1 subtype, represented by the A6 sub-subtype. Subtype B was detected in 4.0% (n = 8) of HIV-infected patients, and in 1 case (n = 0.5%) subtype G. In 2.0% of cases circulating recombinant forms were found in particular CRF 02_AG (1 , 0%, n = 2) and CRF_03_AB (1.0%, n = 2).

In 28 (14.1%) patients, drug resistance was identified due to the presence of mutations that are significant for surveillance, according to the WHO mutation list (surveillance drug resistance mutations, 2009 SDRM). In 26 (13.3%) patients, drug resistance was identified due to the presence of MDRM mutations (major drug resistance mutations, 2019). The most common mutations for NNRTIs: K103N (11 patients), G190S (5 patients), K101E and Y181C/V (2 patients each); to NRTIs: M41L and M184V (3 patients each), in isolated cases, replacements were identified in positions D67N, T69N, K70R, K219Q).

Conclusion: Obtained data indicate a high level of prevalence of mutations in the PDR group in Belarusian cohort with a tendency to increase drug resistance of a high level to NNRTI class drugs. Considering the widespread use of ART in the country, further representative studies are required, according to WHO recommendations, at the national level, for resolving the issue of optimizing first-line ART after drug resistance studies prior to treatment and the need for increased access to new antiretroviral drugs.

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Late presentation and HIV drug resistance in patients followed in Portugal between 1984-2017

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Background: Late presentation for care is a significant and persistent issue for success of therapy outcomes individually. However, is also a problem for public health systems, since late presenters (LP) may modulate negatively the slowing down of HIV transmission chains. Late diagnosis associated with drug resistance, either transmitted (TDR) in drug-naïve individuals, or acquired (ADR) for individuals exposed to ARV treatment, is a problem, since may involve a substantial reduction in virologic success and a decrease in the control of HIV infection in patients which may at the outset present greater vulnerabilities to the HIV treatment.

Objective: The aim of this study is to analyse the prevalence of mutations associated with transmitted and acquired drug resistance in a population of late presenters compared with non-late presenters followed in a Portuguese hospital between 1984 and 2017.

Method: Socio-demographic characteristics and genomic sequences from 520 HIV-positive patients from a Portuguese hospital diagnosed between 1984 and 2017 were analysed and divided into late presenters (LP) and non-late presenters (NLP) according to the The European Late Presenter Consensus working group. TDR was defined by the presence of one or more surveillance drug resistance mutations (SDRM) according to the World Health Organization (WHO) 2009 surveillance list. To evaluate TDR, nucleotide sequences were submitted to the Calibrated Population Resistance tool -HIV drug resistance database version 8.0. Phenotypic resistance to ART drugs was evaluated using the Standford HIVdb v8.9.

Results: 50.8% of the study population was defined as late presenters, being mostly men (72%), with an age older than 40 years old (51.9%), infected by heterosexual contact (58.3%). Late presentation was higher in patients coming from sub-Saharan countries (32.2%) compared to patients of Portuguese origin (59.5%). LP were predominantly infected with subtype B (43%) and subtype G (27.4%) and presented lower rate of TDR compared with NLP (4.9% vs 8.9%), associated with high level of resistance to NNRTIs (3.8%), mostly due to the K103N/S mutation (22%). On the other hand, LP presented higher rate of ADR compared to NLP (62.7% vs 37.2%), associated to high level of resistance to NRTIs (49.2%), mainly due to M184I/V mutation (40.7%).

Conclusion: Early diagnosis is an essential tool for successful therapy and for slowing down the HIV epidemic. Screening polices need to be urgently implemented, particularly in most-at-risk categories for late presentation and drug resistance, such as migrants, older patients and those with heterosexual intercourse as risk factors for HIV acquisition

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Genetic characteristic of HIV-1 in patients with treatment failure in Kyrgyzstan in 2017-2018.

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Background. A total of 5887 persons were living with HIV in Kyrgyzstan in 2018 and 3718 of them were on therapy. Previous analysis (published in 2015) of samples collected in Kyrgyzstan in 2009-2010 showed the domination of CRF02_AG and A1-subtype (IDU-A variant) and revealed the cases of new AG-recombinant forms' formation. According to the same study, IDUs was the dominating route of infection (84.2%). However after 2015, the HIV-1 taxonomy was modernized and the interpretation of viral sequences variant was changed (IDU-A belongs to A6 and the most CRF02_AG samples – to CRF63_02A1).

Here we present the results of HIV-1 pol-gene region analysis (REZEDA-study) in samples obtained from Kyrgyz patients receiving antiretroviral therapy and with virologic failure.

Material and Methods. We analyzed HIV-1 pol-gene sequences (positions 2253-3368) coding protease and reverse transcriptase fragment in plasma samples obtained in 2017-2018. The sequences alignment and phylogenetic analysis were performed with MEGA6.0. Additional analysis using HIV-Blast was carried out for discordant phylogenetic results. The drug resistance analysis was carried out using HIVdbProgramv.7.0.

Results. Totally, 100 samples were studied. 35 samples were collected in 2017 and 65 samples – in 2018. The samples were collected in different regions mainly in Chuy Region and Bishkek (n=57) and Osh Region (n=31). 45 samples (45%) were collected from women; the median age of patients was ≈36 years (5-66). The main route of infection was heterosexual (n=49) and IDU (n=31). Other routes of infection: parenteral including infection in medical facilities (n=16), mother-to-child (n=2). Only 2 patients were MSM. The routes of infection had a strong correlation with patient sex: 31/49 samples of heterosexual route of infection were obtained from women and 27/31 of IDU samples – from men. There was no correlation between geographic region and infection routes. The main ART drug combinations of first-line therapy were TDF/FTC/EFV (n=52) and AZT(PhAZT)/3TC/EFV (n=23).

56 samples formed a cluster with CRF63_02A1 references. 41 samples belonged to subtype A6. One of two MSM-samples harbored subtype B specific to this vulnerable group. Also, two samples (from man and woman with heterosexual route of infection) were harbored recombinant CRF_94 which was previously detected in Kyrgyzstan and France.

There were no mutations to protease inhibitors in samples studied. The most frequent NRTIs mutation (n=39) was M184V associated with high-level resistance to 3TC and FTC. Also viruses in 38 samples harbored K103N/S, NNRTIs mutations which resistance effect to NVP and EFV. Finally, we found G190A/S in 12 samples associated with resistance to NVP и EFV.

Conclusion. Apparently domination of heterosexual route is associated with a high ratio of women in patients group studied compared with previously published studies. Considering the HIV-1 taxonomy changes, our results are consistent with data about AG-recombinants' dominating in Kyrgyzstan earlier. At the same time, CRF94-samples indicate this form spreading in the country.

The drug resistance in samples is typical for patients treated by 3TC, FTC, and EFV. For the estimation of this drug resistance effect in global HIV-epidemic in Kyrgyzstan, it is important to investigate the spreading of the same mutations in naïve-patients group.

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Case of detection of drug resistance mutations hepatitis C virus to the direct-acting antivirals

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Background. The advent of direct antiviral drugs (DAAs) caused a major breakthrough in chronic hepatitis C. Thanks to this, the antiviral drug significantly reduced the duration of treatment, and the virological response reached about 98%. Nevertheless, therapy with this group of drugs causes the relapse of patients with diseases associated with the appearance of mutations in the drug resistance of hepatitis C viruses to DAAs.

Objective. To identify mutations in the drug resistance of hepatitis C virus in a patient with relapse during treatment with DAAs.

Materials and Methods. A blood sample from a patient with chronic hepatitis C was used in the work. This patient has been on treatment since 11/11/2018. According to the Unified European Recommendations, Sofosbuvir 400 mg + Daclatasvir 60 mg (1tab.) Was administered once a day, with meals at the same time. One month after the prescribed therapy, HCV RNA was not detected, ALT-18 ME / L, total bilirubin-14.2 µmol / L. Three months later, a screening examination revealed an increase in viral load to 1.1 × 10 ^ 6 and an increase in ALT to 50 ME / L.

In the laboratory of immunology and virology of HIV infection Pasteur determined the genotype 1b of the virus, obtained the nucleotide sequences of three target regions NS3, NS5a, NS5b of satisfactory quality and analyzed these sequences for resistance mutations.

Results. An analysis of the NS3, NS5a, NS5b regions revealed the replacement of the tyrosine amino acid with the histidine amino acid at position 93 in the NS5a region, which causes a decrease in the sensitivity of the virus to Daclatasvir.

Conclusions. Despite the achievement of significant success in the treatment of chronic viral hepatitis C, cases of relapse in patients with DAAs therapy continue to increase. This is due to the occurrence of nucleotide substitutions in certain regions (NS3, NS5a, NS5b), which leads to a decrease in the sensitivity of the virus to the drug. At present, there are no domestic kits for detecting these mutations, but they are being developed. The possibility of detecting certain nucleotide substitutions will help to determine the cause of the recurrence of chronic hepatitis C during treatment with DAAs and to choose an alternative treatment regimen.

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Prevalence of pretreatment HIV-1 drug resistance among treatment-naïve patients in the Russian Federation, 2014–2018

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Background: The roll-out of antiretroviral therapy (ART) has been a major breakthrough in the global response to the epidemic of HIV. Access to ART has been increasing in Russia since a national program was initiated in 2006. In the last decade can see a significant expansion of ART coverage from 12.6% in 2008 to 44% in 2018.

Unfortunately, the success of ART is frequently limited by the onset of HIV drug resistance (DR), included DR among naïve patients as a result of the transmission of DR-variants of the virus.

The World Health Organization (WHO) recommends a method to estimate pretreatment HIV drug resistance (PDR) among patients who are going to initiate ART for evaluating the effectiveness of the first -line regimen in countries.

Unfortunately, national studies of PDR surveillance don't conduct in Russia.

The aim of this study was to determine the prevalence of PDR among treatment- naïve patients from Russia before ART-initiation.

Materials and methods: We analyzed protease (PR) and reverse transcriptase (RT) sequences from 1171 treatment- naïve Russian patients, before initiation of ART with data of diagnosis in 2014 (n=195), 2015 (n=231), 2016 (n=320), 2017 (n=205), 2018 (n=220).

PR, RT-sequences were obtained by AmpliSens® HIV-Resist-Seq kit. Viral subtype and drug resistance were determined using the HIVdb Program v.8.9-1.

PDR was estimated using the Stanford algorithm, defining viruses with a Stanford penalty score ≥ 15 to protease inhibitors (PIs): ATV, DRV, LPV, any nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs): EFV, NVP as resistant, as recommended by WHO standardized protocols.

Results: The most frequent determined clade was sub-subtype A6 (77.6%), subtype B was detected for 11.1% viruses, subtype G for 1.9% and the circulating recombinant forms (CRF): CRF63_02A1 (4.7%), CRF 02_AG (3.3%), CRF03_AB (1.0%), CRF 01_AE (0.2%), CRF 20_BG (0.1%), CRF06_cpx (0.1%).

The prevalence of PDR to any drugs classes was determined in 4.6%, 4.3%, 3.8%, 4.9% and 7.3% patients with years of diagnosis 2014, 2015, 2016, 2017 and 2018, respectively.

Least of all, PDR was found to drugs of IP class: 0% among patients with data of diagnosis in 2016-2018 and 0.5%, 0.4% in 2014, 2015, respectively.

PDR to NRTI also was pretty low level and had a trend from 0.5 % among 2014-patients to 0.9% in 2018-patients.

As expected more often PDR was found to NNRTI drugs because of widespread use and low genetic barrier of this class. DR to NNRTI was detected in 3.6%, 3.0%, 3.4%, 3.9% and 6.8% sequences from patients with years of diagnosis in 2014, 2015, 2016, 2017 and 2018, respectively. It should be noted that a high level of DR was determined more often to NNRTI drugs. Thus among patients with 2018 years of diagnosis prevalence of overall DR to EFV and NVP were defined as 5.9% and 6.8%, respectively, including high level of DR to EFV and NVP – 2.3% and 5.5%.

Conclusion: We observed an increase in overall PDR from 4.6% to 7.3% among patients with years of diagnosis 2014-2018, driven primarily by DR to NNRTI (3.6%-6.8%). However, the prevalence of PDR to NNRTI had not reached 10%, the threshold at which the WHO recommends urgent public health action. It should be noted that we analyzed the prevalence of PDR only among naïve patients, so it should also evaluate PDR among patients with prior ART exposure.

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The HBV treatment outcome to cART in HBV/HIV co-Infected patients who failed lamivudine containing regimens

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Background: All HIV/HBV co-infected patients in Serbia have been treated with cART containing and anti-HBV drugs, irrespective of CD4+ T-cell counts and HBV disease status in order to prevent more active liver disease. Like in many developing countries, lamivudine containing HAART was used in all HBV/HIV co-infected individuals.

Material and Methods: A cross sectional cohort study was conducted in order to analyse the optimal treatment response of both HIV and HBV infections among HBV/HIV co-infected patients who underwent lamivudine containing HART, and if experienced lamivudine failure, switched to tenofovir (TDF) based cART.

Results: After the mean duration of lamivudine containing cART of 7.3 ± 3.2 years (range: 1 - 15 years), lamivudine failure was recorded in 35/67 patients (52.2%). Out of twenty-two remaining subjects with favourable virologic response to lamivudine, all achieved HBs Ag loss, out of whom 2 patients developed anti-HBs antibodies, after 4.1 ± 3.1 years (range: 1 - 15 years), and 9 ± 2.8 years (range: 7 – 15 years), respectively. After additional 2.1 ± 1.1 years of tenofovir containing cART, hepatitis B plasma viral load was 1.3 ± 1.1 log10 IU/mL HBV DNA. After TDF introduction, the probability of achieving optimal treatment response, which included either suppression of HBV DNA to less than 20 IU/ml, and/or HBs Ag loss, was 20%, 60% and 90% after additional 2, 3 and 5 years of TDF containing cART, respectively.

Conclusions: The outcome of tenofovir containing cART among HBV/HIV co-infected patients, who previously failed HBV therapy with lamivudine containing cART, suggests that a prolonged treatment with TDF containing HAART is mandatory among those with suboptimal virological suppression and with small risk of cART failure.

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Hepatotoxicity and the hepatic panel analysis among HIV-positive Ukrainian patients receiving efaverentz and dolutegravir-containing modes

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Background: Hepatotoxicity is a serious complication in patients taking ART. Co-infection with hepatitis B and C viruses viruses increases the risk of liver toxicity while taking antiretroviral therapy. This study focuses on the risk of liver complications using measurements of transaminases and bilirubin before prescribing ART, taking a regimen containing efavirenz (EFV), and after switching to dolutegravir (DTG) regimen among HIV-infected Ukrainian patients.

Materials and Methods: 271 HIV-positive patients who applied for medical help between March 2015 and May 2019 were included in this study. Liver panel - enzymes (including alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT) and bilirubin was interpreted in accordance with international recommendations for assessing the levels of enzymes and bilirubin. The 1st section of the study included patients prior to prescribing ART (the 1st group of patients - without co-infection HIV/HCV or HIV/HBV, the 2nd group - with co-infection). The 2nd section of the study included patients who had previously taken EFV for 1-4 years (the 3rd group - without co-infection, the 4th group - with co-infection). And the 3rd section of the study included naive patients receiving DTG or patients after switching from EFV-containing to DTG-containing regimen for various reasons during the 2 year (the 5th group - without co-infection, the 6th group - with co-infection). Patients with decompensated liver cirrhosis were not included in our study, because they previously received an LPV/rtv-containing regimen. Thus, levels of enzymes and bilirubin were compared by direct counting and Pearson's chi-square tests. SPSS version 22.0 was used for statistical analysis.

Results: Males accounted for 34.7%, females accounted for 65.3% of the cohort (average age of patients was 37.2±10.2 years). When the 1st and 2nd groups of the study were compared, the results were obtained: 2% and 21% of patients had an increase in ALT, 4% and 17% - in AST, 2% and 9% - in GGT, 2% and 11% - in bilirubin. Comparison of the 3rd and 4th study patients' groups who had previously taken EFV for 1-4 years showed results: 7 % and 41 % of patients had an increase in ALT, 8 % and 37% - in AST, 8% and 27 % - in GGT, 4 % and 16 % - in bilirubin. Comparison of the 5rd and 6th study patients' groups who receiving DTG showed results: 3% and 23 % of patients had an increase in ALT, 5 and 18 % - in AST, 4% and 14 % - in GGT, 1 % and 10 % - in bilirubin ($p<0,05$).

Conclusuion: Among patients taking EFV, the level of enzymes was much higher, especially for patients with co-infection HIV/HCV. Among patients receiving DTG, or patients switching to DTG-containing regimen, the level of enzymes decreased significantly. Therefore, when choosing ART regimens, it is logical to give preference to InSTI, since these are ARV drugs that reduce the development of liver complications and are less hepatotoxic.

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Feasibility and efficacy assessment of hepatitis B vaccination among adult PLWH and vulnerable population in Vinnytsia region, Ukraine

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Background: Hepatitis B vaccination is a "recommended vaccination" for people living with HIV (PLWH) and vulnerable population (VP) according with Ukrainian national vaccination schedule and national HIV guideline. Nevertheless, vaccination implementation is limited. The aim of current study was to investigate the hepatitis B prevalence and vaccination coverage among PLWH and VP in Vinnytsia region and Ukraine as well as to assess the efficacy of conducted vaccination cases.

Materials and methods: The retrospective analysis of the hepatitis B virus (HBV) epidemiological data gathered from the national source (Public health center) was conducted. The efficacy analysis of the 3-doses HBV vaccine among PLWH and people from vulnerable groups have been done. The vaccination efficacy criterion is a presence of protective anti-HBs titer >10 IU/ml. The data were analyzed with the Statistica 13.3 package.

Results: There were 15578 new HIV cases in 2017 in Ukraine, and 12070 people were tested for HBsAg among them with 1844 (15,3%) positive results. The HIV prevalence was 136378 at the 01.01.2018 in Ukraine with 4480 (3,3%) chronic hepatitis B (CHB) cases included. HIV prevalence in Vinnytsia region was 2816 people with 107 (3,7%) CHB cases included.

There is only possible way to estimate HBsAg prevalence among VP – the seroepidemiologycal monitoring (SEM) within the harm reduction programs. When to compare SEM results – the prevalence among sex workers (SWs) was – 9,3% and 2,6%; among men having sex with men (MSM) – 4% and 0,6%; among people who inject drugs (PWID) - 13,6% and 5,4% in 2004 - 2007 and 2013 – 2017 years' intervals respectively. The routinely HBsAg screening is performed for the opioid substitution therapy (OST) program clients. It has been registered 10189 OST clients in Ukraine at the 01.01.2018 and 1364 (13,4%) HBsAg positive people among them. This percentage is significantly different than in the 2013-2017 years among PWID (p<0,01). There are 407 OST clients in Vinnytsia region with 20 HBsAg-positives (4,9%).

Worth to note, that VP and PLWH have possibility to be vaccinated for free only with the NGO assistance. There were 3755 people from VP who get 3 doses of hepatitis B vaccine across 2013 – 2017 period. Taking into account the lack of data of the total number of the group – it is not possible to assess the percent of vaccinated.

There are 379 (0,3%) of HBsAg-negative PLWH vaccinated with 3 doses of hepatitis B vaccine around Ukraine and 13 (0,5%) from HBsAg-negative PLWH vaccinated in Vinnytsia region (p=0,02) at the 01.01.2018. There are also 34 people with completed vaccination from VP in Vinnytsia. Thus the vaccination coverage is low in the region and in the whole country.

The protective anti-HBs titer >10 IU/ml was detected in 11 of 13 PLWH (84,6%) and 31 of 34 vulnerable population people (91,2%), (p=0,55) without significant difference between groups in Vinnytsia region.

Conclusions: VP seroepidemiological monitoring and PLWH medical observation data showed intermediate and high HBV prevalence in these groups. In the same time the vaccination coverage is very low.

Gained experience suggests us about efficacy of hepatitis B vaccination among PLWH as well as among VP, which allows to make CHB burden in Ukraine much lower.

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Natural course of hepatitis C virus infection: which factors affect spontaneous clearance of HCV among the Ukrainian population?

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Background: Around 30% of patients undergoing acute HCV infection are able to resolve their Hepatitis C (HCV) infection spontaneously. Recent studies have suggested that spontaneous clearance (SC) rates of HCV can vary considerably between 3.7-53% in different parts of the world depending on patient characteristics and genetics. Identification of patients who are likely to achieve SC is of utmost importance in deciding who to treat for HCV and where to wait for SC. The impact of the polymorphisms (SNP) in the human IL28B gene encoding the interferon of lambda type 3 (IFN λ 3) on SC in HCV/HIV-coinfected patients still needs to be better defined in Eastern Europe.

Methods: This retrospective observational study included 117 Ukrainian adults with HCV/HIV-coinfection who had not yet been treated for HCV but had started a first-line regimen ART > 6 months. All study subjects were tested for SNP in the IL28B gene. SC was defined as being HCV RNA-negative at least 2 years after the estimated seroconversion date (the 1st group) in 19 persons. Chronic hepatitis C was diagnosed in the other 98 patients (the 2nd group). Genetic markers and baseline characteristics from the two groups were compared. For genetic analysis we used two basic SNP in human IL28B gene: rs 12979860 with possible genotypes - SS, ST, TT and rs 8099917 with possible variants - TT, TG, GG. Potential factors associated with SC of HCV were identified by using multivariable logistic regression models. SPSS version 22.0 was used for statistical analysis.

Results: In our study SC of HCV was determined in 16.2%. Female gender (OR=3.23; 95% CI [1.47-7.11]), young age (OR= 2.39; 95% CI [1.2- 7.34]), icteric form of acute hepatitis C in the anamnesis (OR=3.44; 95% CI [1.14-11.67]), co-infection with hepatitis B virus (OR= 2.39; 95% CI [1.1- 7.34]), genotype variants: CC rs 12979860 (OR=1.6; 95% CI [1.11-3.7]) and TT rs 8099917 in IL28B gene were identified as the main factors that contribute to SC of HCV.

Conclusuion: This study suggests an association between the presence of genotypes CC rs 12979860 and TT rs 8099917 in the IL28B gene and SC of HCV among HIV/HCV-coinfected Ukrainians which may be helpful in making early treatment decisions.

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Achieving the goals of the Fast-Track 90-90-90 strategy in the context of an anti-terrorist operation

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Background. The United Nations HIV / AIDS Strategy has developed a Fast-Track 90-90-90 strategy aimed at eliminating the epidemic as a public health threat, to which the Donetsk region, along with other regions of Ukraine, has been involved.

The prevalence of HIV infection in Donetsk region is 2.2 times higher than the national one and amounts to 676.9 per 100 thousand people, and traditionally occupies 3-4 places in Ukraine.

Due to the active phase of the anti-terrorist operation in 2014-2016, the population of the territory subordinated to the Government of Ukraine increased by 20% at the expense of internally displaced persons, which may include a significant number of undiagnosed cases of HIV infection.

Materials & Methods: The study analyzed the statistics obtained from the regional bodies of state statistics, the available statistical and reporting data of sero-epidemiological monitoring of the spread of HIV. The territories subordinated to the Government of Ukraine were involved in the assessment, for which the primary data for 2014-2016 were sampled, with further retrospective analysis of indicators, their generalization and trends

Results. As of 01.01.2017, 1965510 people (46% of the total population of the region) live in territories subordinated to the Government of Ukraine.

Since 2014, the regional center for AIDS prevention and control in Donetsk remained in the territory not subordinated to the Government of Ukraine with all equipment, electronic database and reporting.

In May 2016, a new regional center in Slavyansk, which solves only partially the issue of organization of clinical and laboratory monitoring of the effectiveness of HIV treatment for the population of the region, starts operating in the territory subordinated to the Government of Ukraine.

During the ATO, the number of HIV positive patients under medical supervision increases from 62.3% in 2014 to 71.6% in 2016, 46% receive ART, 47% examined for viral load, and have an undetectable VH 43,2%.

As of January 1, 2018, 67.6% of the estimated number are under medical care in the oblast, they receive HAART - 56.8%, of which 47% have been surveyed, and they have an undetermined level of HE - 71.6%.

As of January 1, 2019, they know about their HIV-positive status, 66.0%, receive HAART - 75%, surveyed at HV - 67.7% and have an undetermined level of HV - 86.5%.

Conclusions. Based on the results obtained in the framework of the health services cascade to ensure the first “90%” of the implementation of the Fast - Track Strategy, another 4887 HIV-infected persons should be identified and registered. To reach the second 90%, it is necessary to involve another 5987 HIV-infected persons on ART, and to reach an undetectable viral load of 8401 persons to reach the last 90%.

With the current rates of medical supervision, it takes at least 6 years to reach 90% of the estimated number.

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Effectiveness and tolerability of bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) in HIV-1-infected adult patients in German routine clinical practice – 12-month results of the BICSTaR cohort

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Background: In clinical studies, B/F/TAF is highly efficacious and well tolerated in both ART-naïve and ART-experienced HIV-1-infected participants, without emerging resistance. The present analysis from the ongoing multinational, BICSTaR cohort study evaluated the effectiveness, safety and tolerability of B/F/TAF in clinical practice over 12 months in Germany.

Materials and Methods: Data from treatment-naïve (TN) and treatment-experienced (TE) participants from 20 German sites (cut-off date: October 2019) were included. Outcomes included HIV-1 RNA (missing=excluded analysis), drug-related adverse events (AEs) and persistence (% participants on B/F/TAF) at 12 months (M12).

Results: A total of 278 participants (38 TN, 240 TE) initiated B/F/TAF and had a M12 visit or discontinued the study prematurely. Most (93%) participants were male. Median age (Q1–Q3) was 47 (38–55) years, with 41% of participants aged ≥50 years (21% TN, 44% TE). At baseline, 0/38 TN and 194/218 (89%) TE participants had HIV-1 RNA <50 copies/mL and the median (Q1–Q3) was 4.7 (3.8–5.3) and 1.6 (1.3–1.7) $\log_{10}$ HIV-1 RNA copies/mL, respectively. Median CD4+ count (Q1–Q3) was 469 (283–726) and 697 (458–916) for TN and TE participants, respectively. The total number of comorbidities or coinfections at baseline was 201/278 (72%); these included neuropsychiatric disorders (27%), arterial hypertension (21%), hyperlipidaemia (19%) and cardiovascular disorders (12%).

The main reasons for starting/switching to B/F/TAF for TN participants were 'early treatment according to guidelines' and 'participant's wish' (58% and 53%, respectively); for TE participants these were 'simplification' and 'participant's preference' (60% and 40%, respectively).

Of participants with available data, HIV-1 RNA was <50 copies/mL in 33/33 (100%) TN and 187/200 (94%) TE participants at M12, and 196/200 (98%) TE participants had HIV-1 RNA <200 copies/mL. In a discontinued=failure analysis, 33/36 (92%) TN and 187/223 (84%) TE participants had HIV-1 RNA <50 copies/mL at M12. No major resistance substitutions to the components of B/F/TAF emerged. Within 12 months, median CD4+ cell counts had increased from 469 to 857 (TN) and 697 to 721 cells/µL (TE). Persistence with B/F/TAF was high at 89%, with 4 TN and 26 TE participants discontinuing B/F/TAF prior to M12 (17 due to AEs, 4 lost to follow up, 4 investigator discretion, 2 lack of efficacy, 2 deaths, 1 patient decision). There were no discontinuations due to renal/bone AEs. There were two discontinuations due to weight gain. Overall, drug-related AEs and drug-related serious AEs were reported in 35 (13%) and 2 (<1%) participants (1 nausea, 1 depression), respectively. The most common drug-related AEs were psychiatric (15 [5%] participants) and gastrointestinal symptoms (12 [4%] participants).

Conclusions: Consistent with randomised controlled trials, 12-month data from this observational cohort analysis, which included a large number of participants with comorbidities, support the high effectiveness and overall acceptable safety and tolerability profile of B/F/TAF in routine clinical practice in TN and TE participants.

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Long-Acting Cabotegravir + Rilpivirine for HIV Treatment: FLAIR Week 96 Results

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Background: Long-acting (LA) injectable regimens of the integrase strand transfer inhibitor (INSTI) cabotegravir (CAB) and the nonnucleoside reverse transcriptase inhibitor (NNRTI) rilpivirine (RPV) may address challenges of chronic daily oral antiretroviral therapy (ART), including risks of nonadherence and treatment failure. First Long-Acting Injectable Regimen (FLAIR; NCT02938520) is a randomized, phase III, open-label, multicenter study investigating noninferiority of switching to monthly CAB+RPV LA vs daily dolutegravir/abacavir/lamivudine (DTG/ABC/3TC [current ART regimen {CAR}]) in virologically suppressed adults infected with HIV-1.

Materials and Methods: ART-naive participants received induction therapy with oral CAR for 20 weeks. After 16 weeks, participants with HIV-1 RNA <50 c/mL could enter the maintenance phase (MP) and were randomized (1:1) to switch to LA or continue CAR. Those in the LA arm received an oral lead-in of CAB 30 mg + RPV 25 mg once daily for 4 weeks before receiving monthly injectable CAB+RPV LA. The primary endpoint was viral load (VL) ≥50 c/mL at MP Week 48 (W48) by US Food and Drug Administration (FDA) snapshot algorithm (inferiority margin, 6%). Endpoints assessed at MP Week 96 (W96) included viral loads ≥50 c/mL and <50 c/mL per FDA snapshot algorithm, confirmed virologic failure (CVF; 2 consecutive viral loads ≥200 c/mL), safety, tolerability, and patient satisfaction.

Results: From 629 participants who initiated induction therapy, 566 entered the MP (n=283 for each arm); 22% were female and 74% were white. At W96, 9 (3.2%) participants in each arm had HIV-1 RNA ≥50 c/mL (adjusted difference, 0.0; 95% confidence interval [CI], −2.9 to 2.9), underscoring the noninferiority established at W48. HIV-1 RNA <50 c/mL at W96 was observed in 245 (86.6%) participants in the LA arm and 253 (89.4%) participants in CAR arm (adjusted difference, −2.8; 95% CI, −8.2 to 2.5), a result that met the noninferiority criterion for this endpoint (inferiority margin, −10%). For the LA arm, the rate of CVFs was unchanged from W48 at W96 (4 participants [1.4%]); 3 had mutations in the NNRTI and INSTI domains). The CAR arm had 4 CVFs through W96 (vs 3 through W48); none had mutations. Across both treatment arms, adverse events (AEs) leading to withdrawal were infrequent. Injection-site reactions (ISRs) were the most common drug-related AE (86% in the LA arm); their frequency decreased over time. Median ISR duration was 3 days, and 99% were grade 1/2. Serious AEs were reported in 8.5% and 7.8% in the LA and CAR arms, respectively; no deaths occurred during the MP. At W96, the LA regimen was associated with greater treatment satisfaction vs oral CAR (adjusted difference, 2.3; 95% CI, 1.1 to 3.5), as measured by the HIV Treatment Satisfaction Questionnaire (status version).

Conclusions: CAB+RPV LA maintained viral suppression without further CVFs between W48 and W96 and was noninferior to oral standard-of-care ART. Although ISRs were frequently reported with CAB+RPV LA, they seldom led to withdrawal, and overall treatment satisfaction was higher than with oral standard-of-care ART. These results attest to the durability of CAB+RPV LA.

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Optimisation of First-Line Antiretroviral Therapy (ART) for Children Living with HIV in Uganda: Translation from policy to action

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Background: Uganda has been optimising ART for children: in 2014 the Nucleoside Reverse Transcriptase Inhibitor (NRTI) backbone changed from Zidovudine/Lamivudine (AZT/3TC) to Abacavir/Lamivudine (ABC/3TC); introduction of Lopinavir/ritonavir pellets (LPV/r) in 2016 and Dolutegravir in 2018 for children ≥20kg. Despite these efforts, 52.2% of children aged 3-10 years were still receiving AZT/3TC/NVP(Nevirapine) as first-line ART by June 2018. The aim of the current optimisation strategy (July 2018 to date) is to transition children with viral load

< 1000 copies/ml from AZT/3TC to ABC/3TC, and Nevirapine or Efavirenz (high burden of pretreatment drug resistance) to LPV/r pellets/tablets or Dolutegravir-containing first-line ART. Children with viral load > 1000 copies/ml on Nevirapine or Efavirenz are immediately switched to second line ART without waiting for a repeat viral load result. We describe below, lessons learned during implementation of the ongoing strategy.

Description: Between June-2018 to September-2019; an optimisation checklist, line-listing tool, standard operating procedures and job aides were developed. The check-list was used to identify eligible children for optimisation at the health facilities. These were transferred to the line listing tool for tracking. Using data from the national reporting system (DHIS-2) and web-based ART ordering system (WAOS), supply chain planning was done. Weekly national planning meetings, cascaded trainings and post-training mentorships were conducted. ART optimisation indicators were incorporated into the weekly PEPFAR surge dashboard to monitor implementation.

Lessons Learned: 71% (6,085/8486) of children 3months to <3 years were initiated on LPV/r pellets. Due to global shortage, 1.14% (250/21,898) children 3-<10 years were transitioned to LPV/r tablets from June 2018 to September 2019. 64.7% (6,381/9854) of children < 15 years weighing ≥20 Kgs were initiated on Dolutegravir in the same period. Proportions of children on AZT/3TC backbone reduced from 76.8% to 48% by June 2019. At patient level, changing regimens comes with additional challenges of effectively communicating changes in dosing schedules or administration procedures. A real-time reporting platform has been instrumental in monitoring the process.

Conclusions: The key barrier to ART optimisation for children was inadequate stock of antiretroviral drugs. Dispensing messages for health care workers and a caregiver literacy manual are being developed to address the communication challenges. Continuous mentorship is needed to operationalise changes in guidelines at facility level.

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Dual therapy including dolutegravir plus boosted-darunavir ensures high rate of virological control and rare resistance selection at failure in heavily treatment experienced PLWH

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Background: We evaluated virological response and resistance profile in cART-experienced people living with HIV-1 (PLWH) starting for the first time a dual therapy based on dolutegravir (DTG) and ritonavir/cobicistat boosted-darunavir (DRV).

Materials and Methods: Patients included in the study were either viremic or virologically suppressed. Survival analysis was used to assess probability of virological success (VS: first viremia <50 copies/mL after therapy start) in viremic patients and virological rebound (VR: two consecutive viremia >50 copies or one viremia >1000 copies/mL after therapy start) in virologically suppressed patients. Major resistance mutations (MRMs) and genotypic susceptibility score (GSS) were evaluated at baseline (as cumulative plasma resistance) and after switch.

Results: Overall, 130 individuals were analyzed (62 [47.7%] viremic; 68 [52.3%] virologically suppressed). The majority of patients were male (70.8%) and infected with B subtype (81.5%). Patients had a long treatment history, with a median (IQR) time under cART of 20 (10-22) years and a median (IQR) number of 9 (4-12) previous regimens; most of them had previously received DRV (75.4%) and INI (60%; DTG: 9.2%). Viremic patients showed a median (IQR) baseline viremia of 3.2 (2.1-4.4) log10 copies/mL, while virologically suppressed patients were under virological control since a median (IQR) time of 57 (27-100) months.

Even though at baseline 81.5% of patients had accumulated ≥1 MRM (PI: 35.7%, NRTI: 77.5%; NNRTI: 69.0%; INI:10.1%), 77.7% of patients harbored strains fully susceptible to DTG+DRV (DTG: 93.8%; DRV: 82.3%). Compared to viremic patients, virologically suppressed patients showed a higher level of baseline DRV-resistance (≥3 DRV MRMs: 13.2% vs. 0%, P<0.001; fully susceptible DRV-GSS: 44.9% vs. 95.2%, P<0.001), while DTG susceptibility was high in both groups (INI resistance: 6.7% vs. 12%, P=0.703; fully susceptible DTG-GSS: 92.6% vs. 95.2%, P=0.720).

In viremic patients, by 12 months after treatment start, the overall probability of VS was 91.7%; the median time (95% C.I.) of VS was 2 (1-3) months. The few patients receiving a non-fully active regimen had a lower probability of VS (80.0%) compared to those who received a fully active treatment (93.3%), even though statistical significance was not reached (P=0.660), probably due to the low sample size.

In virologically suppressed patients, by 24 months after therapy switch, the probability of VR was 10.5%, with only 6 VR events at a median viremia of 266 (104-142,761) copies/mL. Patients with a previous time under virological suppression ≤6 months showed a higher VR probability compared to others (37.5% vs. 6.7%, P<0.001). No significant association with resistance was found due to the low number of events.

Among the 27 patients who did not respond (8 never achieved VS; 19 experienced VR), 13 (48.2%) were performed a plasma GRT in a median (IQR) time of 12.4 (9.2-27.4) months after switch. Eight (61.5%) of them were previously exposed to raltegravir or DTG. Two patients (15.4%), both with non-fully susceptible baseline-GSS, accumulated further resistance in integrase and protease (Patient-ID 357: Y143C/H/R; Patient-ID 392: S147G, N155H and V32I, L33F, I54L), while in 11 (84.6%) patients the majority of MRMs accumulated before starting DTG+DRV were no longer present at virological failure.

Conclusions: Dual therapy with DTG+DRV in highly treatment experienced patients ensures a high rate of virological control. In the few failures recorded, the majority of previous resistance mutations are no longer present in plasma genotypic resistance test; selection of new resistance is a rare event.

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Dual therapy with Dolutegravir plus Lamivudine in virologically suppressed patients

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Introduction: as dual therapy (DT) with Dolutegravir (DTG) plus Lamivudine (3TC), has become recently a first-line therapeutic option in HIV-infected patients, real world data begin to appear to confirm results of clinical trials.

Material & methods: a retrospective analysis was performed in this setting to evaluate the performance of this DT in HIV suppressed patients, in which ART was changed due to several reasons. We enrolled patients that completed at least a six month period with this therapy. Several parameters were analyzed, namely HIV-RNA, TCD4 cell count, TCD4/8 ratio, blips, presence of M184V as well as weight.

Results: ninety-three individuals were included, all negative for HBsAg and on stable ART. They were mostly men (68,8%), with an average age of 59 years. Mean time of HIV infection was 10,2 yrs and they were on ART for 8,6 yrs on average (min 1; mx 24 yrs). Previous ART included INSTI (52,8%), bPI (23,7%) or NNRTI (19,4%), and the main reason for switch was toxicity (metabolic/cardiovascular risk- 31,2%; renal-21,5% or bone-4,3%) or simplification (35,5%). Mean time on DT was 14,3 months (min 6; mx 51), and before the switch 86% had undetectable RNA and current evaluation showed 94,6% and 92,5% of undetectability, after 6 and 12 months respectively; the remaining patients had HIV-RNA <63 cp/ml. Blips occurred in 15,1% of patients, but there was no relation with nadir TCD4 cells count (11-697 cell/mm3)(p=0,856) or baseline HIV-RNA (15300-1292339 cp/ml)(p=0,858), when compared to those in no blips. In those suppressed patients with DT for more than a year, 39,3% had a nadir TCD4 cells < 200 cell/mm3. In only one patient M184V was present (1/47 tested), although currently suppressed. Globally six months after switching, there was an increase of TCD4 cells count (p<0,001) and in those with previous bPI, an increase was observed both in TCD4 cell count (p=0,004) and in TCD4/8 ratio (p=0,027). Regarding overweight there was no statistically significant diferences at six (+2,4 kg) or twelve months (+1,8 kg) after DT.

Conclusions: This strategy of switching to DT based on DTG+3TC mantains virological efficacy, allowing the use of co-medications and easy management of co-morbidities, with the beneficial effect of increasing TCD4 cell count and TCD4/8 ratio. In spite of the small increase of weight, these results were not statistically diferent, even between those patients with previous ART including INSTI or not. These results need further and longer studies to confirm some controversial issues.

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Real-world effectiveness of elbasvir/grazoprevir in the treatment of patients with hepatitis C virus genotype 1a infection in Norway

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Background and Aims: In Europe, recommended treatment duration of genotype 1a (GT1a) with elbasvir/grazoprevir (EBR/GZR) is 12 weeks. The 16-week treatment with ribavirin is recommended if resistance-associated substitutions (RAS) exist and/or if the patient has a high viral load at baseline. In Norway, many clinicians pragmatically treat all GT1a-infected patients using 12 weeks of EBR/GZR without ribavirin, regardless of viral load and baseline RAS testing. The primary objective of this study is to evaluate the real-world effectiveness of 12 weeks EBR/GZR treatment in a Norwegian cohort in this patient group.

Method: This is a retrospective, observational cohort study utilizing computerized databases and electronic medical records at five Norwegian hospitals treating HCV that did not systematically offer RAS testing. We included consecutive adult patients with chronic HCV GT1a and compensated liver disease who received 12 weeks of EBR/GZR without ribavirin regardless of viral load and baseline RAS testing. Patients with available HCV RNA data at least 4 weeks post-treatment were included in the analysis. Cirrhosis was defined by liver stiffness measurement of ≥12.5 kPa or an APRI score > 2. The primary outcome was sustained virologic response at week 12 (SVR12), or if not available, SVR4.

Results: We included 433 patients of whom 67.2 % were male and median age was 46 years (22 -73 years). HIV coinfection was present in 3.8% (16/424) and cirrhosis in 4.0% (17/428). The viral load was ≥ 800 000 IU/ml in 55.0% (235/427) of patients. 45 patients had no virological response data post-treatment. Overall SVR was achieved in 97.2% (377/388) patients (360 patients tested 12 weeks post-treatment and 17 patients 4 weeks post-treatment). SVR was 98.3% (169/172) in patients with a viral load of <800 000 IU/ml and 96.2% (202/210) in those with a viral load of ≥800 000 IU/ml.

Conclusion: In Norway, patients with HCV GT1a infection achieved high cure rates using 12 weeks of EBR/GZR without ribavirin or baseline RAS testing regardless of baseline viral load.

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HIV A1 or B Do Not Differentially Impact Cabotegravir In Vitro Potency or Durability

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Background: The phase III FLAIR study evaluates monthly administration of long-acting (LA) cabotegravir (CAB) and rilpivirine (RPV) as maintenance therapy in suppressed HIV-infected adults over 48 weeks and demonstrated non-inferiority to 3-drug daily oral ART. A total of 3/283 (1%) participants (pts) who received CAB+RPV LA had confirmed virologic failure (CVF). All 3 CVF pts were among the 8 pts in the study with subtype A1 virus and all 3 had the baseline integrase (IN) substitution L74I, as did 2/5 pts who maintained viral suppression. All 8 pts with subtype A1 virus in the study were sensitive to CAB at baseline. 174/283 (61%) pts in the LA arm had subtype B, 7% with L74I without CVF. Given the apparent clustering of CVF among A1 and presence of L74I, we sought to determine the impact of L74I and subtype A1 compared with subtype B IN on CAB sensitivity.

Materials and Methods: IN genotypes and phenotypic sensitivity to CAB were generated at Monogram Biosciences. Site-directed mutants were generated in subtype B NL4-3 and a consensus A1 IN sequence derived from the 3 CVF baseline IN sequences. In vitro susceptibility to CAB was assayed and compared across virus subtypes. In vitro durability of CAB was tested against bulk-infected cultures at various CAB concentrations for 3 weeks.

Results: All baseline, A1 IN sequences (8/283 pts) were sensitive to CAB with IC50 fold-change (FC) ranging from 0.7 to 1.0. The 3 CVF sequences at the failure timepoint had CAB FC IC50 values of 5.22-9.36 and substitutions at L74I and G140R or Q148R. The site-directed mutants L74I/G140R (FC 0.87 A1 vs 0.58 B) or L74I/Q148R (FC 4.1 A1 vs 4.4 B) in the A1 background resulted in similar IC50 FC compared with subtype B background. Across both subtypes, time to viral breakthrough was similar at the lowest CAB concentration (1 nM) and no viral breakthrough was detected at 3 weeks for CAB concentrations of 5 nM or 410 nM (1xPAEC90). The genotypes of the breakthrough viruses will be presented.

Conclusions: The FLAIR study demonstrated CAB+RPV LA was noninferior to oral ART at Week 48 with all 3 CVFs harboring HIV subtype A1 with baseline L74I. In vitro virologic assessments do not indicate a differential sensitivity to CAB between subtypes A1 or B in viruses containing IN mutations observed in the CVFs. However, our evaluations cannot determine if HIV subtype A1 with L74I has greater likelihood of selection of additional INSTI mutations under selection pressure. Other factors may contribute to the risk of CVF and require further investigation.

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HIV EVOLUTION IN PATIENTS INITIATING ANTIRETROVIRAL THERAPY DURING PRIMARY INFECTION

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Background: Little is known about HIV-1 molecular evolution on effective antiretroviral therapy (ART). A majority of studies have shown the lack of viral evolution during ART, although dissenting results have been documented. We therefore aimed to look for evidence of HIV-1 evolution for the first time in patients diagnosed with a primary HIV-1 infection (PHI) and with subsequent effective long-term suppressive ART.

Methods: Patients treated at time of PHI diagnosis with effective long term suppressive ART (HIV Viral Load, VL <20 cp/mL during at least 5 years without any blips) were retrospectively studied (n=20). Cell-associated HIV-1 DNA was quantified by real-time PCR. Longitudinal analysis of the diversity of HIV-1 reservoir was conducted: RT and gp 120 genes were sequenced by Ultra-Deep sequencing (UDS) using Illumina technology (Cut-off=2%). Drug-resistance-associated mutations (DRAMs) and tropism were interpreted using the latest ANRS resistance and Geno2Pheno algorithms. Sequences evolution was studied by multiple sensitive methods: maximum-likelihood phylogeny trees were generated and average pairwise distance (APD) was calculated for sequence diversification.

Results: Twenty patients were included: men 18/20 (90%), median age 47 years (IQR 34-53), HIV RNA zenith 5.82 log10 copies/ml (4.94-6.26), CD4 nadir 417 cells/mm3 (325-522), time to ART initiation 5 days (1-12) and time to HIV VL<20 copies/mL under ART 95 days (40-119). The median total cell-associated HIV-1 DNA load at PHI and after 5 years of follow up was 3.24 log10 copies/106 cells (IQR: 2.72 to 3.49) and 1.6 log10 copies/106 cells (IQR 1.6), respectively, with a significant decrease in total HIV-1 DNA during follow up period (p=0.05). At baseline, DRAMs were detected in RT gene for 3 (15%) patients with 2 major resistant variants: 2 K103N (98%) and 1 minor resistant variant: Y188H (2%). New DRAMs were detected in 9 patients (45%) despite sustained virological control: 4 patients had new archived DRAMs at the first point of VL < 20 copies/mL and 7 individuals had at least 1 emerging DRAM in peripheral blood cells after 5 years of follow up (most of them were detected at less than 10%). Four patients had at least one G-to-A mutation resistance associated mutation. The genotypic prediction of C2V3 co-receptor tropism did not vary over time in all patients (n=15, 75% CCR5 tropism). Phylogenetic analysis showed that in all patients, sequences obtained from three different time point were highly homogenous and were intermingled. The comparison of the APD showed the absence of significant viral diversity in primary infection and during 5 years of the study.

Conclusion: Despite a slight variation (emergence or disappearance) of minority resistance-associated mutations variants, there was no clear evidence of viral evolution during a prolonged period of time in this population of highly controlled adult patients treated at time of PHI.

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Molecular Epidemiology of the HIV-1 Infection in Cyprus (2017-2019): A Polyphyletic Infection with an Increasing Prevalence of Drug Resistance

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Background: The HIV-1 infection remains a public health concern despite the decrease in AIDS-related mortality because of CART, yet the virus is still continuously transmitted throughout the world. Specifically, in the context of Cyprus, there were 262 newly infected individuals from 2017 to 2019, which has more than doubled over the course of 10 years. Thus, the ongoing, prospective Cyprus HIV-1 Transmission Cohort Study (CHICS) was founded to monitor the spread of the HIV-1 infection in Cyprus.

Materials and Methods: For this study, 165 blood samples, along with demographic data (5 study subjects have data pending) were collected from consenting HIV-1 infected patients in Cyprus from 2017 to 2019. DNA sequences encoding whole pol (protease, reverse transcriptase and integrase) were amplified by RT-PCR from plasma HIV-1 RNA, and then were sequenced using Sanger-sequencing. Genotypic drug resistance was inferred through the Stanford drug-resistance tool, and HIV-1 genetic subtypes were determined using REGA-3. The sequences were then aligned using CLUSTALW, followed by the construction of a maximum-likelihood phylogenetic tree (GTR model, 1000 bootstrap value), on MEGAX. Transmission clusters of above 70% similarity were identified using Cluster Picker.

Results: Between 2017-2019, 165 HIV-1 infected study subjects participated in this molecular epidemiology study. The newly infected study subjects corresponded to 56% (146/262) of the total number of new HIV-1 infections. Sequences originated from Cyprus (n=84, 51%), sub-Saharan Africa (n=32, 19%), Eastern-Europe (n=19, 11%), other Mediterranean countries (n=12, 7%), United-Kingdom (n=8, 5%), South/South-east Asia (n=3, 2%). The majority of sequences were reported from MSM (n=76, 46%), followed by HC (n=57, 34%), HBC (n=19, 11%), IDU and blood transfusion (each n=2, 1%). Nine study-subjects were unsure of the route of infection (7%). Thirty-nine different group M subtypes, CRFs and recombinants were identified, the most prevalent were A1 (n=38, 23%), B (n=32, 20%) and CRF02_AG (n=23, 14%). Through phylogenetic analysis, 15 transmission clusters were identified. The majority of the clusters were A1 (4 clusters) and B (3 clusters); however, clusters of CRFs and recombinants such as CRF02_AG and Rec B, A1, G were also identified. The largest active cluster (subtype A1) that was identified consisted of 20 MSM mostly Cypriot. There were 16 major and 18 accessory drug resistance mutations identified for NRTIs, NNRTIs, PIs and INSTIs. Six major mutations were identified associated with NRTI: K65R (n=1 study-subjects), T69S (n=1), K70G (n=1), M184VI (n=2), T215FL (n=2), K219R (n=1). Six for NNRTIs: K101E (n=2), K103NQ (n=9), V106I (n=2), E138EAG (n=7), Y181C (n=1), G190A (n=4). Two for PIs: M46L (n=2), V82I (n=7). Two for INSTIs: G140D (n=1), Q148R (n=1).

Conclusions: The results of this study indicate that the HIV-1 infection is a polyphyletic infection characterized by a plethora of genotypes, with A1 being the most prominent, unlike in other European regions. The main route of infection is MSM. Analyses reveal that there are active transmission clusters, most notably of Cypriot MSM subtype A1, highlighting a target for preventive treatment. Importantly, these data demonstrate a higher prevalence of mutations associated transmitted drug resistance relative to earlier studies performed in Cyprus.

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A CHANGING OF HIV-1 GENETIC DIVERSITY IN RUSSIA DURING THE LAST TWO DECADES: INCREASE OF THE RECOMBINANTS PREVALENCE

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Background: During the last two decades, HIV-1 has been spreading rapidly in the Russian Federation with subtype A (sub-subtype A6) dominating in almost all territories. The new recombinant form of virus was firstly detected in Kaliningrad in 1996–1997. It was a result of recombination between the subtype A strain prevalent among injecting drug users (IDU) in Russia and subtype B. This new recombinant form was named CRF03_AB. Later this type of recombinant form of HIV-1 caused an outbreak outside the Kaliningrad region, in Cherepovets (2002–2006).

Around the period 2005–2010, the genetic diversity of HIV-1 in Russia began to increase. Subtypes B, C, and recombinants CRF02_AG, CRF63_02A1 had been identified. This phenomenon created the conditions for the new unique recombinant forms (URFs) emergence as an important component of the HIV-infection epidemic development. The objective of this study was to make the first estimates of the prevalence of HIV-1 recombinant forms (CRFs and URFs) in Russia during the last two decades.

Materials and Methods: Laboratory database of HIV-1 nucleotide sequences together with the GenBank (https://www.hiv.lanl.gov) were used as a source of 6699 sequences from different regions of Russia and included into analysis. These sequences were obtained during 2000–2019. Genotyping and recombinant analyses were carried out using the tools COMET HIV-1, REGA HIV-1 Subtyping Tool (V 3.0), RIP and jpHMM. Data processing was carried out using the custom R script.

Results: The results of data processing revealed that there were three local outbreaks of CRFs: in 2002 the prevalence of CRF03_AB was 38.52% (109/283) in Cherepovets, in 2006 – 5.61% (24/428) in Saint Petersburg, CRF06_cpx and during 2009–2011 – 17.82% (147/825) in Novosibirsk, mainly CRF63_02A1. Since 2012 the prevalence of recombinant forms of HIV-1 has tended to increase. The prevalence of HIV-1 recombinant forms among the studied viruses in the Russian Federation in 2012 was 8.94% (72/805); in 2013 – 11.52% (47/408); in 2014 – 12.01% (49/408); in 2015 – 19.64% (151/769); in 2016 – 19.64% (121/616); in 2017 – 24.54% (146/595); in 2018 – 26.24% (74/282) and in 2019 – 81.52% (75/92). The CRFs were represented by following variants: CRF01_AE, CRF02_AG, CRF03_AB, CRF06_cpx, CRF11_cpx and CRF63_02A1. Since 2008 unique recombinant forms began to make an increasing contribution into HIV-1 genetic diversity in Russia and now URFs are represented by the following genetic variants: URF_A6/B, URF_A6/G, URF63_02A1 and mosaic variants (URFA6_02AG, URFB_02AG, URF03_AB/A6 and URFA6_CRF63).

Conclusions: The results of this study point to the increasing genetic complexity of the HIV-1 epidemic in the Russian Federation, including due to the active spread of recombinant forms. A trend towards an increase in the prevalence of HIV-1 unique recombinant forms and their genetic diversity is noted.

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Phylogenetic analyses applied to the study of transmission variants in a HIV-1 positive couple

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Background: During infection, HIV-1 typically accumulates a high level of genetic diversity. Such high mutation rate plays a pivotal role in conveniently escaping the immune system as well as pharmacological approaches, while adapting to the host. It is well established that in the vast majority of the infections it is just one out of potentially thousands of HIV-1 variants to colonize the new host, named transmitted/founder (T/F) variant. T/F variants are modeled by both stochastic and selective pressures, and the bottleneck of the mucosa plays a major role.

Based on these premises, the present study aims to characterize T/F variants tracing the HIV-1 infection in a young couple who become infected through sexual activity.

Methods: Blood was collected from a fourteen-year-old couple (M and G) upon HIV-1 diagnosis and after six months of anti-retroviral treatment. The HIV-1 protease, RT, V1V2 and gp41 fragments were sequenced with NGS (next generation sequencing) technique using Miseq Illumina. Phylogenetic analyses were conducted with Bayesian inference methods.

Results: Both partners were infected with an HIV-1 B subtype. No evidences of viral recombination were observed in any portion. The lower intrapersonal genetic distances were observed at baseline, before initiation of therapy, and in particular in the V1V2 fragment (M=0.012 and G=0.011) compared to samples during treatment at six months (distances ranging from 0.102 to 0.148). In all different HIV-1 regions analyzed one HIV-1 single variant resulted dominant, although some minor variants could be observed. The same tree structure was observed both at baseline and after 6 months of therapy.

Dated tree indicated a TMRCA for the cluster encompassing all patient isolates of 22.25 months (95%HPD: 8-43.6 months) corresponding to February 2017. The node related to M sequence dated 19.3 months (95%HPD: 6-20 months) corresponding to April 2017.

Conclusions: This unfortunate event offered us the unprecedented opportunity to study the T/F variants in the naïve couple and upon administration of the antiretroviral therapy. Confirming the literature data, our results indicated a limited number of transmitted variants, ranging from one to two, from the boy (G) to the girl (M) highlighting that the viruses circulating in the newly infected subject were closely related to viruses present in the donor. Understanding which HIV-1 variants are most likely to be transmitted would allow a better understanding of viral evolution, playing a relevant role in vaccine design and prevention strategies.

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Unexpected rising in the circulation of complex HBV variants enriched of HBsAg vaccine-escape mutations in HBV genotype-D: potential impact on HBsAg detection/quantification and vaccination strategies.

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Introduction: HBsAg vaccine-escape mutations can alter HBsAg recognition by antibodies thus challenging vaccine efficacy, promoting immunosuppression-driven HBV-reactivation, and impairing HBsAg detection by immunoassays. In HBV genotype-D infection, limited information is available on the circulation of vaccine-escape HBsAg mutations overtime.

Here, we investigate the circulation of vaccine-escape mutations, the burden of complex mutational profiles and their impact on serological parameters in a large cohort of patients (pts) infected with HBV genotype-D.

Methods: This study includes HBsAg sequences from 947 viremic pts infected with HBV genotype-D, collected for routine clinical practice from 2005 to 2019. 21 vaccine-escape mutations (T116N, P120E/S, T126A/I/N/S, Q129H/R, T131I/N, M133I/L, C139S, K141E, P142S, D144A/E, G145A/R, A159G by Lazarevic, 2014) are analyzed.

Results: Median (IQR) HBV-DNA and ALT are 3.5(2.6-5.0)logIU/mL and 39(26-73)U/L, respectively. 4.2% is HBsAg-negative despite HBV-DNA positivity. Overall, 17.7% (168/947) of pts harbor >1 vaccine escape mutation with the highest prevalence in subgenotype-D3 (23% for D3 vs 13.6% for other subgenotypes, P<0.001). Among them, 17.3% (29/168) show complex profiles of vaccine-escape mutations characterized by the co-presence of 2 or more vaccine-escape mutations.

Notably, the proportion of pts with complex profiles of vaccine escape mutations increased over time: from 0.4% (1/237) in 2005-2009 to 3.0% (12/396) in 2010-2014 and to 5.1% (16/314) in 2015-2019, P= 0.007, suggesting an increased circulation of viral strains endowed with enhanced capability to evade humoral responses.

Moreover, the presence of complex profiles of vaccine-escape mutations correlates with lower HBsAg levels: median (IQR) 40(0-2905)IU/mL for pts with complex mutational profile vs 1688(348-6090) without them (p=0.0007), suggesting their role in altering HBsAg quantification.

Focusing on HBsAg-negativity, the presence of complex profiles of vaccine-escape mutations also correlates with an HBsAg-negative result despite HBV-DNA positivity (34.8% of pts with >2 vaccine-escape mutations vs 6.7% and 2.3% of those with a single or no vaccine-escape mutations are HBsAg-negative, p=0.007 and <0.0001). Interestingly, HBsAg-negativity is strongly associated with the presence of T126I/A in combination with >1 additional vaccine-escape mutation (50% of pts with T126I/A-containing profiles vs 3.3% without them were HBsAg-negative, p<0.0001). In HBsAg-negative pts, T126I/A frequently co-occurs with P120S and Q129H.

Conclusions: Complex profiles of vaccine-escape mutations are detected in a not negligible fraction of HBV genotype-D infected pts, and correlate with lower HBsAg quantification and HBsAg-negativity despite ongoing viral replication. These mutations should be considered for a proper clinical interpretation of HBsAg results and their circulation should be taken into account for the development of novel vaccine formulation.