Reviews in Antiviral Therapy & INFECTIOUS DISEASES
5 2019
JOURNAL OF ABSTRACTS AND CONFERENCE REPORTS FROM INTERNATIONAL WORKSHOPS ON INFECTIOUS DISEASES & ANTIVIRAL THERAPY

Abstract Book

17th European Meeting on HIV & Hepatitis

Treatment Strategies & Antiviral Drug Resistance

22 - 24 May 2019, Rome, Italy

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Abstracts

1

First results after 52 weeks of informal PrEP use in a cohort of MSM in Southern Spain.

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Background: Despite the fact that Southern Spain has similar HIV incidences than Eastern Europe, PrEP use is blocked, both nationwide and by the Andalusian Regional Government. Between January 2018 and December 2018, a sample of 167 MSM with high risk practices has begun to be part of a cohort of informal PrEP users in this region. With the objective of making safer the use of PrEP and offer a basic tool in this MSM cohort, Sevilla Checkpoint implemented PrevenPrEP, a new program that assess regular checkouts and follow up to people using this biomedical strategy on their own.

Methods: From the opening of Sevilla Checkpoint in November 2017, 419 MSM showed interest in the use of PrEP. Furthermore, 67% of them declared unprotected anal sex and 61 were chemsex users. In January 2018, Sevilla Checkpoint began to work on this MSM cohort to enforce the UNAIDS combined preventive strategies against HIV.
The educators firstly evaluate the real risk of the different potential prevenPrEP users. Those selected, were first of all tested against HIV/STIs by using PCR technology and they were required a general blood analytic from their GPs to evaluate the kidney and liver function. While users were taking PrEP, Sevilla Checkpoint tested them against HIV/STIs.

Results: A total of 167 MSM were included in the program; 152 of them use daily PrEP and 15 event-driven. All of them showed a normal biochemistry parameters and no initial HIV/STIs infections. The general characteristics of our PrevenPrEP users was similar to those shown by previous studies; an MSM of 34 years of age, 58% of them with a university degree and the rest -42%- secondary studies. 85% of users showed previous HIV tests, with an average of 9 times per user. Regarding anal sex, 78,4% never used condoms during sexual intercourse with an average of 32 different sexual partners in the last 6 months. Moreover, 15,6% of them were chemsex users. Finally, regarding HIV, no infection was detected in this cohort and 6,6% of them were reactive against Chlamydia, 5,4% against Gonorrhoea, and 1,2% against Syphilis.

Conclusion: These results show that there is a cohort of MSM in Seville who are interested in PrEP and whose sexual practices justify the use of it to protect themselves of HIV infections. As we expected, there was not any new HIV infections in this cohort. On the other hand, the percentage of STIs diagnosed was approximately 50% lower than those people tested in our community center who are not using PrEP, showing the importance of STIs monitorization to prevent new infections.
The community centers as Sevilla Checkpoint have the capacity to offer proffessional advise and monitoring services to informal PrEP users and prove to be strong candidates to offer these services when PrEP will be implemented by the Spanish Government.

2

European surveillance of HIV drug resistance to NRTI, NNRTI and INSTI in newly diagnosed individuals using next-generation sequencing

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Background: Although European guidelines have shifted towards INSTIs as first line therapy, NNRTIs are still frequently used in particular in Central and Eastern Europe. Due to its multicenter and representative nature, the surveillance SPREAD program is an ideal framework to investigate the prevalence of the minority resistant variants to NRTI, NNRTI and INSTI in newly diagnosed individuals in Europe.

Material and Methods:A sample of 275 individuals newly diagnosed therapy-naïve in 2012/2013 were randomly selected from the European SPREAD database from 12 countries: Belgium n=20; Bulgaria n=40; Croatia n=25; Cyprus n=18; Finland n=20; France n=20; Greece n=30; Israel n=15; Lithuania n=22; Luxembourg n=22; Poland n=23; Slovenia n=20 and from Russia (n=24). Ultradeep RT sequencing was performed using a 454 FLX Genome Sequencer (Roche, Manheim) and IN Sequencing was performed on an Illumina MiSeq platform (Illumina, USA). Sequencing files were imported into CLC GenomicWorkbench (v.10.1.1). Sequences were checked for quality and trimmed using a minimum PHRED score 20. Low frequent variant calling was performed with a significance of 1% and a minimum coverage of 500 reads. For RT interpretation the WHO list for surveillance of drug resistance mutations was used. For IN all substitutions relative to HXB2, and included the mutation scoring of Stanford HIVdb v8.7 were listed.

Results:A total of 254 samples were successfully sequenced for RT and 214 for IN. The majority was male and of European origin, infected with various HIV subtypes. Based on the WHO list, the prevalence of NRTI and NNRTI resistance mutations with an abundance above 20% of the viral population was 3.5% and 7%, respectively, in agreement with Sanger sequencing data from the SPREAD program. The total of low abundance variants (<20%) was 26 (10.2%) for NRTI-resistance mutations and 17 (6.7%) for NNRTI-resistance mutations. The majority was detected at a prevalence level below 5%: 15 (5.9%) and 13 (5.1%) for NRTI and NNRTI mutations, respectively. Resistance mutations between 5 and 20% prevalence that may have an impact on the virologic outcome include: K65R (n=5), M184V (n=2), T215S (n=3), K219R (n=1) for NRTI and K103R (n=1), V179E (n=1), G190E (n=1) for NNRTI. No INSTI signature mutations were observed above the cut-off of 20%. Only at a detection level below 5%, S147G was detected in 2 individuals and Y143C in one individual. Presence of Y143C was found in combination with L74M, E157Q and S230N. Non-polymorphic mutation E138K was observed as minority variant in 4 individuals, only once in combination with L74M. Polymorphic mutation T97A was detected in 3 individuals but always as single mutation. Other frequent detected polymorphic mutations were E157Q (n=12; 9/12 subtype B), L74M or L74V (n=46; 31/46 subtype A) and S230N (n=36; 35/36 subtype B).

Conclusions:A significant proportion of low abundance transmitted drug resistance to NRTI and NNRTI was detected in this representative set of newly diagnosed therapy-naïve HIV-patients from Europe. We found no evidence of increasing TDR for INSTIs that would support the need to perform integrase genotyping before initiating INSTI therapy. Continued surveillance of INSTI resistance is nevertheless warranted.

3

Molecular analysis suggests post-migration HIV-1 acquisition among migrants in Paris, France

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Background: A considerable proportion of people diagnosed with HIV-1 in Europe the last few years, are migrants. We aimed to trace the geographic origin of HIV-1 acquisition for migrants in Paris, France, using current state-of-the-art molecular epidemiology methods.

Materials & Methods: We studied 1,582 and 302 sequences of CRF02_AG and subtype A1, respectively, available in the pol region, isolated from HIV-1 diagnosed patients in Paris during 2002-2016. These data were derived from a total sample of 5,553 sequences. HIV-1 subtyping was carried out using automated subtyping tools (COMET, REGA). We analyzed phylogenetically the subtype A1 sequences from migrants (N=58) along with all the available A1 sequences from non-migrants in Paris (N=244), a random set of globally sampled A1 sequences (N=1,400) and the most closely related sequences to our study population (migrants & non-migrants from Paris) using the HIV BLAST tool (N=744), used as references. We also analyzed 273 and 1,309 CRF02_AG sequences from migrants and non-migrants from Paris, respectively, along with all the available globally sampled CRF02_AG sequences (N=4,013), as references. Local transmission networks (LTNs) were phylogenetic clusters including sequences from France at proportions >70%, receiving bootstrap value >75% or SH-support >0.9. Phylogenetic trees were estimated by the maximum likelihood method (RAxML, FastTree). The origin of HIV-transmissions was traced by phylogeographic analysis using the criterion of parsimony (Mesquite).

Results: Subtype A1 (N=302; 5.4%) and CRF02_AG (N=1,582; 28.5%) were the most prevalent non-B clades in Paris. The number of LTNs for subtype A1 was 42 with a range of 2 to 8 sequences. For CRF02_AG we found 166 LTNs consisting of 2 to 15 sequences and two large ones including 26 and 139 sequences. Phylogenetic analysis also revealed that 18 (31.0%) A1 sequences from migrants and 60 (30.5%) from non-migrants clustered within LTNs. For CRF02_AG, 101 (37.0%) sequences from migrants, and 411 (38.7%) from non-migrants fell within LTNs. The distribution of transmission risk groups in migrants infected with A1 strains was: heterosexuals (N=47; 81%), MSM (N=4; 6.9%), and others/unknowns (N=7; 12.1%). For CRF02_AG, it was: heterosexuals (N=191; 70.0%), MSM (N=42; 15.4%), and others/unknowns (N=40; 14.6%). Notably, the proportion of migrant MSM within CRF02_AG LTNs was significantly higher (85.7%) than the corresponding proportion for the heterosexuals (28.3%) ($p<0.001$). Phylogeographic analysis showed that 23.3% and 33.3% of the subtype A1 and CRF02_AG HIV-transmissions within migrants, respectively, occurred locally.

Conclusions: We found that 23.3% and 33.3% of subtype A1 and CRF02_AG HIV-transmissions within migrants, respectively, originated in Paris/France. For CRF02_AG we found that transmissions within LTNs were associated with MSM transmission risk group. The proportions of putative local transmissions for these subtypes were similar for the non-migrant population, pointing out that local transmissions within migrants and non-migrants occur at similar rates. Given the low coverage of our sampling we expect that the proportion of local transmissions will be higher for both subgroups. This is one of the few molecular studies highlighting that even non-B transmissions occur locally at similar rates for migrants and non-migrants in Paris.

4

TRENDS OF TRANSMITTED AND ACQUIRED HIV-1 DRUG RESISTANCE IN PATIENTS FOLLOWED IN PORTUGUESE HOSPITALS BETWEEN 2001 AND 2017

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Background: By the end of 2017, there were approximately 36.9 million people living with HIV and 21.7 million people were receiving antiretroviral (ARV) therapy. Drug resistance to ARV is still a limitation for the control of HIV-1 infection. Despite new ARVs having higher genetic barriers, some patients continue to virologically fail due to transmitted (TDR) or acquired (ADR) resistance that can harm the efficacy of ARV regimens. The goals of this study were to describe and analyse the trends of TDR and ADR in patients clinically followed in Portuguese hospitals between 2001 and 2017.

Methods: A total of 11911 HIV-1 patients followed in Portuguese hospitals were included in the study. Patients were categorized into drug naïve (DN) and treated patients (TP). TDR was defined by the presence of one or more surveillance drug resistance mutations according to the World Health Organization (WHO) 2009 surveillance list. Genotypic resistance to ART drugs was evaluated using the Standford HIVdb v8.04. Logistic regression was used to characterize prevalence by calendar year, drug class and demographic and clinical factors (SPSS software).

Results: Most participants were male (64.6% for DN and 67.3% for TP). The median age of patients at the time of genotyping was 38.0 (IQR: 31.0-48.0) and 39.0 (IQR: 33.0-46.0) years for DN and TP, respectively. DN and TP were predominantly infected with subtype B virus (36.7% and 42.6%, respectively), followed by subtype G (25.2% and 32.8%, respectively). Most of the patients were born in Portugal (34.7% for DN and 29.9% for TP) followed by countries in Sub-Saharan Africa (13.3% for DN and 11.1% for TP). Overall, the prevalence of TDR was 9.4% (95%CI: 8.8-10.1) and of ADR was 69.0% (95%CI: 67.6-70.5). The prevalence of TDR increased from 7.9% in 2003 to 13.1% (p for trend<0.001) in 2017, whereas the prevalence of ADR decreased from 86.6% in 2001 to 51.0% (p for trend<0.001) in 2017. The prevalence of nucleotide reverse transcriptase inhibitors (NRTIs) and non-nucleotide reverse transcriptase inhibitors (NNRTIs) mutations for drug naïve patients increased from 5.6% to 6.7% (p for trend=0.002) and 2.9% to 8.9% (p for trend<0.001) between 2003 and 2017, respectively. For protease inhibitors (PIs), TDR decreased from 4.0% in 2003 to 2.2 (p for trend=0.985) in 2017. ADR mutations declined for all ARV classes over time (pfor trend<0.001). The most frequently detected TDR mutations were K103N/S (3.2%), M41L (1.6%) and M184V/I (1.3%). For treated patients, the most common mutations found were M184V/I (45.3%), K103N (26.0%) and T215Y/F (17.4%). Predicted phenotypic high-level resistance to first line ARV drugs presented the highest value to NNRTIs with Nevirapine (4.7%) and Efavirenz (4.0%), for drug-naïve patients and the highest values to NRTIs with Emtricitabine and Lamivudine (45.3%) for treated patients.

Conclusion: While ADR is decreasing since 2001, TDR has been increasing, reaching moderate rate of 13.1% in 2017. It is urgent to develop public health programs to monitor levels and patterns of TDR in newly diagnosed patients.

Collaborators of the Portuguese HIV-1 Resistance Study Group: Kamal Mansinho, Ana Cláudia Miranda, Isabel Aldir, Fernando Ventura, Jaime Nina, Fernando Borges, Emília Valadas, Manuela Doroana, Francisco Antunes, Nuno Marques, Maria João Aleixo, Maria João Águas, Júlio Botas, Patrícia Pacheco, Micaela Caixeiro, Teresa Branco, José Vera, Inês Vaz Pinto, José Poças, Joana Sá, Luís Duque, António Diniz, Ana Mineiro, Flora Gomes, Carlos Santos, Domitília Faria, Paula Fonseca, Paula Proença, Luís Tavares, Cristina Guerreiro, Jorge Narciso, Telo Faria, Eugénio Teófilo, Sofia Pinheiro, Isabel Germano, Umbelina Caixas, Nancy Faria, Ana Paula Reis, Margarida Bentes Jesus, Graça Amaro, Fausto Roxo, Ricardo Abreu and Isabel Neves.

5

Seminal HIV1 RNA and drug concentrations in DTG+3TC dual therapy (ANRS167 lamidol)

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Background: Intermittent HIV-1 RNA detection in seminal plasma may occur in patients with undetectable plasma viral load (pVL) on standard triple-drug therapy. Few data are available regarding HIV-1 RNA detection in seminal plasma samples from virologically-suppressed patients receiving a maintenance dual therapy, and DTG+3TC in particular.

Patients & Methods: In this ANRS167 LAMIDOL sub-study, a non-comparative open-label, single arm, multicenter trial, semen samples were collected at D0 and W24 of DTG+3TC. HIV-1 RNA was quantified in seminal plasma using COBAS® TaqMan® HIV-1 Test, v2.0 (limit of quantification [LOQ]=100 c/mL). Ultra-sensitive pVL (USpVL) was performed with centrifugation of the maximum volume of available plasma to reach a LOQ of 3 c/mL. The limit of detection (LOD) was defined as an undetected PCR signal. Plasma and seminal plasma drug concentrations (Cmin) were measured using UPLC-MS/MS.

Results: Among the 104 enrolled patients, seminal plasma samples were collected from 18 participants, including 16 paired samples at D0 and W24 of DTG+3TC. Median (IQR 25-75%) total DTG blood plasma Cmin and DTG seminal plasma Cmin were 1880 ng/mL (1377-2337; n=29) and 198 ng/mL (94-239; n=34), respectively. While the unbound/total DTG blood plasma Cmin ratio was 0.21% (0.17-0.25%; n=29), the seminal plasma/blood plasma total DTG Cmin ratio was 12% (8-15%; n=29), suggesting a DTG accumulation in the male genital tract. HIV-1 RNA was detected in seminal plasma of 3 patients: 1 at D0 (5.9%, 95%CI: 0.1-28.6) and 2 other at W24 (11.8%, 95%CI: 1.5-36.4). Seminal viral load was 475, 440 and 365 copies/mL and concomitant USpVL was below the LOD in all three cases. All three participants, except one, presented a DTG Cmin in seminal plasma above the in vitro protein-binding adjusted IC90 values (i.e. 64 ng/mL). These three patients did not experienced virological failure or plasma viral blip along the study and had no concomitant sexually transmitted infection.

Conclusions: No differences were observed regarding seminal plasma HIV-1 RNA detection in patients under triple therapy and at W24 of a maintenance DTG+3TC dual-drug therapy.

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Predicting 2-drug antiretroviral regimen efficacy by genotypic susceptibility score: results from a cohort study

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Background: HIV drug resistance has a deleterious effect on the virological outcome of antiretroviral therapy (ART). The aim of the study was to evaluate the ability of genotypic susceptibility score (GSS) to predict virological outcome following an ART switch to a 2-drug regimen in virosuppressed HIV-1 infected patients.

Material and methods: From the ARCA database we selected HIV-1 infected patients virologically suppressed switching to 2-drug ART (2006-2018, time of switch=baseline), with pre-baseline resistance genotype and at least one HIV-1 RNA determination during follow up. Primary endopoint was virological failure (VF: an HIV-RNA, VL, >200 cps/mL or 2 consecutive >50 cps/mL). Survival analysis was used to investigate predictors of VF. The GSS predicted by the latest and the cumulative genotype (CGSS) was calculated using the Stanford hivdb (v.8.5) with respect to the 2-drug regimen started. CD4 changes from baseline at weeks 24, 48 and 96 were assessed using Student's t-test for paired samples.

Results: We included 773 patients: 522 (68%) were males, 186 (24%) heterosexuals, with median age of 50 years (IQR, 43-56), 10 years of HIV (5-20), 7 years of ART and 5 (3-8) previous antiretroviral (ARV) lines. At baseline patients had been virologically suppressed for 6.4 years (2.5-14), allowing isolated blips. The median zenith VL was 4.9 log10 (4.4-5.5), CD4 cells count at nadir 222 (108-324) and at baseline 640 (477-860). Median GSS was 2 (1.5-2), with GSS <2 in 213 (28%) pts, median CGSS was 2 (1-2), with CGSS <2 in 250 (33%). The previous ARV classes used were NRTI in 770 patients (99%), NNRTI in 416 (54%), boosted PI in 639 (83%) and INSTI in 218 (28%). Current ARV regimens included: PI+3TC in 455 pts (59%), of which 3TC+ ATV unboosted or ATV/r or ATV/c in 181 (23%) and DRV/r or DRV/c in 274 (36%), DTG+3TC in 260 (34%) and DTG+RPV in 58 (7%). During a median observation time of 75 wks (37-120) the estimated probability of VF at 48 weeks was 6% (95% CI 5-7) among patients with GSS=2, 4% (3-5) among patients with GSS 1-1.99 and 11% (4-18) among those with GSS <1 (Log Rank p=0.21). According to CGSS, the estimated probability of VF at 48 weeks was 5% (95% CI 1-6) among patients with CGSS =2, 6% (4-8) among patients with CGSS 1-1.99 and 8% (3-13) among those with CGSS <1 (log Rank p=0.006,). Observed median changes of CD4+ counts from baseline were +24 cells/µL (IQR -67;+132) at 24 weeks, +49 cells/µL (IQR -31;+159) at 48 weeks and +74 cells/µL (IQR -30;+197) at 96 weeks (p<0.001 for all comparisons). At multivariate analysis, adjusting for years of ART, CD4 cell count at nadir and at baseline, CGSS strata, number of previous ARV lines, only longer time since last VL>50 cps/mL was associated with lower risk of VF (+ 1 year, aHR 0.89, 95% CI 0.82-0.98; p=0.01).

Conclusions: Despite an effect of CGSS, the duration of virosuppression was the only independent predictor of virological efficacy of switching to 2-drug regimens.

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High prevalence of the integrase resistance associated accessory mutation L74I in the Russian Federation

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Background: Cabotegravir is the newest integrase strand transfer inhibitor (INSTI) that is being developed as a long-acting injectable for monthly or quarterly administration and as an oral tablet for daily use for the treatment and prevention of HIV-1 infection. During CROI 2019, the FLAIR study was presented which showed that a long-acting regimen of cabotegravir and rilpivirine is non-inferior for HIV maintenance therapy as compared to a dolutegravir, abacavir and lamivudine. Three patients had confirmed virological failure to cabotegravir. These three patients were all from Russia, had a HIV infection with subtype A and a L74I mutation in their baseline integrase sequence. In-vitro studies found that substitutions in the 74 position of the integrase gene, especially together with major (G140A/C/S, Q148 H/K/R) and other accessory mutations (V75I, T97A), can significantly reduce susceptibility to cabotegravir. Although the Stanford HIV drug resistance database states that L74I occurs as a polymorphism in a minority of 21% of subtype A sequences, the database includes sequences from across the World. The aim of this study was to assess the prevalence of L74I in INSTI-naïve sequences from Russia.

Methods: We analyzed integrase (IN), protease (PR) and reverse transcriptase (RT) sequences from 412 HIV-infected INSTI- naïve patients, collected between 2008 and 2018 in Russia: 227 treatment-naïve patients and 185 patients with virological failure to antiretroviral drug therapy. IN and PR-RT sequences were obtained by AmpliSens® HIV-Resist-Seq kit. Viral subtype (PR-RT sequences) and the presence of resistance mutations (IN sequences) were determined using the HIVdb Program v.8.8. (https://hivdb.stanford.edu/).

Results: The most frequent clade in the Russian IN sequences was subtype A (85.9%), followed by subtype B (7.3%), subtype G for 3 (0.7%) and the circulating recombinant forms (CRF) CRF02_AG (4.1%), CRF63_02A1 (1.7%). In our study 196 sequences from 227 treatment-naïve patients contained the accessory mutation L74I. Among subtype A, L74I was detected in 97.9% samples, B - 6.25%, CRF02_AG - 44.4% and CRF63_02A1- 20%. Furthermore, we detected 5 sequences with additional major and/or accessory mutations (sample 1 (Q146P), sample 2 (Y143C/S147A/Q148H + G140L/P145G/S153F), sample 3 (Q146P + G163R), sample 4 (N155A+ S153Y/G163R) and sample 5 (G163R). Similarly, among patients with virological failure of therapy without INSTI, L74I was determined in 157/185 sequences (84.9%). The prevalence of L74I in subtype A, B and CRF02_AG viruses was 95.7%, 7.1% and 25% respectively. In addition to L74I additional relevant substitutions were found in 3 samples, including major drug resistance associated mutations (R263K, S147T) and an accessory mutation (T97A).

Conclusions: Our results demonstrate that in Russia, L74I is present in almost all subtype A sequences, and in a large proportion of CRF02_AG sequences. This high prevalence of L74I should be considered when introducing cabotegravir in Russia.

8

Fostemsavir (FTR) Week 48 efficacy and evaluation of treatment emergent substitutions in the BRIGHTE study

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Background: FTR is a prodrug metabolized to temsavir (TMR), a first-in-class, investigational attachment inhibitor that binds directly to HIV-1 gp160, preventing initial interaction with, and attachment to, CD4 receptors on T-cells and other host immune cells. Previous studies have identified amino acid substitutions at four gp160 positions (S375H/I/M/N/T, M426L/P, M434I/K and M475I) that may influence susceptibility of the envelope to TMR. We present Week 48 efficacy and the potential impact of treatment emergent substitutions in protocol defined virologic failures (PDVF).

Study Design: BRIGHTE is an ongoing Phase 3, randomized, placebo-controlled, double-blind trial evaluating fostemsavir in heavily treatment-experienced patients with multidrug resistant HIV-1. Two cohorts were studied: randomized cohort (RC) with 1 or 2 remaining ARV classes, and non-randomized cohort (NRC) with zero fully active and approved ARVs. Week 48 efficacy was assessed using the FDA snapshot analysis. Full genotypic and phenotypic analysis was performed on all participants at Screening, to evaluate eligibility and to assess options for OBT, and at failure. TMR susceptibility was assessed using the Monogram PhenoSense Entry assay and gp160 substitutions were assessed at these timepoints. There was no TMR IC50 entry criteria. Changes in TMR IC50 FC (fold change) <3x were considered within the variability of the testing assay.

Results: At Week 48, 54% of RC and 38% of NRC achieved virologic suppression (HIV-1 RNA <40 c/mL). The proportion of participants with prior exposure and pre-existing genotypic substitutions to approved ARV agents was extensive (NRC >RC). Genotypic polymorphisms in gp160 at 1 or more positions of interest were present at baseline in 45% of treated participants. Median baseline TMR IC50 FC was 0.99-fold; 87% of participants had IC50 FC ≤100 (range from 0.04 to >9,000-fold from reference). Day 8 median decreases in HIV RNA were 1.032 log vs. 0.652 log for participants without and with baseline polymorphisms at positions of interest, respectively. Presence of these polymorphisms at Screening did not affect virologic response (VL< 40 c/mL) at Week 48 in the RC. Rates of PDVF at Week 48 were 18% (49/272-RC) and 46% (46/99-NRC), including some participants who re-suppressed beyond Week 48. Overall, in evaluable PDVFs, 52/93 (56%) had treatment emergent genotypic substitutions at positions of interest. Median increase in TMR FC in the RC was 2.34. As expected, more participants in NRC experienced a greater median increase in TMR FC (469.7). The most frequent emergent changes were M426L (33 participants-35%), and S375N (29 participants-31%). The emergence of genotypic and phenotypic resistance to OBT agents was consistent with observations in similar studies.

Conclusions: In BRIGHTE, Week 48 rates of virologic response and PDVF were comparable to other studies performed in similar patient populations. Emergent substitutions mapped to the 4 prespecified amino acid positions, while their presence at baseline in the RC did not impact Week 48 response. The clinical evaluation of PDVF and TMR susceptibility remains context dependent; therefore, more data will be required to reach a clinical cut-off for FTR. The Week 48 data continue to show the benefit of FTR in the HTE population.

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Epidemiological study of Doravirine associated resistance mutations in HIV-1-infected antiretroviral-experienced patients from two large databases in France and Italy

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Background: Doravirine (DOR), a novel HIV-1 non-nucleoside reverse transcriptase (NNRTI), in combination therapy, has non-inferior efficacy to darunavir/r (800/100 mg) or efavirenz (600 mg) in treatment-naïve patients. DOR has an in vitro resistance profile that is distinct from other NNRTIs retaining activity against viruses containing the most frequently transmitted NNRTI mutations, K103N, Y181C and G190A. DOR selects for distinct mutations in vitro, including mutations at reverse transcriptase (RT) positions 106, 108, 188, 227, 230 and 234. The aim of this study was to examine the prevalence of DOR-associated resistance mutations in HIV-1-infected antiretroviral-experienced patients.

Materials & Methods: Resistance genotypic tests were performed at five reference laboratories, 2 in Paris (Pitié-Salpêtrière and Bichat Claude Bernard hospitals) and 3 in Italy (University/polyclinic of Rome Tor Vergata, INMI Spallanzani-IRCCS, Modena Hospital). A total of 9199 HIV-1 RT sequences obtained between 2012 and 2017 from HIV-1 antiretroviral-experienced patients in routine clinical care were analysed. Among this set of sequences, 381 sequences were originated from NNRTI-failing patients. DOR-associated mutations identified in vitro or in vivo were considered: RT V106A/M, V108I, Y188L, G190S, F227C/L/V, M230I/L, L234I, P236L, K103N+Y181C, K103N+P225H, K103N+L100I. The sequences were also interpreted according to the ANRS algorithm to predict genotypic resistance to DOR.

Results: Among the 9199 sequences, 4056 and 5143 were performed between 2012-2014 and 2015-2017, respectively. The distribution of subtypes was: 45.3% B, 27.3% CRF02_AG, 3.7 % A1, 2.5% C, 1.7% CRF06_cpx and 19.5% other various non-B. Among the DOR-associated mutations, the frequencies of mutations (total set vs NNRTI-failing patients) were V106A/M (0.8% vs 2.6%), V108I (3.3% vs 9.2%), Y188L (1.2% vs 2.6%), G190S (0.3% vs 2.1%), M230I/L (2.8% vs 0%), K103N+Y181C (3.9% vs 3.9%), K103N+P225H (2.9% vs 4.7%) and K103N+100I (1.7% vs 3.9%) with a significant higher proportion of these resistance mutations in the NNRTI-failing group ($p<0.05$), except for K103N+Y181C. The overall prevalence of sequences with at least 1 DOR-associated mutation was 12.2% and 34.9% in total set and NNRTI-failing patients, respectively. Among NNRTI-failing patients, the prevalence of common NNRTI mutations V90I, K101E/P, K103N/S, E138A/G/K/Q/R/S, Y181C/I/V, G190A/E/S/Q were 8.9%, 7.9%, 28.6%, 12.6%, 14.2%, 8.9%, respectively. Thus, in the NNRTI-failing group, according to the ANRS algorithm, 18.1% (n = 69) of sequences were genotypically resistant to DOR whereas 36.5% (n=139) were genotypically resistant to nevirapine ($p<0.0001$), 51.7% (n = 197) to efavirenz ($p<0.0001$), 21.9% (n=88) to etravirine ($p=0.1067$) and 55.6% (n=212) to rilpivirine ($p<0.0001$).

Conclusions: These results suggest that DOR resistance in antiretroviral-experienced patients generally and specifically also NNRTI-failures is significantly lower than resistance to NNRTIs currently used, supporting the use of DOR in experienced patients, considering its distinguishing resistance pattern.

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The integration of Hepatitis B virus in relevant regions of human genome is a common event in the setting of HBeAg negative chronic infection despite limited liver disease: implications for an altered cell metabolism

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Background: HBV integration into human genome has been primarily described in HCC and cirrhotic patients with long-term chronic hepatitis B, shedding light on the contribution of integrated HBV DNA in tumorigenesis. Differently, limited data are still available on the occurrence of HBV integration in HBeAg-negative patients with a limited evolution of HBV-related liver disease.
In this light, we aimed to analyze intrahepatic reservoir and the occurrence of HBV integration events in the setting of HBeAg-negative chronic infection with no/mild liver fibrosis, including patients with low levels of viraemia.

Material and Methods: Liver tissues from 40 HBeAg-negative patients were studied. Patients were classified according to viraemia: group-1 (HBV-DNA<2,000IU/ml; n=8), group-2 (HBV-DNA 2,000-20,000IU/ml; n=14), group-3 (HBV-DNA>20,000IU/ml; n=18). cccDNA, intrahepatic total (it)-HBV-DNA were evaluated by Real-Time PCR and pgRNA by digital-droplet PCR. Whole Exome Sequencing [Illumina, median(IQR) coverage: 115x (90x-140x)] was performed in all 40 patients. HBV integration into human exons and into the flanking intronic regions was identified by recognition of chimeric HBV-human sequences applying a bioinformatic pipeline based on Virus-Clip software. The role of genes involved in HBV integration was analysed by GeneCards and Protein Atlas Databases. The threshold of parameters predicting HBV integration was defined by AUROC.

Results: Overall, median(IQR) serum HBV-DNA and HBsAg are 3.9(3.4-5.3) logIU/ml and 3,980(1,260-10,000)IU/ml and 80% has no/mild liver fibrosis (Ishak score< 2).
Group-1 and -2 show a comparable intrahepatic reservoir in terms of cccDNA, it-HBV-DNA and pgRNA. Conversely, compared to group-2, group-3 is characterized by higher cccDNA (median[IQR]:2.6[2.3-2.7] vs 2.0[0.9-2.3]log copies/1000cells, P=0.01), it HBV-DNA (median[IQR]:3.9[3.5-4.4] vs 3.1[2.2-3.9]log copies/1000cells, p=0.005) as well as by more elevated levels of pgRNA (190[7-770] vs 3.3 (1.5-12) copies/1000cells, p=0.02).
HBV integration is detected in 35.4% of pts, more frequently in group-3 than in group-1 and -2 (55.6% vs 18.2%, p=0.03). Among the 17 overall recognized integration events, 11 involves HBx-encoding region, followed by preCore-Core- (N=3) and Pol/S-regions (N=3). In most cases (64.7%), HBV integration events are observed within intronic regions, mainly close to RNA splicing-site, at a distance <100 nucleotides from the proximal exones (47.1%). These regions are known to be critical for a proper splicing and, in turn, for mRNAs production. Notably, HBV integration often localizes in human genes, regulating cell proliferation and, thus, directly involved in hepatocarcinogenesis (NUP85, COL18A1, AGBL5, ANKRD52 and ELAC-2). Furthermore, HBV is also found integrated in genes regulating lipid or drug metabolism (CYP2UI, LMF-1) or in those regulating antiviral or inflammatory response (IFITM-1, NR3C1).
Finally, a higher amount of serum HBsAg is the only factor positively correlated with the occurrence of HBV integration (p<0.001). By AUROC, HBsAg>5,000IU/ml identifies HBV integration with the best diagnostic-accuracy (83.5%), 92% sensitivity and 73% specificity.

Conclusions: In HBeAg-negative infection, HBV integration occurs frequently in highly-viremic patients and in a not negligible percentage of low-viremic patients. Localization of HBV integrations suggests that this event can be involved not only in carcinogenesis but also in mechanisms regulating hepatocyte metabolism, antiviral immunity and inflammatory response.

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The transmission dynamics of acute HCV infections in HIV-positive men who have sex with men in the Netherlands is suitable for targeted risk reduction strategies

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Background: Recently, several outbreaks of acute hepatitis C virus (HCV) infections have been observed among HIV infected men who have sex with men(MSM) in Europe. In the Netherlands, prior to the unrestricted availability of direct-acting antivirals(DAA) a stable and high incidence rate of 10/1000 person-years was reported. In addition, reinfection occurred in 20-25% within two years. Fortunately, the overall HCV incidence among HIV-positive MSM declined with 51% after the widespread use of DAAs in 2015. Yet, reinfection rates remained soaring in this population. HIV-positive MSM whom acquire HCV are often engaged in high-risk behaviour as for example chemsex and unprotected anal intercourse with multiple partners. In order to target risk reduction strategies or to establish a search and destroy strategy as with HIV, more in-depth knowledge is required regarding the transmission dynamics of acute HCV. Therefore, we aim to identify how the acute HCV infections among HIV-positive MSM are linked to specific transmission networks.

Methods: We included a total of 45 HIV-positive MSM, whom were diagnosed with an acute HCV genotype 1 infection in one of the ten HIV treatment centres across the Netherlands and were enrolled in the Dutch Acute HCV in HIV study(DAHHS 1) between 2013 and 2014. All patients received a DAA regimen containing boceprevir. Target enrichment for viral nucleic acid separation and deep sequencing with the Illumina MiSeq platform with 500bp v2 reagent sets were run multiple times to recover full-length HCV genomes. All generated sequences were aligned and for each query sequence the 100 highly similar sequences were selected through BLAST. After preforming a model test with IQtree and removal of insufficient and duplicate sequences, a maximum likelihood tree was constructed in MEGA X64 using the general time reversible nucleotide substitution model allowing for among-site variation. The robustness of phylogenetic clustering was tested by a bootstrap analysis with 1,000 replicates. Transmission networks were evaluated using ClusterPicker, with a cluster being defined as a group sequences with a genetic distance of at most 1.5% and a bootstrap support value of 100%. The most recent common ancestor was estimated with a coalescent-based HKY model with a Bayesian statistical framework.

Results: We are the first study in which all new HCV infections among HIV-positive MSM in the Netherlands were included. Four major genotype 1a transmission clusters were identified including 37(84%) patients. The largest cluster accounted for 13(30%) of the new genotype 1a HCV infections among HIV-positive MSM. Interestingly, all clusters were indicative of recent outbreaks, highlighted by small genetic distances and a most recent common ancestor after the year 2000, when the first cases of HCV infection in HIV-positive MSM were reported.

Conclusion: We used well-defined data from the Netherlands to identify the acute HCV distribution among HIV-positive MSM. Our data showed indeed that the HCV epidemic is a young epidemic and that mostly all of the acute infections are linked within major transmission networks. This is crucial information that highlights the possibility of targeted risk reduction strategies. In addition, this information can contribute into curbing the epidemic.

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Clinical and virological characteristics of patients with chronic hepatitis C and failure to a Glecaprevir and Pibrentasvir (G/P) treatment in real world

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Background: Treatment of chronic hepatitis C virus (HCV) infection with Glecaprevir and Pibrentasvir (G/P) results in high rates of virologic success both in clinical trials and real world. However, the number of patients with virologic failure to G/P in phase II/III studies was limited (n=22), and there is also little information on how patients fail to this combination.. Here we present real world data on failure to G/P from three international cohorts across different genotypes.

Methods: Samples were obtained from resistance databases in Germany, Spain, Italy, Austria and Switzerland containing samples from more than 1200 DAA failure patients. Population sequencing of NS3, NS5A and NSB5 was conducted and RASs conferring a >2-fold increased DAA susceptibility were analyzed. Limited clinical parameters were collected retrospectively.

Results: Altogether 63 patients had a virologic treatment failure to G/P, 93% (52/56) treated for 8 weeks and 5% (3/56) for 12 weeks. 47 patients were male (82,5%) with a mean age of 52 years (IQR, 44-61). The majority of patients was infected with genotype (GT) 3a (n=29, 46%), while 15 individuals had GT1a, 11 had GT2c, 5 had GT1b, 2 had GT2a and 1 was infected with GT4d. Cirrhosis was detected in 6 patients, 2 patients had previous exposure to interferon and one had failed to a previous DAA combination treatment. Overall, 17 patients (26%) failed a G/P regimen without any RAS to the regimen. After failure to G/P, 47% (36/58) patients developed RASs in NS5A, 19.3% (11/57) in NS3, and 13.0% (7/54) in both NS3 and NS5A. The most frequent RASs were Y93H (22/58; 37.9%) in NS5A and Q168L (4/57; 7.0%) in NS3. Interestingly, a combination of two or more RASs in NS5A was frequently detected (one n= 5; two n=26; three n=5). P32 deletion in NS5A was detected in a genotype 1b infected patient.

Conclusion: Even if around one third of patients failed to G/P without resistance, RASs in NS5A were frequent and more prone to develop than in NS3. NS5A RAS were frequently observed as dual and triple patterns, with a high impact on NS5A inhibitor activity. Our results support the use of resistance findings to aid decision making on how to retreat G/P failures.

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Retreatment of HCV infected patients with a previous failure to a NS5A inhibitor-containing regimen after performing a genotypic resistance test: the Italian Vironet C real life experience

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Background: Up today, there is a limited documentation about the efficacy of retreatment in patients who previously failed a recommended NS5A-containing regimen in Italy.

Materials & Methods: Within the Italian network VIRONET-C, a total of 412 NS5A-failing patients infected with different HCV-genotypes (GT) (GT1a/1b/2a-c/3a-b-g-h/4a-d-n-o-v=96/125/26/127/38) were analyzed. The retreatment of 108 failures was also investigated. Sanger sequencing of NS3-protease and/or NS5A and/or NS5B was performed at the time of virologic failure, with in-house protocols in 14 different Italian centers.

Results: Failures following seven different NS5A-containing regimens were studied: 3D/2D (paritaprevir/ombitasvir+dasabuvir)+ribavirin (N=77/4), daclatasvir/ledipasvir/velpatasvir +sofosbuvir+ribavirin (N=113/131/24), grazoprevir/elbasvir+ribavirin (N=34), glecaprevir/pibrentasvir (N=29). Notably, 19.2% (79/412) of NS5A-failing patients did not show any resistance-associated-substitutions (RAS), while 80.8% (333/412) showed at least one NS5A-RAS, with multiclass-resistance in 33.7% (139/412). A different distribution of NS5A and NS3 RAS was observed at grazoprevir/elbasvir and glecaprevir/pibrentasvir failure, respectively. In particular, NS5A-RAS were more

prevalent in grazoprevir/elbasvir failures (94.1%, 32/34) than in glecaprevir/pibrentasvir failures (51.7%, 15/29; p<0.001). Also NS3-RAS were frequently detected in grazoprevir/elbasvir failures (29.4%, 10/34) compared to glecaprevir/pibrentasvir failures (6.9%, 2/29; p=0.02). Notably, all patients with NS3-RAS showed also NS5A-RAS at failure in both these regimes. At velpatasvir/sofosbuvir failure, NS5A-RAS were found in 15/24 (62.5%) patients, particularly in GT1a (28.6%, N=7) and GT3a (90.0%, N=10).

To date, 108 failures have started a retreatment: sofosbuvir/velpatasvir+ribavirin (N=30), sofosbuvir/velpatasvir/voxilaprevir+ribavirin (N=70), glecaprevir/pibrentasvir (N=4), grazoprevir/elbasvir+sofosbuvir+ribavirin (N=3), grazoprevir/elbasvir+ribavirin (N=1).

The majority of patients were cirrhotic (50.9%) and relapsers (93.1%). The prevalence of NS5A-RAS before retreatment was 79.6%, and multiclass-resistance 27.8%. Among patients completing post-retreatment follow-up, a sustained-viral-response at week 12 (SVR12) was observed in 66/74 (89.2%). SVR4 was documented in 76/83 (91.5%). SVR12 was 77.8% with sofosbuvir/velpatasvir+ribavirin (N=27). Differently, SVR12 was 100% with glecaprevir/pibrentasvir for 8/12/16 weeks (N=4), grazoprevir/elbasvir+sofosbuvir+ribavirin for 12/24 weeks (N=4), despite the presence of NS5A-RASs.

Of 70 patients who started sofosbuvir/velpatasvir/voxilaprevir+ribavirin recommended-retreatment for 12 weeks, 56/70 (80.0%) showed at least one baseline NS5A-RAS, 23/70 (32.8%) multiple-NS5A-RASs, and 22/70 (31.4%) multiclass-resistance. Of 39 patients with available outcome, 94.9% had SVR12. Virologic failure was observed in two patients. One was a GT1b infected patient non-responder, without any RAS before and after retreatment. The other failure was a GT1a infected patient relapse, who showed the NS5A resistance pattern Q30R+L31M at baseline of retreatment. This resistance pattern was confirmed at retreatment-failure, without any new additional RAS in NS3 and NS5B genes.

Conclusions: In this Italian real-life experience, NS5A-RASs were frequently detected in NS5A-failing patients, and multiclass-resistance was around 30%. Overall, SVR after resistance-test-guided retreatment was >94%, with the exception of the sofosbuvir/velpatasvir retreatment. Our results show how HCV genotypic resistance test after failure may be useful to optimize the retreatment strategies.

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Specific NS5A polymorphisms correlate with hepatocellular carcinoma in cirrhotic patients infected with HCV genotype 1b

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Background: Hepatocellular Carcinoma (HCC) generally arises after a stage of advanced liver fibrosis and cirrhosis with an incidence about 1-8% per year. The HCV protein, NS5A, is known to interact with different cellular proteins, including P53, thus inducing intracellular signaling pathways associated with cell proliferations. In this light, we explored the genetic variations in NS5A associated with HCC.

Materials & Methods: This study includes 188 patients chronically infected with HCV genotype 1b, all cirrhotic and DAA-naïve: 34 diagnosed with HCC and 154 controls without HCC. NS5A domain-1 (amino acid: 1-183) sequences were obtained for all patients by Sanger method from plasma samples. Association of mutations with HCC was assessed by Fisher Exact test. Shannon Entropy (SE) was used to describe residues with significantly higher (P<0.05) variability (SE>0.2) in HCC compared to No-HCC.

Results: HCC patients were characterized by comparable median (IQR) log serum HCV-RNA [5.6 (5.3-6.1) vs 5.8 (5.3-6.1) IU/mL], ALT [65 (37-86) vs 71 (50-112) U/L], and significantly higher liver stiffness [28 (20-33) vs 19 (15-26) KPa, P<0.001] compared to No-HCC patients.

By mutational analysis, four specific NS5A polymorphisms significantly correlated with HCC: S3T (8.8 vs 1.3%, P=0.01), T122M (8.8% vs 0.0%, P<0.001), M133I (20.6 vs 3.9%, P<0.001), and Q181E (11.8 vs 0.6%, P<0.001). By SE, other three residues were more variable in HCC: C13R/S (SE=0.264; P=0.03, located in highly conserved N-terminus NS5A domain), F127L/S (SE=0.225; P=0.03), and N137D/K (SE=0.264; P<0.001). Furthermore, an enrichment of additional mutations is observed at residue 181 (Q181E/G/H/P, SE=0.410; P=0.01). Notably, all the above mentioned residues are localized in regions of NS5A domain-1 known to interact with cellular proteins as P53 (aa:1-149), involved in the apoptosis regulation, and/or with P85-PIK3 (aa:1-112), involved in Wnt/β-catenin signaling pathway regulating the cell growth.

Conclusions: The association of specific NS5A polymorphisms with HCC provide a focus for further investigations aimed at elucidating the molecular basis of HCV-mediated oncogenesis. These viral signatures, if confirmed in a larger population, could play a crucial role as prognostic markers of HCC, especially in cirrhotic-HCV patients, helping to identify patients at higher HCC-risk, deserving more intense liver evaluation and/or early treatment.

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Longitudinal analysis of proviral HIV-DNA

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Background: HIV replication can be measured as viral load in plasma (VL), which is the preferred approved marker for treatment success monitoring. Sustained plasma viral load below the limit of detection (<50 copies/µL) is the goal in treatment strategy of HIV patients. However, in time of successful long-term therapies and upcoming cure strategies, there is need for a further marker to analyse the effects on the proviral reservoir. In order to analyse the dynamics of proviral load (pVL) and evolution of proviruses in patients with viremia and undetectable plasma viral load we are collecting and examining longitudinal collected samples from more than 70 patients on ART. Here we present the updated results of this ongoing analysis.

Materials and methods: Sequential blood samples (at least 3) during a period of 2.5 years were collected from each patient. For pVL measurement, cellular DNA was extracted from PBMCs. Proviral HIV load and PBMC count were measured in a real-time PCR to calculate pVL (log cop/Mio cells). The data were analysed with regard to VL, pVL, CD4 cell count, HIV treatment combinations and also the period of undetectable VL.

Results: Three subgroups were defined depending on the VL: SVL (suppressed viral load, <50 copies/µL), LLV (low level viremia, 50-499 copies/µL) and viremia (>500 copies/µL). The highest pVL was in the LLV subgroup and the lowest in the SVL subgroup.
We saw a slight decline of the pVL over time in all patients on successful treatment. A regression line was calculated from all available pVL values in the observed period for each patient. The slope of the regression line indicates the extent of increasing or decreasing proviral load. The LLV group showed the strongest decline of the proviral load in median. In contrary, in patients with constant suppressed viral load the lowest decline was observed.
There were no significant differences neither in mean nor in the slope of proviral load between the patients with an INI-containing regimen and without INI.
Comparing the course of pVL and CD4 cell counts of the consequent time points we could observe three different groups of trends. One group consisted of patients with similar courses of CD4 count and pVL (concordant ascending/descending curves). Second group was characterized by disconcordant curves, e.g. descending pVL in spite of increasing CD4 counts and vice versa. Third group was built by patients, which could not fit neither of the characteristics of previous groups.

Conclusions: Our analysis showed that a sustained suppressed viral load leads in the long term to the reduction of the proviral reservoir. The decrease of the pVL can serve as a marker for success of the antiretroviral therapy. An increase of the pVL may indicate a minimal virus replication and thus progressing the damage of the immune system. Furthermore, there is need to examine in detail which factors contribute to the dynamics in proviral DNA load in spite of suppressed plasma VL. Proviral load could be an easily to perform and useful readout in routine diagnostics.

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Single genome sequencing of near full-length HIV-1 RNA

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Background: Studies on virus evolution are mostly restricted to short fragments of the HIV-1 genome, often located in the fast evolving env or gag genes. Amplification and sequencing of the whole 9.2 kb long genome of the virus remains technically challenging. The infeasibility to reliably link short sequence fragments to individual variants impedes research aimed at characterization of the HIV-1 quasispecies, tracing of immune evasion or in vivo recombination. As we were particularly interested in the study of recombination, we developed and validated a method for amplification of individual viral variants over an as long as possible length. We then assessed the possibility to execute this amplification on single viral RNA molecules and study recombination in patients with dual HIV-1 infection.

Material & Methods: The near full-length HIV-1 genome is covered by 2 overlapping amplicons with a length of respectively 4,511 and 4,627 nucleotides. The efficiency and sensitivity of the amplification was assessed on EDTA blood plasma from 41 patients, sampled early after infection. Subsequently the usefulness, sensitivity and accuracy of the method was evaluated by limiting dilution using a plasma pool of 3 different HIV-1 subtypes. Either viral RNA or in-bulk generated cDNA were diluted to the limit after which multiple replicates of the end point dilution were analyzed. The distribution of the 3 subtypes and the occurrence of in vitro recombination was assessed through construction of phylogenetic trees and highlighter plots. The protocol for limiting dilution single genome sequencing was also applied on samples from 5 patients with HIV-1 dual infection.

Results: Amplification and sequencing of the full HIV-1 genome was successful for 38 of the 41 samples that comprised a broad range of HIV-1 subtypes and a range of viral load between 330 and 10^7 copies/mL. For the plasma pool of 3 HIV-1 subtypes equal sensitivity and accuracy in identifying the 3 subtypes and their distribution was obtained after RNA and cDNA limiting dilution. One evidence of in vitro recombination was demonstrated in 20 sequences obtained after cDNA dilution. No indications for in vitro recombination were found in the 25 sequences obtained after RNA limiting dilution. Analysis of the single genome sequences of samples from 5 patients with dual infection revealed a heterogeneous virus population with co-circulation of at least 3 recombinant forms in 1 patient and clear indications for the presence of multiple recombinant variants in 2 other patients.

Conclusions: This study presents a method for the sequencing of individual near full-length HIV-1 genomes using a 2 amplicon approach and limiting dilution of either viral RNA or cDNA. The possibility to dilute viral RNA to the extreme before reverse transcription was successfully explored as a way to reduce in vitro recombination. Using the method for the analysis of HIV variant composition in patients with dual infection showed co-circulation of multiple recombinant forms.

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Maraviroc as a Latency Reversing Agent in cell line models

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Background: HIV-1 latency is the hallmark of persistent infection, because silently infected cells can escape antiretrovirals or immune attack. The use of compounds aimed at reversing HIV-1 latency coupled with fully suppressive antiretrovirals is the basis for the shock and kill strategy. Recent studies suggested that maraviroc (MVC), the only anti-HIV-1 agent targeting a cellular factor, may exert an HIV-1 latency reversal effect. The aim of this study was to evaluate MVC mediated induction of HIV-1 in two in vitro immortalized cell models of HIV-1 latency.

Materials and Methods: HIV-1 promoter activation was evaluated in TZM-bl cells expressing luciferase under the control of HIV-1 LTR. Induction of extracellular and cell-associated HIV-1 RNA (CAR) was evaluated by real time PCR in U1/HIV-1, an HIV-1 latently infected promonocyte cell line, which can be induced to produce virus upon stimulation with transcription activators. NF-kβ induction was evaluated in TZM-bl and U1/HIV-1 nuclear extracts by the NF-kB (p65) Transcription Factor Assay Kit. Expression of CCR5 in the cell lines tested was assessed by flow cytometric analysis.
Cell lines were treated with 4-fold dilutions of MVC (from 80 to 0.31 µM) for 24 hours. Phytohemagglutinin (PHA, 10 µg/ml) and Ionomycin (ION, 1 µg/ml) in combination with Phorbol-12-myristate-13-acetate (PMA, 50 ng/ml) were used as control latency reversal agents (LRA). Induction was expressed as fold-activation (FA) with respect to untreated cells. MVC cytotoxicity was measured by the Cell Titer-Glo cell viability assay.

Results: Cell surface CCR5 was detectable in 40% and 94% of TZM-bl and U1/HIV-1 cells, respectively. MVC was not cytotoxic in the tested range (from 160 to 0.31 µM). No HIV-1 promoter activation was observed in TZM-bl cells at any MVC concentration (0.93±0.07 FA), nor with PHA (1.04±0.06 FA). ION+PMA induced luciferase expression by 4.51±0.15 FA.
HIV-1 CAR was weakly and equally induced in U1/HIV-1 cells at different MVC concentration (80, 20, 5 and 1.25 µM) with values (1.28±0.08 FA) comparable to PHA (1.30±0.37 FA), but considerably lower than ION+PMA (317.53±120.32 FA). Extracellular HIV-1 RNA FA was 3.11±0.92, 1.35±0.50, 1.90±0.46 at 80, 20 and 5 µM MVC, respectively, higher than PHA (0.83±0.19) but much lower than ION+PMA (1768.03±1055.31). NF-kB expression was not upregulated at any MVC concentration in either U1-HIV-1 (0.74±0.16 FA) or TZM-bl (1.11±0.05 FA) cells.

Conclusions: We showed a weak induction of extracellular HIV-1 RNA in U1/HIV-1 cells at MVC concentration ranging 5 to 80 µM, comparable to standard 10 µg/ml PHA. However, MVC failed to activate the HIV-1 promoter in the TZM-bl model and NF-kB expression in TZM-bl and U1/HIV-1 cells. Based on this and previous studies, MVC appears to act as a weak LRA in some but not all cell line models. While further investigation could unveil the reasons for such differential effects, ex-vivo studies of patient derived latently infected CD4 cells are required to define a possible role of MVC as clinically relevant LRA.

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Viral Dynamics During Suppressive ART - Towards HIV-1 Elimination From Reservoirs

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Background: With increasing long-term control of viral replication and with HIV-related health issues rising globally, new approaches for sustainable therapy and efforts towards a cure have reached research agendas worldwide. Cure strategies like latency reversal have garnered much interest, but largely lack selectivity.
Our research strategy takes a different approach by following the changing viral properties of HIV-1 over the course of infection and during times of suppressive therapy. Recently, our laboratory demonstrated that envelope properties of HIV correlate with immunological recovery and disease outcome. In particular, while the presence of CXCR4-tropic (X4) virus correlates with poorer outcomes in therapy-naives, effective therapy seems to facilitate a superior control of X4 viruses[1].
Based on this unexpected activity, this study aims at a detailed characterization of HIV inside the key T-cell populations during ART to identify critical lymphoid compartments responsible for the selective elimination of X4-tropic proviruses and cells.

Materials and methods: Peripheral blood from HIV-positive patients within the Swiss HIV cohort study was used for selective cell fractionation, applying MACS technology. Non-relevant CD8+(CTLs) and CD19+(B-cells) were depleted, and CD8-CD19- cells were selected for CD4 and Integrin B7 (gut homing) or CCR7 (lymph node homing). Proviral load (pVL) was determined by qPCR. For multi-dimensional data visualization a customized tSNE tool was applied.

Results: Taking total HIV DNA per 10e6 cells as a proxy for reservoir size, MACSorted fractions achieved the expected significant proviral enrichment in CCR7+/ß7+(CD8-CD19-) cell fractions, enabling a detailed analysis of CD4+ and CD4- fractions. Our study focuses in the retention/re-establishment of crucial immune compartments.
Selective FACS sorts, using highly-specific marker antibodies, revealed that ≥90% of cells with gut homing also have properties compatible for Lymph node homing. First analyses applying tSNE identify homing properties in detail and a depth down to individual cells where we find a significant tissue contribution beyond the circulation, evidencing active compartments.

Conclusions: Only a small fraction of the total HIV RNA-positive cells are present in the peripheral blood at any time[2], highlighting the importance of assessing the viral distribution in lymphoid compartments like lymph node or gut. Our first cell selection approach demonstrates the ability to assess central markers for viral sanctuaries and cell-homing. Currently, we are expanding the marker panel to co-analyze HIV Envelope (surface) and intracellular Gag-expression as proxies for viral intactness, and by longitudinally following patient samples (suppressed but with high pVL) during cART therapy-episodes. Relevant markers identified by tSNE are being used for live cell sorts. Virus reactivation from sorted cell fractions will determine viral phylogeny and tropism and potential links to compartmentalization and viral dynamics. We believe that our strategy will contribute to the formulation of new strategies towards targeted cellular virus elimination and ultimately HIV eradication.

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CD3+CD8low T cells harbored dysfunctional functions in patients at the earliest stage of HIV infection

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Background: In acute HIV infection in humans, HIV-specific CD8+ T cells are critical for the initial control of HIV infection. CD3+CD8low T cells are recognized as a subset of CD8+ T cells with down-regulated CD8 expression, whose increase is observed in patients infected with chronic HIV infection, but little is known about whether HIV-1 suppression benefits from low CD8 expression on CD8 T subpopulation during acute infection stage in the absence of antiretroviral therapy (ART).

Materials & Methods: A total of nineteen acute HIV-1-infected individuals in 1st, 3rd month, and 1st year after infection were enrolled from the Beijing Primo Cohort in this study to evaluate HIV-1-specific effector functions of CD3+CD8low T cells. Samples of 20 individuals in chronic HIV infection were also analyzed, and without ART. Immunophenotypic and functional characterization of CD3+CD8low and CD3+CD8hi T cells were analyzed by the multicolor flow cytometry. HIV-specific CD8 T-cell responses were measured by quantifying interferon gamma (IFN-γ) release with an intracellular cytokine staining assay, and the degranulation (CD107a) of CD8 subpopulations were also measured in untreated individuals with acute/chronic HIV-1 infection.

Results: We found for the first time that CD3+CD8low cells quickly expanded after HIV-1 infection and lasted for a short time, and then decreased until to the chronic phase of infection, while CD3+CD8hi T cells were significantly increased from the 1st year of HIV infection to chronic infection over 2 years. Interestingly, the immune activation of CD3+CD8low cells was significantly higher than that in CD3+CD8hi T cells at different stage of HIV infection (all $p<0.05$). In addition, we observed that a comparable proportion of CD3+CD8hi and CD3+CD8low T cells produced HIV-1-specific IFN-γ on the 1st, 3rd month and 1st year of infection, while the levels of CD3+CD8low T cell expressing CD107a degranulation were lower in untreated individuals after 3rd month of HIV-1 infection than those induced in CD3+CD8hi T cells.

Conclusions: Our findings suggest that a better understanding of the involvement of CD3+CD8low T subpopulation at the earliest stage of HIV infection would significantly improve our knowledge of the impaired T-cell responses in HIV-1-infected patients, which has important implications for HIV vaccine development.

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Lack of HIV-1 integrase inhibitor resistances among 392 antiretroviral-naïve subjects in a tertiary care hospital in Beijing, China

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Background: Integrase strand transfer inhibitors (INSTIs) have high potency, high barriers to resistance, and good tolerability, and INSTIs have become an important part of antiretroviral therapy (ART) since the introduction of raltegravir (RAL) in 2007. Currently, RAL and dolutegravir (DTG) are included in the first-line regimen recommended by the "Society of Infectious Diseases of Chinese Medical Association". No major INSTI mutations were found among primary HIV-1 infected individuals in America, Europe, and Australia, but few data are available about the prevalence of HIV-1 INSTI resistance among ART-naïve patients in China. In this study, we characterized HIV-1 INSTI resistances among ART-naïve patients from a tertiary care hospital in Beijing, China.

Materials & Methods: Individuals with primary HIV-1 infection were enrolled in an observational Primo cohort in a tertiary care hospital in Beijing. We analyzed the HIV int gene from plasma of 392 antiretroviral-naïve patients with primary HIV-1 infection. All HIV-1-infected patients in the study were without HBV/HCV coinfection and other comorbidities, and none of them were drug users.

Results: No major INSTI mutations were identified among ART-naïve individuals in the study. However, two subjects harbored INSTI accessory mutations E157Q/T97A were detected for the first time. Thus, INSTI resistance mechanisms and to what extent these resistances impact the clinical effectiveness of INSTI need to be investigated in further studies.

Conclusions: Our results emphasize the need to consider testing for INSTI resistance at baseline as this class of drugs is increasingly used in clinical routine.

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HCC development in HCV-infected patients with SVR and diagnosis challenges in resource-limited settings

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Background: In some low- and middle-income countries, include Armenia, directly acting antiviral agents (DAAs) are still not registered and health care budgets are not cover treatment expenses. Take into consideration published studies and expert consensus in 2018, it was estimated that 4.0% (2.9-6%) of the adult population of Armenia were anti-HCV positive. Using a viremic rate of 70% (65-72%) there were 68,000 Armenians have chronic hepatitis C in 2018, correlating to a prevalence of 2.8% among all ages.
Based on the mathematical model of disease progression, which was calibrated using reported Armenian specific epidemiologic data, if there is no change through 2030 under the current treatment paradigm liver related deaths, HCC, and decompensated cirrhosis will increase by 1-8%. New cases of hepatocellular carcinoma will increase by 6% to 330 in 2030. Management of HCC is huge challenge, because of problems with diagnostic and therapeutic procedures (RFA, TACE, liver transplantation) and insurance system covered expenses. From August of 2018 we set up checking of PIVKA-II (Protein Induced by Vitamin K Absence or Antagonist-II) or DCP (Des-γ-carboxy-prothrombin) as an early bio-marker of HCC.

Materials & Methods: 80 HCV-infected patients from 18 to 73 years old (62.5%male, 48.6±13.2 years old, BMI 26.3±5.23 kg/m2, viral load 118–27485300 IU/ml) treated with DAA-contain regimens with/without IFN (8/69). Genotype distribution: genotype 1b –43.8%, genotype 1b+2 – 5%, genotype 1b+3 – 1.3%, genotype 2 – 6.3%, genotype 3 – 40.0%. IFN-free SOF contain regimens were following: SOF+RBV in 5 patients; SOF+DCV±RBV in 35 patients, SOF/LDV±RBV in 25 patients and SOF+VLP±RBV in 7 patient. In 7 naïve patients with 1b genotype and viral load <6000000 IU/ml duration of SOF/LDV treatment were shortened to 8 weeks. AFP checked in all patients with F 3 and 4 (normal ≤8.78ng/mL). PIVKA-II checked in patients with elevated AFP or liver nodules on US. Serum levels of PIVKA-II were measured using the chemiluminescent assay (Architect, Abbott, USA) with cut of 50.9 mAU/mL.

Results: F4 diagnosed in 46.3% of patients, F3 in 13.8%. Three patients with decompensated cirrhosis died despite SVR. AFP in average 14.9±3.9 ng/mL (range 1.4-135.3) was elevated in 44% of patients with F3 or F4. In majority of patients AFP decreased after antiviral therapy, except 3 patients. In one of them with Child-Pugh A liver cirrhosis, genotype 1b, obesity (BMI=34.3) AFP elevated from 26 to 51.6 ng/mL, despite SVR on SOF/LDV. Patient despite awareness not pass US control. After 2 years US revealed nodules, biomarkers PIVKA-II 95658.28 mAU/mL, AFP 77308.58 ng/mL. Another experienced patients with Child-Pugh A liver cirrhosis, genotype 3, obesity (BMI=35.7), metabolic syndrome, dyslipidemia, NAFLD/NASH treated with SOF+PEG+RBV with SVR (in 2016) after two years develop 3 nodules in liver. AFP dynamic: 8.56 (12.05.2016.), 5.08 (07.07.2017.), 20.55 (27.11.2018.), 181.72 (12.03.2019.) ng/mL. PIVKA-II in 01.12.2018. – 577.18 mAU/mL, elevated 644.42 (12.03.2019.).

Conclusions: Awareness to HCC development is mandatory in all patients with F3-F4. Patients with HCV cirrhosis required close monitoring even after SVR. Serum levels of PIVKA-II in combination with AFP and imaging technics can help in early diagnosis of HCC.

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The activities of friendly offices in the Republic of Kazakhstan

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In order to ensure access of vulnerable groups of population (IDU, SW, MSM) and young adults to diagnostics and treatment of STIs on a free, confidential and anonymous basis, there are 31 friendly offices (FO) in the country (2017-32), 25 of which are located at the AIDS centers, and 6 are at the other medical organizations (venereal and skin clinics, antenatal clinics, polyclinics).

In 2018, 24641 people appealed to friendly offices (2017 - 28068). From the number of persons who applied to FO, 43.5% are SW (2017–44.7%), 5.9% - MSM (2017–4.6%), 22.5% - IDU (2017–26.4 %), 22.2% are young people (2017–20%) and 5.7% of PLHIV (2017–4.2%). In 45.6% (2017-47.8%) of the persons who applied, one or more STI syndromes were confirmed by laboratory. 15.8% of clients were sent to the STI clinics to clarify the diagnosis (2017 - 15.4%). The number of clients surveyed for STIs - 22,156 people, of which 50.7% were diagnosed with STIs (2017 - 51.5%), and 97% received treatment (2017 - 94.3%) . Pre-test counseling was carried out for 23053 people, of which 62.7% were tested for HIV by a rapid test method (2017 - 66.8%).

According to the data, it can be observed that the detectability of STIs and HIV infection is increasing; syphilis has significantly increased among MSM, 8.5% of cases (2017- 2.9%), and slightly increased among HIV, 1.8% (2017-1.4%). Among PLHIV, gonorrhea and syphilis increased by almost 2 times compared with last year. HIV infection has increased among young people, which is why strengthening prevention work among this category is especially important.

Thus, in spite of the fact that the number of people covered with medical services has decreased compared to last year, the incidence of STIs and HIV infection among key populations is increasing.

In this regard, it is necessary to strengthen the activities of Friendly Offices, revise the diagnostic algorithms for STIs and treatment protocols in accordance with international recommendations, increase the budget for the purchase of diagnostic consumables and drugs, and take preventive measures in relation to STIs and HIV infection.

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Hepatitis E in HIV-infected patients in Belarus

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Hepatitis E is clinically occurs similarly with other types of acute viral hepatitis. There is a particular risk group with immunodeficiency state, among them are the HIV infected. The purpose of this study was to determine the markers of HEV infection among of HIV positive patients in Republic of Belarus.

Material and methods: Were study 126 patients with HIV infection and icteric form of acute hepatitis. Serum samples were tested for anti-HEV IgM and IgG by ELISA and for HEV RNA (PCR).

Results and discussion: The analysis of the survey allowed to identify the presence of anti-HEV Ig M in 3.17% of patients, indicating acute infection of hepatitis E at the time of the study. In 7.14% of patients were detected anti-HEV IgG, indicating previous contact with HEV and the presence of immunity to the infection. It was revealed that the frequency of anti-HEV Ig M and Ig G were higher in men as compared to frequency of circulation rate of these antibodies among women i.e. anti-HEV Ig G (8: 1) and Ig M (3: 1), respectively. It was also revealed that anti-HEV Ig G was found in combination with anti-HCV in 56% of cases. All patients with acute infection in the past year did not leave the territory of the Republic of Belarus and consumed dried meat of pigs. Only one patient was infected outside the country, in Thailand. The patient was diagnosed with hepatitis E, hepatitis A and acute HIV infection. However, due to the fact that immunity is not lifelong, this category of people is at risk. It can be argued that these autochthonous cases are caused the virus.

Conclusions: The conducted research allow to claim that the cases were caused by autochthonous virus (patients were infected in the Republic of Belarus, resulting from the consumption of dried meat of pigs) circulating on the territory of the Republic of Belarus. All patients, including HIV patients, diagnosed with Hepatitis or with elevated levels of alanine and aspartic transaminase should be tested by ELISA for detection of antibodies to hepatitis E virus. All cases of hepatitis E in the Republic of Belarus were associated with HEV genotype 3.

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Discovery of Previously Unknown Archived M184V/I and Thymidine Analog Mutations and Maintenance of Virologic Suppression in HIV-1 RNA-Suppressed Patients Switching to Bictegravir/Emtricitabine/Tenofovir Alafenamide (B/F/TAF)

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Background: Pre-existing resistance can affect the efficacy of switching antiretroviral regimens in HIV-infected individuals. Studies 1878 and 1844 demonstrated the non-inferior efficacy of switching stably suppressed HIV-1-infected adults to bictegravir/emtricitabine/tenofovir alafenamide (B/F/TAF) versus continuing boosted protease inhibitor (b/PI)-based triple regimens or dolutegravir/abacavir/lamivudine (DTG/ABC/3TC) through 48 weeks. After Week 48, all participants received open-label B/F/TAF. We retrospectively investigated pre-existing M184V/I and thymidine analog resistance mutations (TAMs), frequency of viral blips, and virologic outcomes through >2 years of B/F/TAF treatment.

Materials and methods: Participants included in the analysis had ≥1 post-baseline HIV-1 RNA measurement through September 15, 2018. Pre-existing HIV-1 drug resistance was first assessed by historical genotypes, and documented M184V/I or K65R at screening was exclusionary. Proviral DNA genotyping (GenoSure Archive® assay, Monogram Biosciences) was conducted retrospectively on samples from the baseline visit. Participants with resistance substitutions detected by proviral genotyping after randomization were allowed to continue on study. Transient viremia (blip; one HIV-1 RNA ≥50 copies/mL measurement preceded and followed by HIV-1 RNA <50 copies/mL) was assessed. Virologic outcomes were based on last available on-treatment HIV-1 RNA.

Results: Altogether, 570 participants switched to B/F/TAF on Day 1 and were treated for a median of 116 weeks (IQR 108-120 weeks). Cumulative baseline genotypic data from historical and/or proviral genotypes were available for 78% (445/570) of B/F/TAF-treated participants: 36% (159/445) had ≥1 pre-existing primary resistance substitution to any antiretroviral drug at baseline. M184V/I was uncovered by proviral genotyping in 10% (44/445) of participants, and 1-2 TAMs were detected in 6.1% (27/445). Through Week 48, there were 74 viral blip events: 26 occurred in 4.4% (25/570) of B/F/TAF-treated participants compared to 31 in 8.1% (23/285) of participants in the b/PI group and 17 in 5.3% (15/281) of participants in the DTG/ABC/3TC group. After Week 48, there were 22 blip events in the B/F/TAF group: 18 occurred in 17 participants who experienced their first blip post-Week 48 and 4 occurred in 4 of those who also experienced a blip before Week 48. In total, 48 blips were observed in 7.4% (42/570) of B/F/TAF-treated participants through >2 years of follow-up. When stratified by baseline resistance, blips were observed in 4.5% (2/44) with M184V/I (both occurred before Week 48) and in 11% (3/27) with 1-2 TAMs (all occurred post-Week 48). At the time of analysis, 98% (561/570) of all B/F/TAF-treated participants were suppressed (HIV-1 RNA <50 copies/mL), including 98% (41/42) who had a viral blip on study, 95% (42/44) with M184V/I, and 93% (25/27) with 1-2 TAMs. No B/F/TAF-treated participant developed drug resistance.

Conclusions: In studies 1878 and 1844, high frequencies of previously unidentified baseline M184V/I and TAMs were detected among suppressed patients who were enrolled. In participants with or without pre-existing resistance, viral blips were infrequent and high rates of virologic suppression were maintained through >2 years of B/F/TAF treatment. Long-term suppression and the absence of treatment-emergent resistance indicate that B/F/TAF may be a treatment option for suppressed patients with documented or unidentified M184V/I and/or 1-2 TAMs.

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Baseline factors associated with treatment outcome in HCV-infected patients starting a first- line DAA-treatment containing a NS5A inhibitor: particular focus on natural resistance

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Background: The introduction of direct-acting antiviral agents (DAAs) in clinics has revolutionized the management of HCV infection. Even if today, with the new recommended regimens, we have a very high rate of sustained virologic response (SVR, >95%) and viral failures are much less frequent than in the past, they still represent a concern, especially when resistance is present. This study aimed to evaluate the presence of natural resistance-associated-substitutions (RASs) and other pre-treatment risk-factors for failure in the Italian Vironet C cohort group of HCV-infected patients, starting a first-line treatment with a NS5A inhibitor-containing regimen

Materials & methods: RASs in NS3/NS5A/NS5B (N=1748/1560/1239) were analysed in 2010 DAA-naïve patients. Of them, 769 patients with a baseline NS5A resistance-test and available outcome after a first-line NS5A-containing regimen recommended by the 2016 or 2018 European Association for the Study of the Liver (EASL) guidelines, were also analysed. HCV Sanger-sequencing was performed by home-made protocols in 7 different Italian centers. Potential differences between the SVR and virological-failure group were evaluated by Fisher's exact test. A multivariable logistic-regression analysis was performed to define risk-factors associated to treatment-response.

Results: Overall, 596/2010 (29.7%) patients showed at least one natural-RASs, particularly NS5A-RASs were observed in 18.5% patients. 769 patients (GT1a/b/g[239/222/1]-GT2a/c[87]-3a[153]-4a/d[67]) had an available outcome (720 with a SVR and 49 with a virological-failure) after the following recommended NS5A-containing regimen: daclatasvir/ledipasvir/velpatasvir+sofosbuvir+/-ribavirin (N=125/134/179), 3D/2D (paritaprevir/ritonavir+ombitasvir±dasabuvir)+/-RBV (N=125/44), grazoprevir+elbasvir+/-ribavirin (N=96), glecaprevir+pibrentasvir (N=66). Analysing retrospectively the baseline samples, a higher prevalence of natural NS5A-RASs was observed before treatment in DAA-failures (38.8%) vs SVR-patients (18.6%; p=0.01). Notably, ≥2 risk-factors for failure were more frequently observed at baseline among patients who experienced a virological-failure to a DAA

treatment (69.4%) vs those achieving SVR (34.2%, p<0.001). By multivariable logistic-regression high HCV-RNA, natural RAS, cirrhosis, previous IFN-failure were negatively associated with SVR.
Furthermore, by multivariable logistic-regression, baseline HCV-RNA >800.000 IU/ml, presence of at least 1 natural RAS regimen-related cirrhosis and previous IFN-treatment were all negatively associated to SVR (adjusted odd ratios [95%C.I.]: 0.42 [0.20-0.89], P=0.002; 0.47 [0.23-0.95], P=0.035; 0.43 [0.22-0.86], P=0.017; 0.39 [0.20-0.78], P=0.007; respectively). No other risk-factors were associated to SVR.
Interestingly, all 66 GT1/GT2/GT3 patients treated with glecaprevir+pibrentasvir achieved SVR, with the exception of 1 GT3 breakthrough, having at baseline the NS5A-RAS A30K and HCV-RNA>800.000 IU/ml. Regarding velpatasvir+sofosbuvir+/-ribavirin, all 179 GT1/GT2/GT3/GT4 treated patients achieved SVR with the exception of 2 relapser (GT1a and GT3a), none showing baseline RASs regimen-related.

Conclusions: In this study, around one third of DAA-naïve patients showed at least one natural-RASs. NS5A-RASs were particularly observed with higher prevalence before treatment in DAA-failures. The presence of one or more specific pre-treatment risk factors, such as RAS regimen-related, baseline HCV-RNA >800,000 IU/ml and cirrhosis was associated with virological failure for some specific regimens and genotypes. Further analyses are needed to confirm these observations, particularly for the new current regimens and in the context also of shorter treatment durations (8 weeks).

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Efficacy of DTG-based + PI dual-class therapy in an observational cohort

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Background: Dual-class therapies including the INSTI dolutegravir (DTG) associated with rilpivirine or with lamivudine have shown their efficacy to maintain virological suppression in well-selected virologically-suppressed patients with no previous virological failure. Here, we report an observational cohort study of patients switching to a dual-class therapy containing DTG and a protease inhibitor (PI) (darunavir [DRV] or atazanavir [ATV]).

Patients & Methods: A prospective observational cohort enrolling all patients initiating DTG+DRV/r or DTG+ATV(+/-r) between January 2016 and January 2018. Plasma viral loads (pVL) were performed using Cobas-Taqman HIV-1 V2.0 assay. Plasma drug concentrations were measured using UPLC-MS/MS.

Results: Fifty-four patients were assessed in this observational cohort, 38 received DTG+ATV(+/-r) and 16 received DTG+DRV/r. Median age was 55 years (IQR=48-61) and 37 were men (69%). Thirty-eight patients (70%) had a pVL <50 c/mL at time of DTG-based dual-therapy initiation with a significant longer duration of virological suppression in the ATV group than in the DRV group (7.9 versus 1.2 years, p=0.02). Twenty-seven patients (50%) were already receiving a dual-therapy before DTG-based dual-therapy initiation, including raltegravir (RAL) in 14 cases. An historical genotypic resistance test was available in 49 patients showing viruses with NRTI or NNRTI drug resistance mutations (DRM) in 41 cases and in 34 cases, respectively. A major PI DRM was reported in six cases (12%) including an ATV DRM (N88S) in one case. Three of the 27 integrase available sequences showed INSTI DRM selected at failure of a previous INSTI-based regimen (E138K, Y143R and S147G-N155H-S230R). GSS could be measured for 25 patients and was equal to 2 in 21 cases and to 1 in four cases. One comorbidity has been reported in 35 patients (65%). A comedication was prescribed in 41 patients (76%) with a drug-drug interaction evidenced in three cases in the DRV group and in ten cases in the ATV group. Median time of follow-up of DTG-based dual-therapy was 2.1 years (IQR=1.3-2.9). Three patients (5.6%) discontinued DTG-based dual-therapy due to adverse events. Median CD4-cell counts at baseline and at the last follow-up of DTG-based dual-therapy were 580/mm3 (IQR=418-774) and 586/mm3 (IQR=458-800), respectively. The median gain of CD4 cell count was +30/mm3 (IQR=-47; +120). Two patients (3.7%, CI95%: 0-8.8%) experienced a virological failure (VF) with no selection of additional DRM compared to their historical genotype; one in the DRV/r group and one in the ATV/r group. Dual-class therapy was fully active (GSS=2) in both cases of VF. Eight patients (14.8%, CI95%: 5.2-24.4%) had a viral blip during the duration of follow-up (5 in the DRV/r group and 3 in the ATV/r group). Plasma drugs concentrations were available in 30 and 14 patients in the ATV and the DRV groups, respectively showing adequate DTG concentrations in all patients except two in the DRV group.

Conclusions: We report a good virological response to a dual-class DTG-based therapy with ATV or with DTG in an observational cohort showing a VF in 2 patients among 54 with no resistance selection. ATV+DTG is a favorable dual-class strategy in terms of pharmacokinetics with increased DTG concentrations.

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Real world characterisation of elbasvir/grazoprevir treatment patterns and associated effectiveness in patients with GT1A or GT4 chronic hepatitis C: results from the Alcibiades study in England

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Background: The EMA label for elbasvir/grazoprevir (EBR/GZR) recommends that in chronic hepatitis C (HCV) GT1a patients, to minimise the risk of treatment failure, a 16 weeks plus ribavirin (RBV) regimen should be considered in patients with baseline HCV RNA level >800,000 IU/ml and/or the presence of specific NS5A polymorphisms causing at least a 5-fold reduction in the activity of elbasvir.

Materials and Methods: Retrospective/prospective, observational, multi-centre chart review of patients with HCV GT1a or GT4 who initiated EBR/GZR ± RBV between 01-Dec-16 and 30-Sep-17 in 3 large public hospitals in England. We examined a real-world (RW) cohort of patients to determine if the EMA label recommendations around baseline viral load (BVL) and Resistance Associated Substitutions (RAS) testing were followed and what impact this had on outcomes.

Results: We identified 130 patients eligible for inclusion. Mean age was 54 years, 85% male, 94% GT1a, 6% GT4, 60% high BVL (≥ 800,000 IU/ml), 19% cirrhosis, 27% P/R or NS3/4A treatment-experienced, 70% were ex- or active PWID (people who inject drugs) and 43% drink alcohol.

Overall per protocol (PP) SVR12 (patients who completed therapy and had an SVR assessment) was 93.1% (94/101 patients). The rest 22% of patients were lost to follow-up (did not attend their last-visit or SVR12 appointments).

Among the GT1a patients 57% (74/129) had high BVL, of whom 58% (43/74) did not have baseline RAS testing. 91% (39/43) of these received the recommended 16 weeks ELB/GZR + RBV achieving SVR12 100% (34/34). For 9% (4/43) patients the EMA label was not followed as they had just 12 weeks EBR/GZR with SVR12 100% (2/2).

Of the GT1a patients with high BVL, 42% (31/74) underwent baseline RAS testing. EBR-specific NS5A RAS were detected in 26% (8/31) of patients: M28/A/G/T/S (2/8), Q30/H/K/Y/R (6/8), L31/F/M/I/V (2/8). These patients received: 12 weeks EBR/GZR (1/8) with the patient achieving SVR12; and 16 weeks EBR/GZR + RBV (7/8) with 5 patients achieving SVR12. Both patients not achieving SVR12 were ex-PWIDs, and one had 2 identified EBR-specific RAS and was treatment-experienced.

GT1a patients with low BVL: as per EMA label, 64% (25/39) were given 12 weeks ELB/GZR with SVR12 100% (18/18). Against EMA label, two patients received 12 weeks ELB/GZR + RBV, with SVR12 100% and the remaining 31% (12/39) patients were given 16 weeks ELB/GZR + RBV, with 9/10 achieving SVR12; the patient not achieving SVR12 had prior null response.
Although not mandated by EMA label, 28% (11/39) of GT1a patients with BVL < 800,000 IU/ml underwent RAS testing but no EBR-specific RAS were detected.

SVR12 in patients reporting weekly alcohol consumption above UK-recommended safe limits (>14 units; range 20 –186 units) was 100% (10/10).

Conclusions: In this English RW cohort EBR/GZR was highly effective in GT1a. Treatment extension and addition of RBV was common in GT1a patients with high BVL irrespective of baseline RAS testing, which only identified a few EBR-specific RAS; but often also in GT1a patients with low BVL, in both cases leading to very high SVR12 regardless of alcohol abuse.

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Impact of resistance mutations on virological efficacy of DTG-based maintenance two-drug regimens: a cohort study

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Background: Two-drug regimens (2DR) are largely prescribed in Italy as maintenance therapy, nowadays mainly based on DTG. While many data have been reported about PI-based 2DR, the impact of resistance mutations and duration of virological suppression on DTG-based 2DR remains to be elucidated. The aim of this study was to evaluate the impact of resistance mutations on virological outcome of DTG-based 2DR maintenance ART.

Material and methods: Virologically suppressed patients switching to DTG+3TC or DTG+RPV with pre-baseline (time of switch=baseline, BL) resistance genotype (at least PR/RT) were selected from the ARCA database. Primary endpoint was virological failure (VF: an HIV-RNA, VL, >200 cps/mL or 2 consecutive >50 cps/mL). The probability of VF was estimated by Kaplan-Meier analysis. Resistance to 2DR was defined as occurrence of at least Stanford HIVdb (v.8.5) low-level resistance (LLR) to at least one drug included in the current 2DR, based on cumulative genotype. CD4 changes were assessed using Student's t-test for paired samples. A secondary analysis comparing 2DR with DTG-based 3D regimens was also performed.

Results: A total of 318 2DR patients were analysed: 260 (82%) switching to DTG+3TC, 58 (18%) to DTG+RPV; 68% were males, median age was 51 (44-56) years, 12 (6-23) years of HIV infection, 5 (3-8) years of virological suppression, nadir CD4 cell count 231 (121-329), 5 (3-9) previous ARV lines, 59% previously exposed to INSTI, 11% with resistance to current 2DR. The integrase sequence was available in 14% of patients, none harbouring resistance to DTG. 20 VF were observed, of whom 4 (3/17 VF in DTG+3TC, 1/3 in DTG+RPV) in patients with at least LLR at BL (M184V+K219Q; D67N+K70R+K219Q; D67N+K70R+T215Y+219Q; E138A), in a median FU of 1.3 years (IQR 0.6-2). The 2-year estimated probability of VF was 8.7% (95% CI 4.4;13); 8.6% (4.1;13.1) in those without resistance and 9.7% (-4.4;23.8) in those with resistance (Log rank: p=ns). No factor was significantly associated with VF at multivariate analysis, however in patients with <6 years of virological suppression, baseline resistance was associated with a higher probability of VF (log rank p=0.003). After 48 weeks, a statistically significant increase in CD4+ cells was detected (+56 cells/mmc, p<0.001), independently from baseline resistance. The 2-year estimated probability of VF in the reference 3DR group (n=564) was not different from that for the 2DR group: 8.8% (5.9;11.7) in the whole case file and 9.7% (6.6;12.8) in the presence of baseline resistance. Longer time of virological suppression was the only factor associated with a lower risk of VF in the 3DR dataset.

Conclusions: DTG-based 2DR maintenance regimens show high virological efficacy, even in the context of predicted incomplete activity, at least within a short-term follow-up. A long duration of virological suppression seems to decrease the impact of resistance on virological outcome, however further studies are warranted to confirm this hypothesis and possibly define a clinically useful threshold.

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High Efficacy and No Emergent Resistance in Participants with HIV-1 Strain A or A1 in Russia Treated with E/C/F/TDF

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Background: WAVES (GS-US-236-0128) was a double-blind, phase 3b study among treatment-naïve HIV-1-infected women that demonstrated that elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (E/C/F/TDF; N=289) was superior to atazanavir+ritonavir+F/TDF (ATV/r+F/TDF; N=286) for HIV-1 RNA <50 copies/mL by FDA Snapshot analysis at Week 48 (87% vs 81%). Participants with HIV-1 strains A or A1 in Russia represented 29% (168/575) of all study participants. Some accessory resistance substitutions have been shown to be highly prevalent in Russian subtype A strains. The substitution A62V in reverse transcriptase (RT) is a fitness compensatory mutation for K65R and other multidrug-resistant viruses, and L74I in integrase (IN) has been implicated in virologic failure on cabotegravir (CAB)+rilpivirine (RPV). Here, a detailed analysis of baseline genotypes and treatment outcomes of the A and A1 subgroups from Russian sites was performed.

Methods: At screening, protease (PR)/RT were analyzed by population sequencing (Monogram Biosciences, Inc.). Baseline samples were also analyzed by deep sequencing for PR/RT and IN with a frequency cut off of 15% (Seq-IT, Germany). Resistance analyses were performed on samples with HIV-1 RNA ≥400 copies/mL at confirmed suboptimal virologic response (HIV-1 RNA ≥50 copies/mL and <1 log10 reduction from baseline at Week 8), confirmed virologic failure (HIV-1 RNA ≥50 copies/mL after achieving HIV-1 RNA <50 copies/mL), discontinuation, or Week 48.

Results: In the WAVES study, 192 participants were located in Russia; 88% (168/192 participants) had HIV-1 subtype A or A1. At baseline, all 168 had PR/RT genotypic data available and 102 had IN data. Some amino acid substitutions at drug resistance sites were highly prevalent and appear fixed in the circulating A/A1 strains in Russia: 46% (78/168) had A62V in RT and 99% (101/102) had L74I in IN. Primary NRTI, NNRTI, and PI resistance substitutions were present in 1%, 11%, and 1% of participants, respectively. Notably, RPV resistance substitutions were present in 9% of participants, all with E138A/G/Q (n=15). Through 48 weeks of treatment, virologic suppression (HIV-1 RNA <50 copies/mL) was observed at similar frequency for the Russian subtype A/A1 participants compared to the overall study population (81% vs. 84%, respectively). By treatment arm, more Russian subtype A/A1 participants were suppressed on E/C/F/TDF (90%; 82/91) than ATV/r+F/TDF (70%, 54/77). A total of 14 Russian A/A1 participants met the criteria for resistance analysis, 7 participants each from the E/C/F/TDF and ATV/r+F/TDF groups. No participant in the E/C/F/TDF group had emergent resistance, and 2 participants in the ATV/r+F/TDF group developed M184V/I in RT, neither of whom had preexisting A62V.

Conclusions: The WAVES study had a high proportion of participants from Russia with HIV-1 subtype A or A1. The HIV-1 from these participants had polymorphic A62V in RT and L74I in IN. These substitutions did not lead to virologic failure with drug resistance in either treatment group.

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Viral dynamics among HCV infected patients with different genotypes treated with genotypic specific or pan-genotypic direct-acting antiviral agent combinations

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Background: New hepatitis C virus (HCV) therapies have improved efficacy, allowed pangenotypic applications, increased barriers to drug resistance and shortened therapy duration.

Methods: Patients infected with different HCV genotypes were divided into two groups: group 1 included 169 patients receiving genotypic specific regimens (GSR), while group 2 included 186 patients receiving pan-genotypic regimens (PGR). Patient's HCV RNA was quantified and sequenced.

Results: Comparable sustained viral response (SVR) rates were observed in both GSR and PGR treated patients. Nevertheless, a greater proportion of non-detectable levels (NDL) of HCV RNA was observed in patients treated with PGR as compared with GSR. Overall, among patients in the GSR and PGR groups with residual viremia, 124/169 (73.4%) and 125/186 (67.2%) at four weeks, and 66/169 (39.1%) and 58/186 (31.2%) at eight weeks, achieved SVR. No difference was observed in the clinical outcome comparing patients in the GSR and PGR groups according to genotype. While, comparing patients between the two groups, the proportion of patients with NDL HCV RNA at four and eight weeks was higher in patients infected with genotype 1b treated with PGR (p=0.05). A significantly higher number of patients infected with 1b had RASs at baseline (p=0.0001). In addition, the proportion of patients with treatment failure was higher in patients with RASs at baseline compared with those without (p= 0.012). Overall, 2.5% patients failed to achieve SVR after DAA treatment.

Conclusions: A sharp HCV RNA decrease was observed in patients treated with both GSR and PGR. However, even if comparable, a slightly greater number of patients treated with PGR achieved NDL HCV RNA as compared with GSR. A significant difference was observed in patients with baseline RASs, both in relation to treatment failure and genotype. In conclusion, the use of new DAA combinations helps patients achieve a more rapid virologic response.

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Prevalence of HBV genotypes, escape mutants and treatment-resistance mutants in a cohort of HBV chronic patients

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Background: Based on genetic divergence, HBV has been classified into 9 genotypes (GT) designated A to I, defined by >8% divergence at the nucleotide level, and several sub-genotypes. Within every genotype, due to the absence of proofreading activity, the HBV polymerase/RT leads to the introduction of random mutations into HBV genome, creating a genetic variability described as viral quasi-species. These variants include escape mutants and antiviral drug-resistance mutants.
The aim of this study was to evaluate the prevalence of genotypes, HBV escape mutants, treatment-resistance mutants in a cohort of HBV chronic patients.

Materials & Methods: From 2014 to 2018, in a cohort of 143 HBV chronic patients treated with Entecavir (ETV) and/or Tenofovir (TDF) from Infectious Diseases Unit, San Martino Polyclinic Hospital, sequencing assay for detection of RT/S-Gene mutations was performed using Trugene® HBV Genotyping kit (Siemens Healthcare Diagnostics Inc., Tarrytown, NY) or Abbott HBV Sequencing (Abbott Molecular) according to manufacturer's instruction.

Results: All HBV chronic patients received ETV e/o TDF. Forty-six/143 (32.6%) had been sequenced prior to treatment, 8/143 (5.6%) after treatment start. GT D was found in 36/54 (66.7%), A in 11/54, (20.4%), B in 3/54 (5.6%), C in 2/54 and E in 2/54 (3.7%).
In 9/54 (16.7%) samples at least one HBsAg escape mutant was found: 8 had pre-therapy escape mutations, while in 1 sequencing was performed after ETV was started. The presence of escape mutations was not GT-restricted (4/36, 11%, vs. 5/18, 27.7%, p = 0.14, escape mutations in GT D vs. nonD). The following escape mutants were found: 129H, 120S, 120P/T, 123A+130R, 120T, 145A, 122K, 100C, 144G.
With regard to RT mutation prevalence, 9/54 (16.7%) sequences revealed the presence of at least one mutation conferring drug resistance to either Lamivudine or Adefovir or, partially, ETV. No mutation impacting susceptibility to TDF was found. The RT mutations were the following: V173V+L180M+M204V in 1/9, 181T in 1/9, L180M+M204I in 1/9, 180V in 1/9, 180M+204V in 3/9 and 181V in 2/9 patients.

Conclusions: The present study showed that D and A GT are currently the most prevalent in our Region, not differently from the rest of Europe.
The regular therapeutic response in patients with escape mutations does not seem to per se justify differences in the approach of treatment.
In comparison to data from literature, this study highlighted that over the last years a progressive decrease of resistance-associated mutations is being observed, likely due to the latest international guidelines that recommend the use of drugs with high antiviral potency and high genetic barrier such as ETV or TDF. Moreover, no mutations conferring relevant resistance to ETV and TDF were found before the start of treatment.

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Prevalence of NS3, NS5A, NS5B resistance associated substitutions through patients who failed a DAA treatment: data collected in Liguria from March 2018 to March 2019.

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Background: The new Direct Acting Antivirals (DAAs) oral regimen demonstrate high efficacy in the treatment of HCV infections, with the achievement of a sustained virological response (SVR) in over 90% of patients. Virological failure is related to the presence of resistance associated substitutions (RAS) in DAAs regimen targets, which may persist for years, affecting the choice of HCV re-treatment options. Here we describe the distribution of NS3, NS5A and NS5B RAS detected in 27 patients who experienced a virological failure during a second generation DAAs regimen in Liguria, from March 2018 to March 2019.

Methods: From March 2018 to March 2019 a total of 27 serum samples collected from HCV infected patients who had failed to achieve a SVR during a DAAs regimen were analyzed for the presence of RAS in the Laboratory of the Hygiene Unit of Ospedale Policlinico San Martino (Genoa), belonging to the VIRONET-C Italian Network. HCV RNA was extracted using NucliSENS easyMAG system (bioMérieux, Boxtel, The Netherlands) and genomic regions were amplified with specific HCV genotype/subtype primers, in two steps PCR. Subsequently, cDNA of the three genomic targets (NS3, 1-181aa; NS5A, 1-213aa; NS5B, 219-347aa) was purified and sequenced by 3130-Avant Genetic Analyzer (Life Technologies, NY, USA). Sequences were aligned by SeqScape Ver. 3.3 Software (Life Technologies, NY, USA). Mutations and predictions of phenotypic resistance were obtained using Geno2pheno tool (latest version available at the time of our analysis). (http://www.geno2pheno.org/).

Results: Serum samples were collected from 27 DAAs failed patients (9 Female,18 Male), median age 61 years, out of 2774 treated in our region (0,97%). Genotypes distribution was the following: 9/27 (33,3%) 3a, 8/27(29,6%) 1a, 7/27 (26%) 1b, 2/27 (7,4%) 4a; two different genotypes (1b, 3a) were detected in 1/27 (3,7%) patient. Treatments administered were the following: Elbasvir /Grazoprevir in 10/27 (37,0%), Sofosbuvir / Velpatasvir in 14/27 (52%), Glecaprevir /Pibrentasvir in 3/27 (11,0%). The pan-genotypic 93H RAS was found in 10/27 (37,0%) patients who failed an NS5A non-structural protein inhibitor with the higher prevalence in patients who received an Elbasvir/Grazoprevir regimen 6/10 (60%). The NS3 Q80K RAS was observed in 2/13 (15,4%) patients after failing a protease inhibitor. No clinically relevant RAVs were detected in NS5B region. No significant difference was observed between genotypes.13/27 (48%) patients failed to obtain a SVR without RAS.

Discussion: This study showed an excellent efficacy of DAAs treatment of HCV infections, with a rate of failure <1%, equally distributed among the three considered drugs. Despite the small number of analyzed patients, in our cohort a more elevated prevalence of NS5A RAS seems to be identified in patients treated with Elbasvir/Grazoprevir respect to those who received either a Sofosbuvir/Velpatasvir or a Glecaprevir/Pibrentasvir regimen, Of note, 48% of virologic failure occur without the presence of clinically relevant RAS, thus suggesting limited implications in term of therapy failure during a second line regimen. Nevertheless, RAS screening at failure may confer some advantage in re-treatment options choice for those patients found to harbor resistant viruses.

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Timely emergence of letermovir resistance in a patient with primary immunodeficiency – the need for resistance surveillance

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Introduction: Human cytomegalovirus (CMV) causes significant complications in immunocompromised patients. CMV reactivation can be prevented and treated with the antiviral drugs (val)ganciclovir, foscarnet, and cidofovir. However, frequent treatment is complicated by drug toxicity, antiviral resistance and hampered by intravenous drug administration. Letermovir, a novel CMV antiviral which was recently approved by the FDA and EMA for prophylactic use in hematopoietic stem cell transplantation recipients, has several advantages over currently used CMV antivirals. Letermovir can be administrated either orally or intravenously and shows mild toxicity. In addition, there is no risk of cross-resistance with existing anti-CMV drugs due to a different mechanism of action.

Case: Here we report off-label prophylactic use of letermovir in a patient with a severe B- and T-cell deficiency and multiple episodes of CMV end-organ disease (colitis and retinitis) and repeated CMV reactivations with selection of a ganciclovir resistant virus with the M460I in pUL97. Thus, the patient received several courses of foscarnet i.v over 18 months, which were complicated by foscarnet related side-effects (anaemia, leukopenia, hypomagnesaemia, and renal impairment.). Therefore, the patient was switched to off-label oral letermovir prophylaxis of 480mg per day while CMV replication was suppressed. Letermovir was well tolerated. Unfortunately, the patient experienced several CMV breakthroughs during letermovir prophylaxis and the predominant CMV variant in blood acquired the C325Y letermovir resistance mutation in pUL56 after 119 days of treatment, which confers high-level resistance to letermovir. Letermovir was stopped and replaced with foscarnet i.v. CMV DNAemia returned to below 50 IU/mL.

Conclusions: This is the first report on the use of letermovir in primary immunodeficiency. Although letermovir was well tolerated, a viral breakthrough with the development of the C325Y mutation in pUL56 conferring resistance after 119 days occurred. More insight is needed regarding the resistance profiles of letermovir encountered in clinical practise. Therefore, we propose to gain more in-depth knowledge on letermovir resistance profiles by starting a letermovir resistance registry, to aggregate letermovir resistance data across multiple centers in Europe. Furthermore, efficacy of letermovir in other patient groups such as solid organ recipients should be investigated as well as its use for pre-emptive therapy and treatment of CMV end organ disease.

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Causes of death in HCV positive patients successfully treated with DAAs

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Background and Aims: HCV infection can lead to the development of complications such as cirrhosis and hepatocellular carcinoma (HCC). Currently, direct-acting antiviral drugs (DAAs) are the main treatment for HCV + patients, including cirrhotic patients, allowing the achievement of sustained virological response (SVR) in 90-95% of cases. Actually, there is unclear association between treatment with DAAs and risk of increased occurrence of HCC. No information is available on the causes of mortality of HCV + patients after treatment with DAAs.

Method: The study was designed to evaluate all the causes of mortality in HCV + patients undergoing DAAs at AOU of Sassari, between January 2015 and September 2017.
Patients were evaluated in an observational manner and subjected to various controls: at baseline, during therapy and during follow-up. Liver fibrosis was measured by elastometry with FibroScan before and after treatment.

Results: Out of a total of 355 patients (206 M/149 F), mean age 49.5 ± 4.95, with HCV-related chronic hepatitis, DAAs therapy induced an SVR in 94% of cases. Of these, 38 had a basal stage of fibrosis F0/F1, 31 stage F2, 88 stage F3 and 198 stage F4.
During the first 52 weeks of follow-up 12 deaths were detected (3.38%, 10 M/2 F): 4 related to HCC (3 relapses, 1 de novo) and its complications, while the remaining 8 were due to other causes (lymphoma, cerebral hemorrhage, prostate adenocarcinoma, breast cancer, lung neoplasm, suicide, femoral fracture complications, cachexia). Among the deceased patients, 10 had a F4, 1 stage F2 and 1 stage F0F1 stage. During the observation period, HCC was detected in 9 (2.54%, 95% CI: 0.903-4.176) patients: 4 cases occurred in patients (57%, 95% CI: 20.33-93.67) with previous HCC and in 5 patients (1.44%, 95% CI: 0.188-2.692) HCC presented itself de novo.

Conclusion: Numerous studies have shown that the majority of HCV + patients who underwent antiviral treatment with DAAs obtained SVR with virus eradication. No association was found between treatment with DAAs and recurrence/occurrence of HCC, although patients previously treated for HCC have a higher risk of short-term tumor recurrence. All this suggests that close follow-up in cirrhotic patients remains mandatory during and after antiviral therapy.
Our results confirm that there is no greater risk of de novo hepatic neoplasia after antiviral therapy with DAAs. The initial condition of cirrhosis in the eradicated patients does not represent the cause of greater mortality in the studied population.

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Efficacy and safety of anti-HCV direct acting antivirals in HIV-HCV co-infected patients: a real-life experience in Sardinia.

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Introduction: Hepatitis C virus (HCV) infection is the most important cause of liver failure and the second cause of death in cART (combination antiretroviral therapy) treated HIV patients. Although direct acting antivirals (DAA) have revolutionized HCV treatment, HIV-HCV co-infected patients are still considered a difficult-to-treat population due to comorbidities and potential drug-drug interactions.

Aim. In this study, we reported our real-life experience of DAA treatment in a cohort of Sardinian HIV-HCV co-infected patients.

Materials and Methods, Results: All consecutive HIV-HCV patients who underwent DAA treatment at two Sardinian liver units between 2016 and 2018 were included. Different DAA combination were used depending on HCV genotype, liver disease severity, cART and concomitant drugs, in according with current guidelines. The primary endpoint was treatment efficacy defined as a sustained virological response (SVR) at 12 weeks after therapy. Secondary endpoints were treatment safety and tolerability. We recruited 131 patients: males 74,80%, median age 54 years (range 30-66). The most common genotype was 1a in 41 (31,29%). Cirrhosis was detected in 53 (40,45%) patients. A positive history of injective drug use was present in 108 subjects (82,44%). All patients were receiving antiretroviral treatment, while 32 (25.4%) were on methadone maintenance therapy and 15 (11,45%) were on psychotropic drugs therapy. 39 (29,77%) patients had a history of interferon-based treatment. Among those who completed the 12-week follow-up post DAA (n=128), SVR was observed in 124 (96,87%). Only 1 (0.78%) patients was non responder to the treatment, and 3 (2,34%) relapsed at the end of therapy. The most common side effects included pruritus (8,59.%), headache (7.81%) and fatigue (6.25%). None of our patients discontinued the DAAs for adverse reactions or drugs interactions.

Conclusions: Our data confirm that DAA therapy is highly effective and well tolerated among co-infected HIV-HCV patients, without or with advanced liver disease.

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Epistatic interactions in NS5A of Hepatitis C virus suggest drug resistance mechanisms

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Background: Hepatitis C virus (HCV) causes a major health burden. HCV is classified into 7 major genotypes and further into subtypes (GT). GT1 represents 69% of the infections in the P.E.P.S.I. Study, which analyses HCV infections in several German centres.
HCV can be effectively treated by direct-acting-antivirals (DAAs). The viral NS5A protein, which plays a key role in viral replication, is one of the DAAs targets. Resistance-associated-viruses (RAVs) harbouring NS5A resistance-associated-mutations (RAMs) have been described at baseline and after DAA therapy failure. Q30R is a major RAM in GT-1a. Surprisingly, R30 corresponds to the wild type in the GT-1b, and still, GT-1b strains are susceptible to NS5A-inhibitors.

Material & Methods: 2777 GT-1b sequences of from Los Alamos database and 364 sequences from patients from the P.E.P.S.I. cohort were used in this study. Sequences were aligned in a codon-aware manner with MACSE and mutations that were statistically significantly correlated with the R30Q polymorphism were identified. In order to confirm the epistatic nature of this association, a maximum likelihood phylogenetic tree was reconstructed with RAxML using a GTR + Gamma model of substitution, and ancestral sequences were reconstructed in all internal nodes. Amino acid substitutions correlated with the R30Q polymorphism in several independent branches of the phylogenetic tree were identified and mapped into the three-dimensional model structure of the NS5A dimer in complex with the Daclatasvir inhibitor.

Results: We show that R30Q is a polymorphism in GT-1b appearing in around 5% of the NS5A sequences retrieved from Los Alamos database. These strains often display other specific NS5A substitutions, particularly in positions Q24K and V34I, but also in positions L28M, Q54H and V138L. We demonstrate that secondary substitutions usually happen after initial R30Q development in the phylogeny of the GT-1b NS5A sequences, both in vivo and in vitro, and that the chemical properties of the corresponding amino acids either serve to restore the positive charge in this region or affect the movement of the N-terminal portion of the protein, acting as compensatory mutations.

Conclusions: We have demonstrated epistatic intragenic interaction in the NS5A gene of GT-1b, which lead to compensating amino acid changes affecting physical and chemical properties of the protein after introduction of substitutions in the residue 30. These findings may have implications for RAVs treatment.

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Torque Teno Virus plasma concentration as novel biomarker of delayed immune reconstitution in HIV-infected patients

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Background: The Torque Teno Virus (TTV) is a single-stranded DNA anellovirus and part of the human virome. Clinical manifestations associated with the virus are unknown, but the TTV-DNA plasma concentration has been identified as predictive marker for risk assessment in immunosuppressed patients after transplantation.
In the context of HIV-1 infections, a prediction of the course of immune reconstitution (IR) upon initiation of combined antiretroviral therapy (cART) may contribute to guide individualized treatment. Thus, this work aimed to measure if the extent of TTV plasma concentration correlates with the amount of IR in these patients.

Methods: 301 plasma samples of therapy-naïve HIV-1-infected patients of the RESINA cohort were retrospectively analyzed. TTV-DNA was quantified according to Maggi F et al., J Virol 2003. TTV plasma level were correlated to CD4 cell count, HIV load, age and sex. Patients were subdivided, according to their initial CD4 cell count (<100, 100-200, 200-350, >300 CD4 cells/µl) and the extent of CD4 cell count increase within the first year with cART (<50, 50-200, >200 CD4 cells/µl).

Results: TTV plasma DNA was detectable in 96% of the samples. The median TTV plasma concentration was 235,844 copies/ml. Baseline CD4 cell count correlated significantly negative to TTV plasma concentration (p=0.037).
Analyzing IR, patients with a CD4 cell recovery <50 cells/µl within the first year on cART presented TTV viremia in 100%. Notably, compared to patients with CD4 cell count increase of >200 CD4 cell/µl, patients lacking a robust increase showed significantly higher TTV plasma concentrations (median 481,963 cop/ml versus 97,471 cop/ml; p=0.011).
Overall, CD4 cell recovery within the first year of cART correlated negatively to TTV plasma concentration and baseline CD4 cell count (p=0.001).

Conclusion: There is a high prevalence of TTV viremia within the RESINA cohort. Significantly higher TTV plasma concentrations in patients with low CD4 cell counts prior cART initiation compared to those with higher CD4 cell counts were confirmed. Remarkably, higher TTV plasma concentrations correlated with reduced CD4 cell recovery within the first year on cART, indicating a more severe immune defect. In summary, this study shows that TTV plasma concentration before initiation of cART is predictive for the amount of IR in HIV-infected patients with severe immunodeficiency.

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Design and Performance of the new Alinity m HCV Assay

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Background: The WHO estimates that 71 million people have a chronic hepatitis C infection, and nearly 400,000 individuals die each year from complications of hepatitis C, primarily of cirrhosis and liver cancer. In order to realize the value of the new antivirals, testing strategies must improve. Alinity m HCV assay was developed with a goal of streamlining the diagnostic process. By providing simultaneous confirmation of viremic infection and baseline viral load measurement in one test, Alinity m HCV reduces the number of tests and steps required for the initial diagnosis of HCV infection. Here we evaluate key performance attributes of the Alinity m HCV assay.

Methods: To ensure robustness against the new and emerging HCV variants, the Alinity m HCV assay was developed as a dual-probe assay targeting a highly conserved region of the HCV genome. The Alinity m System utilizes magnetic microparticle sample preparation chemistry, unit-dose lyophilized amplification reagents, and ReadiFlexTM sample processing. Linearity, Limit of Detection (LOD) and precision studies were evaluated using either the 4th WHO HCV International Standard (NIBSC 06/102 genotype 1) or HCV positive clinical specimens diluted in HCV negative serum and plasma. Clinical performance and correlation were evaluated against comparator methods using both plasma and serum specimens.

Results: The Alinity m HCV assay demonstrated linearity from 12 IU/mL to 200,000,000 IU/ml. Probit analysis demonstrated that the Alinity m HCV assay detected HCV RNA with 95% probability at 5.11 IU/mL for genotype 1 in both plasma and serum. The assay also exhibited 95% or greater detection rates with HCV genotypes 2-6 at 12 IU/mL. An overall specificity of 100.0% (95% CI: 99.2 to 100.0%) was determined by testing HCV negative specimens, including 250 plasma and 254 serum. In a 5-day precision study, the Alinity m HCV assay demonstrated a within-laboratory SD of ≤ 0.16 Log IU/mL of HCV RNA from 1.43 to 8.42 Log IU/mL, and an SD of 0.18 Log IU/mL at 1.40 Log IU /mL. Method correlation to Abbott RealTime HCV (n=362) demonstrated a correlation coefficient of 0.978, slope of 1.03 and intercept of 0.02 using the Deming Regression. The Positive (n=363, 178 plasma and 185 serum) and Negative (n=299, 149 plasma and 150 serum) agreement was 100% between Alinity m HCV and a comparator CE-marked confirmatory assay.

Conclusions: The Alinity m HCV assay deliver highly sensitive detection of diverse HCV genotypes and accurate quantitation across a wide dynamic range. This assay performance is further enhanced by rapid turnaround time (time-to-first-result of 115 minutes) and workflow flexibility of the Alinity m System.

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Evaluation of the effectiveness of serological and molecular tests for determination of HIV infection duration

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Background: The time passed since the infection is an important epidemiological and prognostic indicator but often is undefined. Recent Infection Testing Algorithm (RITA) is an approach based on laboratory methods that allow to differentiate recent and established HIV infection. Laboratory tests include detection of antibody titer, avidity index (AI), viral load (VL), and CD4 cell count. A recent infection is defined as the period during the first 6-12 months after infection, depending on the diagnostic tests used. Today, knowledge of molecular biology of the virus gives an opportunity to estimate the time period after infection using additional technique. The proportion of variable positions in HIV genome can be used as a marker of the duration of infection because the heterogeneity of the viral population in human organism increases with time. This parameter can be successfully used in practice to evaluate a recent infection. The aim of this study was to assess the effectiveness of serological and molecular tests for determination of HIV infection duration.

Materials & Methods: Plasma samples (n=91) was obtained from ARV-naïve HIV patients: 34 samples from patients with infection duration up to 6 months (recent infection samples) and 57 samples from patients with duration more than 9 months (established infection samples). The duration of infection was determined by epidemiological and clinical data and indicators of seroconversion. 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). Nucleotide sequences of pol region including protease gene and fragment of reverse transcriptase gene (according to HXB2, positions 2052-3345) were obtained using AmpliSens HIV-Resist-Seq kit (CRIE, Russia).

Results: According to RITA on the first step all samples were analyzed by the Sensitive-Less Sensitive and antibody avidity assays. The concurrence of DS-EIA-HIV-Ab-TERM results and epidemiological data were obtained for 23/34 (67.6%) of recent infection samples and for 54/57 (94.7%) of established infection samples. The concurrence data for Architect HIV Ag/Ab Combo were 25/34 (73.5%) and 43/57 (75.4%) respectively. The concordance of two tests was 82.4% (28/34) for recent infection samples and 80.7% (46/57) for established infection samples. On the second step were done the sequencing of pol region. The reliable differences in a number of variable positions in sequences were found in comparing of patients with infection duration less 6 months and more than 9 months (0.26% vs 0.37%). There was no significant difference between groups in CD4 cells number, but VL in the early stages of infection (up to 6 months) was significantly higher than in the later period (mean 1.9x10^5 vs 3.6x10^4 copies/ml).

Conclusions: Study results showed that serological tests (DS and Abbott) correctly identified the duration of HIV infection in 84.6% and 74.7% respectively. It was also found that cohorts of patients with recent and established HIV infection differ in viral load and degree of heterogeneity of the viral population. The inclusion of these laboratory parameters in diagnostic algorithm will increase the accuracy of determining the recent HIV infection.

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Design and Performance Characteristics of the new Alinity m Hepatitis B Virus (HBV) Viral Load Assay

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Background: Currently 3.5% (257 million) of people worldwide have chronic HBV infection. Chronic HBV infection can cause liver cirrhosis, hepatocellular carcinoma, and other diseases. Current guidelines recommend screening populations with HBV surface antigen and using a viral load assay to identify patients requiring therapy. The Alinity m HBV viral load assay was developed to achieve broad genotype inclusivity (A-I), high sensitivity, and a wide dynamic range. Here we evaluate key performance attibutes of the Alinity m HBV assay.

Methods: The primer/probe target region was developed based on analysis of sequences from genotypes A-I in collaboration with Abbott's Global Surveillance program. The Alinity m System utilizes magnetic microparticle sample preparation chemistry, unit-dose lyophilized amplification reagents, and ReadiFlex™ sample processing. Linearity, Limit of Detection (LOD) and precision studies were evaluated using the 3rd WHO standard. Clinical performance and correlation was evaluated across 279 specimens by using Abbott RealTime HBV assay as a method comparator.

Results: The Alinity m HBV assay is compatible with EDTA and ACD plasma, serum, PPT, SST, and rapid-clot tubes. LOD by probit is 6.72 IU/mL for plasma and 9.62 IU/mL for serum. The assay is linear between ≤1 to ≥9 Log IU/mL for genotypes A-I in plasma and serum. A precision study demonstrated a within-laboratory SD of less than or equal to 0.21 Log from 1.3 to 9.4 Log IU/mL in plasma and serum. Carryover of 0.0% (95% CI: 0.0 to 1.0%) was demonstrated. An overall specificity of 100.0% (95% CI: 99.3 to 100.0%) was determined by testing 257 plasma and 253 serum specimens. A matrix equivalence study using negative and positive samples yielded an overall percent agreement between plasma and serum samples of 100.0% (95% CI: 95.1 to 100.0%) and Alinity m HBV quantitation demonstrated a slope of 0.97, intercept of 0.18, correlation coefficient (r) of 0.996, and mean bias of 0.04 Log IU/mL between plasma and serum samples. Method correlation to HBV by analyzing 279 specimens (139 plasma and 140 serum) from HBV infected patients (including genotypes A, B, C, D, E, F, G and H) demonstrated a correlation coefficient of 0.997.

Conclusions: The Alinity m HBV assay utilizes a dual-probe assay design to deliver highly sensitive detection of diverse HBV genotypes and accurate quantitation across a wide dynamic range. This assay performance is further enhanced by rapid turnaround time (time-to-first-result of 115 minutes) and workflow flexibility of the Alinity m System.

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Next generation sequencing of Hepatitis B virus with the Vela Sentosa HBV genotyping and resistance assay

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Background: With new therapeutics for treatment of Hepatitis B on the horizon there is an increased need for reliable diagnostics to identify genotype, nucleoside/nucleotide inhibitor resistance pattern, escape mutations and mutations in the pre-core and core region of HBV. While in-house solutions are available for many different viral pathogens, commercial NGS solutions are at the moment only available for HIV and HCV. We compared the Vela Sentosa HBV genotyping and resistance assay (Vela) which is in development to previously characterized samples.

Materials & methods: 17 samples were analysed with the Vela assay. We compared the results to the previous characterization of the Hepatitis B virus RT-domain by Sanger sequencing. Viral load of the samples was between 2 200 IU/ml up to >100 000 000 IU/ml. Genotypes included were A, B, C, D and E. A reference set of sequences consisting of 4267 HBV full genome sequences was generated form HBVdb (https://hbvdb.ibcp.fr/HBVdb/HBVdbDataset).
Sequences were generated from the precore, core surface antigen and RT-Domain region using our established NGS pipeline.

Results: Sequencing was performed with high efficacy and nearly 3.2 million final reads could be analyzed. The sample set consisted of 3 A, 5 B, 2 C, 4D and 3 genotype E samples. All previously determined genotyping results could be confirmed. Phylogenetic distance calculation showed a high pairwise homology of the generated sequences with differences mainly resulting from increased sequence quality in the leading and trailing ends of the sequences. Reverse Transcriptase mutations conferring resistance to RT-inhibitors could be recovered in all the appropriate samples with an additional minority mutation in the Vela assay. All mutations in the Hepatitis B surface antigen gene associated with escape could be verified.

Conclusion: The Vela Sentosa HBV genotyping and resistance assay showed excellent performance for all tested genotypes and viral loads. All relevant mutations could be reliably detected. The assay is planned to be combinable with the Vela Sentosa HIV, HCV and CMV genotyping and resistance assays to allow short turn-around times even in laboratories with lower sequencing requests.

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Integrating genotypic HIV tropism testing into the Vela Sentosa HIV genotyping and resistance assay

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Background: Testing for CCR5-tropic virus is a requirement before starting a Maraviroc containing treatment as this CCR5 blocker can only act against CCR5-tropic HIV. Genotypic tropism testing by analysing the V3 loop sequence is the most common method used nowadays as phenotypic tropism testing is time-consuming and expensive and good guidelines and tools exist for interpretation of sequences. To detect and quantify minor non-CCR5-tropic populations the method of choice is to perform tropism testing using next generation sequencing. We established a method to integrateV3 loop analysis into the Vela Sentosa HIV next generation sequencing genotyping and resistance assay to analyse tropism together with Protease, Reverse Transkriptase and Integrase resistance.

Materials & methods: For optimized analyses in the geno2pheno coreceptor tool for NGS data (geno2pheno [454]) full length V3-loop sequences are necessary. We designed a nested PCR (1. round V3_6952f GCACAGTACAATGTACACATGG; V3_7357r CAGTAGAAAAATTCCCCTCCAC; 2. Round V3_7062f AATGCCAAAACCATAATAGTACA, V3_7316r TTCTGGGTCCCCTCCTGAG) with a final short PCR product of around 250 basepairs to avoid too much fragmentation within the V3-loop sequence. The PCR-product was spiked into left blank sample cavities after the PCR in the workflow of the Vela Sentosa HIV genotyping and resistance assay. The following steps (library prep, emulsion PCR, enrichment and sequencing) were performed within the standardized Vela workflow. 6636 full length envelope sequences were downloaded from Los Alamos database and used for mapping the NGS data and identifying sequences spanning the complete V3 loop. Using the geno2pheno [454] pre-processor sequences were prepared for upload and analysis with the geno2pheno [454] tool.

Results: Interpretation by geno2pheno [454] led to quality reads from 3000 – 11000 per sample with up to 1700 variants in a single sample. The predicted X4-tropic viruses at the false positive rate (FPR) cut-off of 3.75% varied between 0% and 99% interpreted as X4-tropic only or R5-tropic virus only. Results for proviral and plasma-viral preparations (for samples with viral loads below 200 c./mL or above 200 c./mL) led to comparably result quality.

Conclusions: Integrating a genotypic tropism test into the Vela Sentosa HIV genotyping and resistance assay showed excellent performance. The V3-loop sequences could be reliably detected and showed comparable results in geno2pheno 454 analysis compared to next generation sequencing using the illumina platform. The combined V3-loop sequencing with the Vela Sentosa HIV genotyping and resistance assay allows short turn-around times and completes the resistance analyses for HIV on the Vela platform.

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The Acceptability and Preferences of Home Self-Collect Kits for Sexually Transmitted Infection Testing: A Qualitative Study

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Background: Everyday, 1 million sexually transmitted infections (STIs) are acquired daily, of which youth aged 20 -24 years account for the highest rates. These statistics are comparable in the United States of America (USA) where youth account for 50% of all new STIs. A significant influence of the incidence of STIs among youth is the low rates of STI testing. The majority of STI testing is recommended by a clinician following assessment of sexual health and sexual risk behaviors. However, this strategy of testing has been ineffective in comprehensively reaching youth due to barriers like low knowledge, cost, access, and fear of judgment and discrimination. Innovative strategies to promote STI testing are urgently needed among youth. To address this gap, the purpose of this qualitative study was to explore the preferences, feasibility, and acceptability of a home-based STI testing self-collect kit.

Methods: Data was collected using a demographic and sexual history questionnaire, STI knowledge questionnaire, and individual audio-recorded interviews using a semi-structured interview guide from 30 youth aged 18 to 24 in Central Pennsylvania, USA. The semi-structured interview guide contained questions on the feasibility and acceptability of using home-based STI testing self-collect kits, and the preferences for access, packaging and instructions, cost of self-collect kits, sending self-collect kits and receiving test results. Descriptive data were analyzed using the SPSS statistical software, and qualitative data (audio-recorded interviews) were analyzed using qualitative content analysis.

Results: The findings from this study revealed that the availability of home-based self-collect kits could reduce experienced youth barriers related to STI testing. Youth expressed willingness to use home-based self-collect kits. Some described preferences include access to pick-up kits without a prior clinician visit, clear instructions on using kits using packaging instructions or video explanations, discrete packaging, self-collecting testing specimen (i.e., urine, rectal swabs, vaginal swabs) at convenient locations, and dropping off kits at specified locations.

Conclusion: With low rates of STI testing as one of the significant prevention challenges, findings from this study provide insights to structuring alternative ways to offer STI testing services for youth. Recommendations from this study include locations where home-based self-collect kits could be accessed by youth, cost considerations and methods of designing user instructions. Suggestions for future research include the examination of facilitators, barriers, and predictors of the use of home-based self-collect kits and evaluating the accuracy of self-collected test specimens outside clinical settings. Furthermore, findings from this study provide guidelines for policy creations that will enhance the uptake of STI testing.

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Expression of HLR-DR on T-lymphocytes, content of CD4+CD25+T-regulatory cells and CD4+CCR5+T-cells in HIV-infected patients, depending on the presence of AIDS and the virus tropism

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Background: Switching tropism of HIV in the course of HIV infection is considered as an unfavorable factor during the course of the disease. It is possible to assume the presence of various immunological mechanisms for the formation of immunosuppression at different tropism of HIV.
Aim of study: to present the expression of HLR-DR like marker of immunity activation on blood T-lymphocytes, the content of CD4+CD25+ T-regulatory cells and CD4+CCR5+T-cells in HIV-infected patients depending on the presence of AIDS and the nature of the virus tropism.

Material and methods: Two groups of HIV-infected patients were included in the study: group 1 - 34 patients infected with the R5-tropic virus (mean age 34.1 ± 5.9 years old, males - 14/41.2%), among them AIDS (the 4th clinical the stage of HIV infection by WHO classification (2006) and/or CD4+T cells less than 200 cells/µl) was in 10 patients. Group 2 - 19 patients (mean age - 33.4 ± 6.3, males - 10/52.6%) infected with non-R5-tropic virus, among them AIDS was identified in 7 cases.
The cell immunophenotype was determined by Flow cytometry (FACS Calibur). Monoclonal antibodies produced by ExBio, Czech Republic and BD, USA were used.
The HIV-1 tropism for CCR5 and CXCR4 coreceptors was determined by a genotypic method based on the sequencing of the V3 loop of gp120 of the HIV env gene. The nucleotide sequences were edited and the consensus nucleotide sequence was obtained using the DEONA software (MediTi Group, Russia). Analysis of the nucleotide sequence was carried out on the website http://www.geno2pheno.org/ of the Max Planck Institute for Computer Science (Max Planck Institut Informatik, Germany). The FPR (False Positive Rate) was assumed to be 20%.

Results: In patients infected with non R5-tropic HIV with the presence of AIDS compared with patients without AIDS an increase the percentage of HLA-DR+CD3+T-lymphocytes: 36.08 (33.9-50.6) and 29.9 (17.5-31.3), p<0.05, respectively, and number of HLA-DR+T-helpers (cells/µl): 37.8 (26.2-51.5) and 62.81 (51.9-84.6), p<0.05, respectively, an increase in the intensity of HLA-DR expression by T-helpers (conventional units): 116.2 (108.4-132.6) and 76.7 (57.2-83.2), p<0.05, respectively, expression of HLA-DR by CD3+CD8+lymphocytes (%): 62.79 (57-70.3) and 46,46 (36,7-56,6), p<0.05, respectively was found. Also, in patients of the 2nd group with AIDS in comparison with patients without AIDS a decrease in the content of CD4+CD25+T-regulatory cells (cells/µl): 8.6 (4.76-22.1) and 23.8 (13.9-41,7), p<0.05, respectively, a decrease in the content of CD4+CCR5+T-helpers (cells/µl): 18.58 (8.52-32.6) and 55.67 (43.65-85.43), p <0.05, respectively was found.
In patients infected with R5-tropic HIV, there were no significant differences in the content of these cells depending on AIDS presence.

Conclusions: In patients with non R5-tropic HIV, the formation of AIDS, unlike patients with R5-tropic HIV, showed a pronounced activation of T-cell immunity, a decrease in the content of CD4+CD25+T-regulatory cells and CD4+CCR5+T-cells, which indicates different immunological mechanisms of AIDS formation at HIV-infection with different virus tropism.

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Use of Rapid HIV Antibody Tests in Chile as prevention strategy

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Introduction: Today Chile has the highest rate of new HIV infections in Latin America. Although in Chile the first rapid test for HIV was registered in 1999, it has never been used massively in the general population as a prevention strategy for HIV infection.

Method: 4th generation rapid HIV test (BTNX, Ontario Canada) and an anonym epidemiologic survey were realized to 1337 people in Santiago between November 28 of 2017 and March 31 of 2018. In accordance with the national HIV/AIDS law, a complete confirmation process was made to the people who were reactive in the rapid test.

Results: A total of 1,337 people were tested of which 20 were reactive for antibodies against HIV. The 20 people were confirmed as HIV positive by the confirmation algorithm carried out by the Institute of Public Health of Chile (ISP-Chile), the technical agency mandated at the national level to confirm all new cases of HIV infection that are being screened in Chile. Of the 20 people detected, 18 were men with an average age of 32.4 years and 2 were women with an average age of 42.5 years. According to these data, the current prevalence of HIV in the people studied is 1.5%. A total number of 1200 surveys were collected. Responders were 52% male and 48% women. During the last year: only 20% of the respondents answered that they had always used condom, only 40% reported having had an HIV test and same period time the average number of sexual partners was 3. An 80% responded that they would be willing to use a drug as pre-exposure prophylaxis. A 90% of the respondents answered that the test was not carried out for the following reasons: difficult access, lack of time, lack of interest, excessive price and fear of the result.

Conclusions: In Chile during the five-year period 2010-2015 new cases of HIV have increased up to 96% in the age group that goes from 14 to 29 years. This reality has the country in a situation of epidemiological alert. The prevalence of 1.5% reported by our work is three times higher than that estimated by UNAIDS for Chile. This discrepancy has its origin in the difficult access that the population has to the diagnosis of HIV, which is performed exclusively in public and private health centers. Our experience tells us that massive testing by means of rapid fourth-generation tests is an excellent opportunity to bring the diagnosis closer to people. The limited use of condoms by the majority of the population studied is evidence that could explain the high number of new HIV cases in Chile.

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Ultra-Deep Sequencing in real-life conditions: highlights from first assays on HIV and HEV - Virology laboratory, University hospital of Nancy, France.

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Backgrounds: Sanger population sequencing has been the reference to monitor HIV1-infected patients' antiretroviral (ARV) therapy, but Ultra-Deep Sequencing (UDS), sensitive and quantitative, may be an alternative. The present report aimed to analyze original features by comparing UDS versus Sanger sequencing on HIV and hepatitis E virus (HEV).

Materials & methods: Plasma samples from 12 HIV-infected patients and 8 HEV-infected patients were studied by Sanger and UDS (MiSeq/Illumina). HIV genome was studied either within Env V3 loop (R5/X4 tropism, n=8 patients), or in protease/reverse transcriptase/integrase, n=4 patients; for HEV, ORF2/3 gene overlap was studied for 8 patients. The data were analyzed by bioinformatics for variability (MEGA/Geneious). For UDS, nucleic acid coverage at a mean of 20000 reads and amino acid cut-off at 1% were considered.

Results: For HIV, similar results or silent mutations were observed for 9/12 patients by Sanger and UDS. For one patient, one minor Env-V3 loop variant was X4-tropic by UDS (16%) while Sanger concluded as R5-tropic. For the two others, UDS demonstrated a wild type residue at position 143 and 263 within integrase in spite of ambiguous results by Sanger. For HEV, similar results were observed by Sanger and UDS concerning the major variants of 7/8 patients. For the last one, suffering from chronic HEV infection, UDS showed a high heterogeneous quasispecies with multiple variants and major variants at only 6.96 % (ORF2) and 10.63 % (ORF3). Elsewhere, for one patient, we observed a major variant at 80.91 % according to ORF2 while at 43.1 % for ORF3.

Discussion: UDS for HIV resistance genotyping can provide useful information with possible consequences for resistance testing. Mutations found on HIV Env-V3 loop or in HEV ORF2/3 gene only by UDS might be explained by previous selection pressure from host-related immunity. Previous ARV therapy can also influence viral quasispecies.

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Diagnosis of HIV proviral DNA in a dry blood spot in Uzbekistan.

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Background: In Uzbekistan twice a year conducts epidemiological surveillance of HIV infection among at-risk groups. The collection of plasma samples is complicated by the hot climate and geographical remoteness of the regions. The aim of the study was to adapt the method of using DBS to conduct epidemiological surveillance of HIV among at-risk groups in Uzbekistan using available diagnostic reagents.

Materials & Methods: 123 samples of a DBS and blood plasma obtained from HIV-positive patients and 200 blood plasma samples and a DBS from HIV-negative patients were examined by PCR. The Interlabservice reagents "AmpliSens® DNA-HIV-FL" were used for detection of proviral DNA of HIV by PCR.

Results: Testing of DBS according to the manufacturer's instructions showed a result of 74.8% (plasma samples were taken as 100%). The addition of extra-time during extraction significantly increase the sensitivity of the method to an acceptable level of 97.5%. Replacing the extraction kit offered by the manufacturer, the "Ribo-prep" with the "DNA sorb B" with the addition of the exposure time of the DBS discs with lysing solution, made it possible to raise the sensitivity of the method to 99.1%.

Conclusions: Changing the extraction method and extra-time for lysis of DBS disks allows using this method for epidemiological surveillance in Uzbekistan.

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Laboratory evaluation of Thermofischer HIV-1 genotyping assay as a HIV drug resistance test in Kenya

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Background: As an effort to meet the UNAIDS 90-90-90 targets, Kenya has made tremendous gains in ART scale up with over 1,000,000 people living with HIV (PLHIV) currently enrolled in HIV care. However, the rise in the number of patients on ART is likely to result in increased risk of emergence and transmission of drug resistance. The National HIV Reference Lab (NHRL) of Kenya is mandated by the Ministry of Health to offer technical support to the national DR surveillances. To achieve this mandate, NHRL was recently equipped with adequate DR equipment . However, before safe utilization of the mentioned DR infrastructure at NHRL, there has to be a laboratory method verification of Thermofischer HIV-1 genotyping assay to verify performance characteristics before being deemed fit for its intended use. We report herein the results of the Thermofischer HIV-1 genotyping assay validation on the plasma sample type.

Methods: EQA panels (n=20) and remnant plasma samples (n=20) with known mutations and subtype previously analyzed at KEMRI HIV-R lab Kisumu were analyzed for DR testing using the Thermofischer genotyping assay at the National HIV Reference Laboratory of Kenya. The results (sequences and DR mutations) were then compared to the available results from the HIV-R lab to determine the accuracy, amplification sensitivity, precision and reproducibility of the Thermofischer assay. The levels of agreement were analyzed using SAS V.9

Results: Of the 40 samples analyzed, Thermofischer assay achieved an accuracy of 99.1% (95% CI: 97.6% - 100%) with an amplification sensitivity of 100% (95% CI: 98.1% - 100%). The precision of thermofischer assay was 100% while reproducibility was 98.1%.

Conclusion: Thermofischer HIV-1 Genotyping assay reported an acceptable performance that would in-turn pave way for its utilization in patient management for the national program. In addition, the NHRL will to support DR testing for protocol-specific studies and national/cohort-based DR surveillances.

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Assessment of immune activation of biomarkers in patients with HIV infection

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Materials and methods: Investigated 224 samples of peripheral blood of patients with HIV-infection. The average age of patients was 36.8±0.6 years. Patients were divided into two main groups: 1st — taking ARVT (n=135), 2nd — not receiving ARVT (n=89). The duration of ART was on average 9.0±0.5 years. The key parameters of the cellular immunity were determined by the cytometric method using labeled monoclonal antibodies. The RealTime PCR method was used to determine the concentration of HIV RNA.

Results: In the 1st-group of patients with HIV-infection, the proportion of naive T-cells CD4+CD45RA+CD45R0- was 14.1±1.4%, in the 2nd - 16.4±2.1% (p>0.05). The population of naive T-cells with a phenotype of 62L (CD4+CD45RA+62L+) also prevailed in the 2nd group compared with the 1st (p<0,05). T-cells of "memory" CD4+CD45RA-CD45R0+ were determined at the level of 51.4±1.9% in patients of the 1st-group and 49.4±1.9% in the 2nd group (p>0.05). The level of activated T-cells (CD45RA+CD45R0+) was significantly higher in the 2nd group of patients compared to the 1st: 0.3±0.1% versus 0.06±0.05% (p<0.05). The marker of late activation of immunity CD3+HLA-DR+ in the 1st group was at the level of 50.8±1.5%, which turned out to be significantly lower than in the 2nd-group - 61.1±2.3%, (p<0.001). Mean levels of activated CD8+Tcells were significantly higher for HIV patients who did not take ART. The share of CD3+CD8+CD38+ in the 1st-group was 56.7±1.7%, in the 2nd-group - 68.6±2.5% (p<0.001); in the 1st-group, the level of CD3+CD8+HLA-DR+cells averaged 17.7±1.0%, and in the 2nd - 32.3±1.9% (p<0.001). As expected, HIV-infected patients who did not receive ART did have a higher proportion of CD3+CD8+CD38+HLA-DR+cells than patients with ART (p<0.001). Moreover, in patients with complete suppression of HIV RNA concentration against the background of ART, the level of CD3+CD8+CD38+HLA-DR+ was significantly lower than in patients with a detectable level of HIV viral load (more than 10,000 copies/ml), p<0.001. Also, the level of CD8+cells with coexpression of CD38+ and HLA-DR+ was significantly lower in patients with a high concentration of CD4+lymphocytes, p<0.05. An inverse correlation between the absolute number of CD4+cells and the level of CD3+CD8+CD38+HLA-DR+cells (r=0.4), as well as a direct relationship between the concentration of HIV RNA in the blood and the levels of CD3+CD8+HLA-DR+ and CD3+CD8+CD38+HLA-DR+cells (r=0.35).

Findings: HIV infection causes a pronounced activation of both innate and adaptive immunity, induces and supports systemic inflammation in the body. In HIV-infected patients with ARVT, there is a decrease in the proportion of naive T-cells and an increase in the level of memory T-cells. ART reduces the activation of T-cells, but does not normalize it. The proportion of CD8+T-lymphocytes carrying CD38 and HLA-DR on their surface remains elevated even with complete (less than 50 copies / ml) viral load suppression. The levels of CD8+CD38+HLA-DR+T-cells correlate with the concentration of HIV RNA, decreasing with the suppression of HIV replication against the background of ART. Thus, persistently high levels of CD38+HLA-DR+cells can be an indicator of HIV replication.

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Forgiveness of Antiretroviral Regimens: In Vitro HIV-1 Viral Breakthrough with 2-Drug versus 3-Drug Regimens Simulating Variable Adherence to Treatment

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Background: Guidelines for modern treatment of HIV-1 infection recommend triple therapy consisting of an integrase strand transfer inhibitor (INSTI) plus 2 nucleoside/tide reverse transcriptase inhibitors (NRTIs). Recently, controlled clinical trials have evaluated the efficacy and safety of an INSTI + 1 NRTI. To simulate regimen "forgiveness" of triple therapy versus experimental 2-drug combinations, in vitro experiments monitoring viral breakthrough and emergence of resistance were conducted under conditions simulating drug exposures at full adherence or suboptimal adherence to treatment.

Methods: Viral breakthrough experiments were performed in MT-2 cells infected with wild-type HIV-1 (IIIb strain). Infected cells were cultured in the presence of fixed doses of bictegravir + emtricitabine + tenofovir alafenamide (BIC+FTC+TAF) or dolutegravir + lamivudine (DTG+3TC), split every 3-4 days with fresh media containing drug, and monitored for viral breakthrough by cytopathic effect for up to 5 weeks. Drug concentrations of BIC and DTG were calculated using their human plasma clinical trough concentrations (Cmin) according to their prescribing information and adjusted for plasma protein binding. The TAF Cmin concentration generated tenofovir-diphosphate at its physiological concentration in PBMCs from TAF-treated individuals. FTC and 3TC concentrations were set at their human plasma-free adjusted clinical maximum or minimum concentrations. In breakthrough experiments that simulate 2 consecutive missed doses, the drug concentrations were adjusted by their plasma half-lives for BIC and DTG and active metabolite half-lives for the NRTIs (TAF, FTC, and 3TC). Emergent HIV-1 variants were characterized using standard genotyping methods.

Results: At trough drug (Cmin) concentrations corresponding to full adherence in adults, no viral breakthrough occurred with BIC+FTC+TAF or DTG+3TC through 5 weeks in culture (0 of 24 replicate wells for each drug combination). At trough drug concentrations corresponding to two consecutive missed doses (Cmin minus 2), no viral breakthrough occurred for BIC+FTC+TAF (0 of 24 replicate wells) through 5 weeks in culture but viral breakthrough occurred as early as 9 days post-infection for DTC+3TC (23 of 24 replicate wells through 5 weeks). Using higher concentrations of FTC and 3TC in the Cmin minus 2 doses experiments, no viral breakthrough occurred for BIC+FTC+TAF (0 of 24 replicate wells) but viral breakthrough occurred as early as 11 days post-infection for DTG+3TC (21 of 24 replicate wells through 5 weeks). When analyzed by population sequencing, all viruses lacked drug resistance mutations at the time of viral breakthrough.

Conclusions: Consistent with high efficacy rates for BIC/FTC/TAF in treatment-naïve and virologically suppressed individuals and for DTG+3TC in carefully selected treatment-naïve adults, no viral breakthrough occurred in vitro using clinical drug trough concentrations corresponding to full adherence. However, our in vitro studies suggest that the higher drug levels provided by triple drug therapy may be more protective than two drug combinations in individuals with suboptimal adherence particularly in the real world where imperfect drug adherence is frequent.

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Prevalence of protease drug resistance in naïve and treated HIV-1 infected worldwide population between 1986 and 2017

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Background: Protease is an effective therapeutic target for the treatment of HIV-1. However, drug resistance mutations challenge the long-term efficacy of protease inhibitors.

Methods: Protease sequences, collected between 1986 and 2017, were downloaded from Los Alamos database, from subtypes A-C, F, G and CRF02_AG. Transmitted Drug Resistance (TDR) was estimated using the World Health Organization 2009 surveillance drug resistance mutation (SDRM) list and genotypic resistance to antiretroviral drugs (ARV) was evaluated using the Standford HIVdb v8.4. Statistical analysis was performed using SPSS software.

Results: 62,676 HIV-1 protease sequences collected from patients were analysed. Of these, 39078 were drug naïve (DN) and 23598 were treated (TR). Among the DN, 836 (2.1%) presented TDR while 7085 (30%) of TR presented ADR. TDR was 3 times higher in men who have sex with men (MSM) than in heterosexuals (HET) ($p<0.001$) and was higher in subtype F1 (3.1%), followed by B (2.7%), A (1.7%), C (0.9%), CRF02_AG (1.2%) and G (1.1%), respectively ($p<0.0001$). Patients from Eastern Europe (5.6%), Oceania (3.1%), North America (2.9%), and Western Europe (2.7%) had higher TDR than patients from Asia (2.4%), South America (2.2%), and Africa (1.1%) ($p<0.001$). ADR was higher in subtypes F, B and G (47%, 44% and 19%, respectively), as other subtypes presented levels lower than 10% ($p<0.0001$). The ADR rate was higher in Western Europe (55%), South America (43%), Central and North America (34%) than in Oceania (18%), Asia (16%), Eastern Europe (9.5%) and Africa (6%) ($p<0.0001$). Concerning risk factor analyses the same profile was observed as for TDR: the rate of ADR among MSM was 3 times higher than HET group ($p<0.0001$). The simple logistic regression models showed that gender, risk factor, subtypes and geographic region could explain the presence of TDR and ADR. Among DN patients, TDR to Darunavir (DRV) (0.18% - 95 IC 0.14%-0.22%) was 5 times lower than resistance to Atazanavir (0.9% - 95 IC 0.8%-0.99%) and almost 10 times lower compared to Lopinavir (1.7% - 95 IC 1.4%-1.83%) ($p<0.05$).

Conclusions: Overall, TDR and ADR to Protease inhibitors is low, but substantially different when comparing risk groups, subtypes and geographic regions. DRV presents very low drug resistance levels and should be considered for first line regimens. Moreover, the findings indicated a higher TDR rate in Eastern Europe and Oceania but lower ADR when compared to other continents.

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Frequent transmission of HIV-1 subtype C harboring L90M mutation in patients followed in Portugal.

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Background: Human immunodeficiency virus type 1 (HIV-1) subtype C accounts for approximately 48% of all people living with HIV, representing the most prevalent HIV-1 subtype in the world. Current advancements in antiretroviral therapy (ART) have turned HIV-1 infection into a chronic and manageable disease. However, treatment is only effective until HIV-1 develops resistance against the administered drugs. The aim of this study was to identify and characterize transmission clusters of subtype C containing the L90M mutation in Portugal.

Methods: Epidemiological, clinical and viral sequence data from 503 HIV-1 subtype C infected patients followed in Portugal between 2001 and 2016 were included, of which 32 had L90M mutation. 13118 unique background control sequences from subtype C were selected by blasting our sequences against Los Alamos database. Phylogenetic tree reconstruction was performed with FastTree software v.2.1. Cluster Picker was used to identify transmission clusters according to a threshold based on a genetic distance > 0.45 and a bootstrap support > 90%. Bayesian phylogenetic inference was performed with BEAST v1.8.4 to analyze the temporal dynamics of HIV-1 subtype C L90M transmission. Statistical analyses were performed to identify possible correlates of clustering.

Results: 204 (41%) portuguese patients were in transmission clusters. Portuguese patients harboring L90M mutation were 2 times more likely to be in clusters than patients from other countries (60%) (IC 95%: 42%-74%) vs 35% (IC95%: 26%-44%; p<0.001). The largest Portuguese C L90M cluster was composed of 14 patients, mainly drug naïve. Bayesian coalescent analyses suggested that this mutation was introduced in Portugal in 1992 while its transmission ignited in 2004.

Conclusion: Our results indicate continuous transmission of HIV-1 subtype C L90M strains in Portugal, with its origin dated back to the early 1990s. However, further analysis is necessary to clarify what determines this higher rate of transmission of L90M in Portuguese patients infected with subtype C.

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Evaluation of HIV-1 gp120 polymorphisms potentially associated with resistance to Fostemsavir

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Background: Fostemsavir (FTV) is a member of a new drug class currently under investigation for highly drug-experienced HIV-1 infected patients. This antiretroviral is an attachment inhibitor that binds HIV-1 gp120, by blocking HIV attachment to host CD4+ T-cells. To date, mutations at seven positions (L116, A204, S375, M426, M434, S475 and V506) of gp120 are known to reduce FTV susceptibility both in vitro and in vivo. In this study, we evaluated the natural prevalence of such FTV-resistance mutations and their potential association with other HIV-1 gp120 polymorphisms.

Material and methods: The study was conducted on 1,997 HIV-1 gp120 sequences of B subtype obtained from plasma samples of individuals naïve to FTV from Los Alamos HIV Database. Sequences were aligned to HXB2 reference and manually edited. Prevalence of mutations at the FTV associated positions was evaluated in overall population and stratified according to HIV-1 tropism (Geno2Pheno algorithm, FPR=10%). A covariation analysis was performed to evaluate potential association between the FTV-resistance mutations and other gp120 polymorphisms present with a prevalence ≥5%. An average linkage hierarchical agglomerative clustering was also performed, analysing FTV-resistance mutations associated with each mutation alongside all the 511 gp120 amino acid positions.

Results: The following FTV mutations were detected: L116Q (0.05%), S375H (0.6%), S375M (1.4%), S375T (17.7%), M426L (7.6%), M434I (4.2%), M475I (1.7%), while mutations L116P, A204D and V506M were completely absent. Among other natural polymorphisms at FTV resistance positions, only the mutation M426R was found with a prevalence >5% (16.3%). Generally, no specific association between viral tropism and FTV mutations prevalence was found, with the exception for S375M (R5 vs. X4: 0.7% vs. 3.9%, p=0.009) and S375T (16.6% vs. 22.1%, p=0.03). By the covariation analysis, specific gp120 mutations were positively correlated with FTV-resistance positions. In particular, S375T correlated with I371V (phi=0.21; p=9.8x10^-15); S375M correlated with the three mutations L134W (phi=0.21; p=2.6x10^-8), I154V (phi=0.20; p=4.5x10^-8), and I323T (phi=0.24; p=9.6x10^-10); and M475I correlated with K322A (phi=0.24; p=1.8x10^-10). Finally, the polymorphism M426R strongly correlated with the mutations G167N (phi=0.33; p=1.6x10^-35), K192T (phi=0.47; p=1.5x10^-66), and S195N (phi=0.24; p=3.6x10^-22). The topology of the dendrogram revealed the existence of two distinct clusters and two pairs of mutations (bootstrap ≥0.98) that confirmed the involvement of divergent pathways of gp120 mutations potentially associated with FTV-resistance. Interestingly, all FTV mutations and polymorphisms present in the clusters are localized in class I/II–restricted T-cell epitopes and antibody epitopes (according to Los Alamos Immunology Database lists, available at https://www.hiv.lanl.gov/content/immunology/products.html), suggesting a potential role in HIV escape from immune response.

Conclusions: Despite the high variability of gp120, FTV-resistance mutations were found with a low prevalence in sequences from individuals FTV-naïve infected with HIV-1 B subtype. The potential contribution of some of these mutations with other specific gp120 polymorphisms to the development of a synergistic effect of resistance to FTV may have viro-immunological implications, thus deserving further in-depth in vitro and structural investigation.

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Differences in codon usage between HIV-1 subtypes and their impact on the calculated genetic barrier for drug resistance to integrase inhibitors

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Background: The World Health Organization recommends dolutegravir in treatment of HIV which can result in increased resistance to integrase inhibitors. Data on drug resistance to dolutegravir is predominantly available from subtype B, whereas in low- and middle-income countries subtypes other than B are the most common. The genetic barrier, defined as the number of mutations required to overcome drug-selective pressure, is a key factor in the development of HIV drug resistance. Because of high variability in codon usage between subtypes, particular HIV-1 subtypes could have different genetic barriers for drug resistance mutations. The aim of this study is to compare codon usage and the calculated genetic barrier for resistance between subtypes.

Methods: We considered the major drug resistance associated mutations (T66A/I/K, E92G/Q/V, G118R, E138A/K/T, G140A/C/S, Y143A/C/G/H/K/R/S, P145S, Q148H/K/N/R/S, N155D/H/S/T, R263K) and accessory drug resistance associated mutations (H51Y, L75F/I/M, T97A, F121Y, Q146P, V151A/I/L, S153F/Y, E157Q, G163K/R, S230R). Drug resistance associated mutations occurring outside of the integrase region were not considered. Codon usage was compared between subtypes at positions where drug resistance associated mutations occurred. The genetic barrier, calculated as the sum of transitions (scored as 1, or 0.2 for the G to A hypermutation) and/or transversions (2.5) required for evolution to any drug resistance substitution, was compared using the Kruskal-Wallis test and Mann-Whitney tests using a false discovery rate of 0.01 to correct for multiple comparisons.

Results: 13,694 integrase sequences from integrase inhibitors naïve individuals (subtypes A-D, F, G, CRF01_AE and CRF02_AG) were selected from the Stanford HIV drug resistance database. The most frequent subtypes were B (6644 sequences/ 49%), C (2517/18%), and CRF01_AE (1824/13%). Several accessory mutations were present as polymorphism in <10% across subtypes (L74M, T97A, E157Q, G163R). L74I occurred as polymorphism in >10% of subtype A (21%) and CRF02_AG (12%). The codon usage was different between subtypes at major drug resistance positions 92 (GAG in a majority of subtype B versus GAA in other subtypes), 118 (GGT in CRF02_AE versus GGC), 140 (GGC in B versus GGA), 148 (CAG in C versus CAA), 263 (AGG in C versus AGA), and at accessory mutations position 151 (GTG in CRF01_AE and subtype A versus GTA), 153 (TCC in D versus TCT), 157 (GAG in subtype G versus GAA), and 163 (extensive differences across subtypes). An increased genetic barrier was only observed for the major mutation G140C in subtype B ($p<0.0001$ versus other subtypes). Compared to subtype B a different calculated genetic barrier was calculated for mutations L74F/I/M, T97A, V151A/I, E157Q, G163K/R and S230R ($p<0.0001$). Using different scores for transitions and transversions did not impact the results of the statistical analysis.

Conclusions: Differences in codon usage between subtypes are common and predominantly affect the calculated genetic barrier of accessory drug resistance mutations and only one major drug resistance mutation, indicating that the rate of development of drug resistance will most likely be similar between subtypes. The impact of differences in codon usage between subtypes on the genetic barrier and drug resistance development has to be confirmed in in-vitro and/or in-vivo studies.

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HCV RECall - Automated Sanger Basecalling providing HCV Genotyping and Sequencing For Drug Resistance Evaluation

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Introduction: Sanger/Population sequencing is widely for resistance testing in clinical settings. Slow, labor-intensive manual sequence editing, subjective variant mixture determinations, and the lack of resistance interpretation can contribute to erroneous reporting. An automated sequence analysis tool is needed to facilitate the use of resistance information for HCV management.

Methodology: The RECall program (https://hcvshared.hcvdb.ubc.ca) originally designed for HIV genotyping was modified and adapted for HCV analyses. Over 200 reference sequences from ICTV were incorporated for GT/subtype determination. Seven HCV "prototype" sequences (GT1a, 1b, 2-6) were used as references for mutation reporting. A rule-based algorithm to interpret clinically relevant resistance mutations was introduced.

To validate accuracy of GT/subtype determination, 1000 HCV sequences with annotated GT/subtype information for NS3 and NS5A were downloaded from LANL and aligned with the reference sequences. To examine accuracy of basecalling, 1046 raw ABI HCV chromatograms were submitted to RECall. The sequencing trace files were processed with the software, Phred, which trimmed sequence ends and excluded regions with <20 quality scores from the contig assembly. Mixture calls were identified based on the primary and secondary peaks of the chromatograms. The resistance-associated substitutions within NS5A were evaluated.

Results: ICTV HCV sequences provide a good reference to accurately determine HCV GT/subtype. For NS5A, the concordance was 100% at the GT level and 99.2% at the subtype level; 8 GT1b were identified as GT1a using the references. For NS3, the concordance was 100% and 99.3% at the GT and subtype levels, respectively: 4 annotated GT1a were identified as GT1b, one GT4b was identified as 4w, one 6u was identified as 6xa, and one 1a was identified as 1 only.

HCV ReCall can automatically select the correct region of HCV (e.g., NS3, NS5A or NS5B) and process raw chromatogram data. For example, a collection of 1046 raw ABI chromatograms were automatically processed for NS5A codons 20 to 95 in a total of 8 mouse clicks, and the aligned FASTA sequence results compared with those generated by an independent reference laboratory. Of the 100 NS5A samples compared, there were a total of 116 basecalling discordances out of 22,500 bases (0.5%); the vast majority of the discordances were caused by differences at "mixed" bases. The correct HCV genotype was chosen in all cases. For samples derived from virologic failures, mutations were observed at the known resistance-associated positions. The HCV ReCall analysis report also provided a summary of quality scores for each of the primers, pairwise assessments of genetic distance of each sample from the others to identify contamination, a list of mutations relative to the prototype sequence, and an assessment of the relative peak height of every mixture.

Conclusions: HCV RECall web application provides a comprehensive analysis for HCV drug resistance; directly from raw ABI sequencing trace files without manual intervention. This tool enables objective and consistent interpretation of HCV genotype data, improves processing speed, and decreases labor and software costs. Consensus on appropriate "reference" strains for genotyping and "prototype" strains for resistance mutation reporting will be important for consistency in inter-laboratory comparisons.

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Prevalence and structure of HIV-1 drug resistance to integrase strand−transfer inhibitors among naïve patients and treatment-experienced patients in Russian Federation

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Background: Achieving the goal "90-90-90" led to a significant increase the number of HIV-1 infected patients on ART in Russia. ART roll-out has improved outcomes but has resulted in increasing acquired and transmitted resistances to the drugs used.
INSTIs are the newest class of antiretroviral drugs to be approved for treatment. In Russia only two drugs have been registered (RAL and DTG) and since 2017 they are part of the first-line therapy.
Thus, to assess the prediction of the effectiveness of the use of INSTI in Russia, it was of interest to analyze the prevalence and structure of resistance mutations in the integrase gene of HIV-1 among: treatment-naïve patients, patients with ineffective therapy, which not included INSTIs, and patients with ineffective therapy, which included INSTIs.

Materials and methods: We analyzed 434 integrase sequences from HIV-infected patients, collected between 2008 and 2018: 227 treatment-naïve patients, 185 patients with virological failure of therapy without INSTI and 22 patients, whom experienced ineffective therapy, which included RAL (median duration of taking was 1,1 years). Integrase sequences were obtained by AmpliSens® HIV-Resist-Seq kit. Viral subtype and drug resistance were determined using the HIVdb Program v.8.4.

Results: HIV-1 subtype A6 was the most frequent clade (86.6%), subtype B was detected for 31 (7.1%) viruses, subtype G for 3 (0.7%) viruses and CRF02_AG (3.9%), CRF63_02A1 (1.6%). Mutations associated with DR to INSTI were detected in 5 (2.2%) treatment-naïve patients. In 5 patients were found mutation to EVG, in 4 to RAL, in 2 to DTG and to BIC. Four patients had major mutations: Q146P (0.9%), Y143C (0.4%), Q148H (0.4%), and 5 patients had accessory mutations: G163R (1.3%) and S153Y/F (0.9%).
Four samples (2.2%) from treated patients with ineffective therapy had resistance mutations: to EVG and RAL, in 1 to DTG and BIC. The only major substitution detected in this samples was R263K (0.5%) and 2 accessory mutations in 3 patients: E157Q (1.1%), T97A (0.5%).
It is important to note the highly polymorphic accessory mutation L74I, which was observed in 353/412 patients without experience INSTI. Although the presence of an L74I mutation alone is not associated with significantly reduced drug susceptibility, in combination with other major drug resistance mutations it could reduce viral susceptibility to INSTIs.
Among 22 raltegravir-treated patients resistance mutations were found in 13 (59.1%) of them. In 13 patients were found mutations associated with DR to EVG and to RAL, in 7 patients to DTG and to BIC. The most commonly selected INSTI major mutations were Y143C/R (40.9%) and N155H (13.6%). The most frequent INSTI accessory resistance mutations were T97A (31.8%), G163R (13.6%).

Conclusions: Our results demonstrate a low rate of DR to INSTIs in Russia among patients without experience INSTI. It proves the efficiency of INSTI-based drug regimens application in treatment-naïve and treatment-experienced patients even with virological failure.
For RAL-treatment patients was obtained high rate of resistance prevalence to this drug. However, DTG has high-genetic barrier and has low degree of cross-resistance with RAL, consequently will effectively inhibit viral variants which resistant to RAL.

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A retrospective analysis of the EuResist data set assessing if NRTI resistance impairs INSTI based treatment with NRTI backbone

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Background: Antiretroviral combination therapy (cART) normally contain two nucleotide reverse transcriptase inhibitors (NRTIs) and an integrase inhibitor (INSTI). But there is still a lack of studies showing how baseline resistance to NRTIs affects the risk of virologic failure in this constellation.

Prospective clinical trials do not enable cases with resistance at baseline. Older retrospective studies in this form exist, but those don't include current INSTI drug combinations, or include only few cases. Now we are at the point to receive meaningful results from retrospective studies for these newer regimens. For a smaller dataset, namely the ARCA database, such data already exists. Here we present the analysis on the Europe wide database EuResist.

We assess whether NRTI resistance can impair INSTI based treatment with NRTI backbone. As noted in the ARCA paper, resistance for NRTI reduces the success of INSTI/2NRTI based therapies which signifies a need for NRTI resistance testing in INSTI based therapies. A suggested, we perform a larger study here.
The study's aim is to determine if this relation of significantly higher risk for virologic failure holds true in the larger and Europe wide context of the EuResist database (i.e. in the scope of multiple European countries and an increased sample size).

Materials and methods: This retrospective study uses the EuResist Integrated Database. EuResist is a meta database of HIV related data. At present, the central database contains data from >81.000 patients, including >100,000 genotypes, >170,000 treatments, >1 million viral load and >1 million CD4 data.
Selected patients had a recorded firstline therapy and a baseline sequence within 1 month of the therapy start. Only therapies after 2009 were selected, the onset of dual therapies in the data set. NRTI resistance mutations where obtained by the Stanford HIVdb program. The cases were then classified as either no NRTI mutation or >1 NRTI mutation. Virologic failure was defined as after 6 months or after first virologic suppression a VL viral load measurement of over 1000 cp/mL. This resulted in 497 INSTI therapies without NRTI resistance and 38 INSTI therapies with NRTI resistance.
Kaplan Meier plots were generated both for the overall risk of viral failure stratified by NRTI mutation and specifically for INSTI based ARTs.

Results: The Log-rank test for the Kaplan Meier curve depicting overall viral failure shows a significant difference between no NRTI mutation and >=1 NRTI mutation p=0.048. The log-rank test for INSTI-based ARTs was p=0.003. For INSTI based ARTs the risk of viral failure was 13% in the resistant group and 4% in the non-resistant group.

Conclusions: Our results point in the direction that NRTI resistance in firstline integrase therapies does indeed increase the risk of viral failure.

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A retrospective analysis of the EuResist data set assessing dual therapy success in a real-life context

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Background: Since the introduction of effective combination ART, combining at least three antiretroviral agents to reduce the risk of treatment failure has been standard of care. The higher barrier to resistance of newer drugs has led to increased interest in simplified treatment strategies. Recent studies show that certain dual regimens are effective in maintaining virologic suppression in treatment-experienced patients. Consequently, both European and US guidelines currently recommend dual therapy to selected virologically suppressed persons. This is still a new treatment strategy, however, and more studies with longer follow-up are warranted to guarantee long-term safety of dual therapy.

Here, we assess the efficacy of dual regimen as switch in patients with undetectable VL as a retrospective study on the EuResist data set. The potential benefits of two-drugs regimens include reduced toxicity, lower cost, fewer drug-to-drug interactions, improved tolerability and possibly increased adherence to treatment. Several clinical trials have shown non-inferiority for dual regimens as maintenance therapy, and the strategy with dual regimen as switch has been tried at several European sites. EuResist Integrated Data Base thus contains real-world data on dual regimen after switch.

Materials & Methods: This retrospective study uses the EuResist Integrated Database. EuResist is a meta database of HIV related data. At present, the central database contains data from >81.000 patients, including >100,000 genotypes, >170,000 treatments, >1 million viral load and >1 million CD4 data.

Patients were selected into either the dual therapy or triple therapy treatment group. Older treatment combinations were disregarded. Both groups need to have a VL (viral load) measurement shorty before switch with VL under 50 cp/mL and multiple viral load measurements while on therapy. Viral failure was defined as two consecutive VL measurements with VL over 200.

The triple therapy cases were then selected via propensity score matching to contain similar covariates gender, age, route of transmission and cd4 at start resulting in 994 cases in either group.

Those data were used to generate Kaplan Meier curves for the dual and triple therapy groups. Afterwards, a TOST (two-one-sided-t-test) was performed comparing the Kaplan Meier estimator at 48 weeks, the endpoint for most prospective studies in this area.

Results: We found no significant difference between the two treatment groups in the Kaplan Meier curve p=0.99. We found a 98% likelihood of success (no virological failure) 48 weeks after switch, regardless of regimen. The two-one-sided-t-test at week 48 showed that Dual-therapy was significantly non-inferior compared to triple-therapy (95% confidence interval for the difference -0.002–0.000; equivalence defined as ±0.005).

Conclusions: Our results showed that in a real-life clinical setting, dual therapy has a similar success rate compared to triple therapy. Therefore, we consider dual therapy as a valuable addition in HIV management.

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Automated deep sequencing for HIV-1 DNA genotypic resistance testing

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Background: HIV-1 DNA resistance genotyping is useful in patients with low or undetectable HIV plasma RNA either to explore a virological failure or to guide a treatment simplification. Ultra-deep sequencing (UDS) techniques have improved the detection of resistant minority variants in HIV plasma RNA. Our aim was to validate the Sentosa platform (Vela DX) for automated deep sequencing for HIV DNA genotypic resistance testing by comparison with direct sequencing.

Materials & Methods: We evaluated the Sentosa SQ HIV genotyping assay on 40 prospective DNA samples isolated from treatment-experienced patients with undetectable or very low plasma RNA load (<200 copies/mL). Automated DNA extraction was performed on MagnaPure 96 (Small volume kit - Roche). HIV-1 DNA was quantified using the Generic HIV DNA cell assay (Biocentric). Direct sequencing was performed using the ANRS protocol (http://www.hivfrenchresistance.org/ANRS-procedures.pdf). The Sentosa SQ HIV Genotyping Assay generated protease (PR), reverse transcriptase (RT) and integrase (IN) sequences. HIV drug resistance was interpreted using the ANRS algorithm v29 (http://www.hivfrenchresistance.org/2018b/Algo-nov2018-HIV1.pdf).

Results: The success rate of Sentosa SQ HIV genotyping assay according to HIV DNA load was evaluated on 20 samples and was 100% for PR and RT and 86% for IN when the HIV DNA load was over 2.5 log copies/million cells. The success rate decreased to 70% for PR and RT when the HIV DNA load was comprised between 1.6 and 2.5 log copies/million cells. The global success rate of prospective UDS on 40 DNA samples was 72%. The prevalence of resistance to at least one antiretroviral drug was 55%. Patients were infected with viruses resistant to protease inhibitors (5%), nucleos(t)idic and non nucleosidic RT inhibitors (42% and 23%, respectively) and integrase inhibitors (15%) according to UDS. PR and RT genes were analysed in parallel using direct sequencing. UDS identified more samples harbouring viruses resistant to nucleos(t)idic and non nucleosidic RT inhibitors (19/40) than direct sequencing (7/40), while both assays were concordant for predicting resistance to protease inhibitors. Resistance to PR and RT inhibitors was consistent with treatment history. Three patients had been treated with integrase inhibitors (prevalence of resistance =3/15 patients previously treated with integrase inhibitors) among the six patients harbouring viruses resistant to integrase inhibitors.

Conclusions: Automated deep sequencing using the Sentosa SQ HIV genotyping assay performed better in predicting HIV DNA drug resistance than direct sequencing. Thus, UDS would be useful for evaluating patients eligible to a strategy of treatment simplification.

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Drug Resistance dynamics in isolates from HIV-1 Infected individuals with Mother-to-Child-Transmission in Italy from 1999 to 2017

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Background: In developed settings, perinatally acquired HIV-1 infection has become a chronic disease of childhood with increasing numbers of adolescents surviving to adulthood. Perinatally infected individuals have been heavily pretreated, and have a long history of antiretroviral treatment (ART), including sub-optimal regimens. This can increase the prevalence of drug-resistance, compromising the success of present and future treatment options. Only few data exist in literature about drug-resistance in HIV-1 individuals infected through mother-to-child transmission (MTCT) in Western countries. Thus, we evaluated the temporal trend of HIV-1 drug-resistance in this vulnerable population.

Materials & Methods: We included ART-experienced individuals HIV-1 infected through MTCT with at least one available plasma genotypic resistance test (GRT) for protease/reverse transcriptase and (when available) integrase, followed up in Central Italy from 1999 to 2017. The trends of resistance to NNRTIs, NRTIs, PIs and INIs, and resistance to 1, 2 and ≥3 classes were evaluated using the Stanford mutations list 2018 according to the following time periods: 1999-2002, 2003-2015, 2006-2008, 2009-2011, 2012-2014, 2015-2017.

Results: We analyzed 554 plasma GRTs from 193 ART-experienced individuals HIV-1 infected through MTCT. Nearly half of the individuals were born in Italy (47.2%) and were male (50.8%); 108 (55.6%) individuals were infected with HIV-1 B; among non-B subtypes, the most prevalent were CRF02_AG (17.1%), F (13.5%) and C (5.7%). Patients were born in a median (IQR) year of 1993 (1989-1998); their median (IQR) age at first-line regimen was 2 (1-2) years, while their median (IQR) age at the moment of GRT was 17 (12-22) years. During their treatment history, around 63% of individuals received a sub-optimal ART based on NRTI dual/monotherapy or unboosted-PI.

Overall, 69.3% of isolates showed resistance to any drug class; in particular, 35.0%, 58.7%, 45.8% 3.1% of isolates showed resistance to PIs, NRTIs, NNRTIs and INIs (N=184), respectively.

Overall, resistance to any drug class dramatically decreased from 90% in 1999-2002 up to 44.2 % in 2015-2017 in conjunction with a remarkable increase of GRTs without resistance (from 10% to 55.8%, p<0.001). More specifically, a significant decrease of resistance to 2 classes (from 48.3% to 5.8%; p<0.001) and ≥3 classes (from 23.3% to 7.7%, p<0.001) was observed from 1999-2002 to 2015-2017. By contrast, resistance to one class was almost stable up to 2014 (from 18.3% in 1999-2002 to 18.4% in 2012-2014, p=0.608), but increased in 2015-2017 (30.8% in 2015-2017 vs. 18.4% in 2012-2014, p=0.085).

Concerning the specific drug classes, the trends of resistance from 1999-2002 to 2015-2017 periods were as follows: PIs (from 53.3% to 11.5%, p<0.001), NRTIs (from 86.7% to 21.2%, p<0.001) and NNRTIs (from 45.0% to 28.8%, p=0.001). Resistance to INIs significantly increased from 0.7% in 2006-2008 to 5.8% in 2015-2017 (p=0.018).

Conclusions: In HIV-1 perinatally infected individuals followed in Italy, a dramatic drop of drug-resistance has been achieved over time. However, drug-resistance to INIs is increasing and resistance to ≥3 classes remains a concern that deserves clinical attention in this fragile population.

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Long term effectiveness of cART in heavily-treated HIV-positive Romanian patients, parenterally infected in early childhood

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Background: Virologic failure and development of HIV drug resistance can hinder the results of combined antiretroviral therapy (cART), causing progression of HIV infection. Our goal was to assess the efficacy of cART in a Romanian cohort of young adults, parenterally infected with HIV clade F in the late 1980s and exposed to cART for more than a decade.

Materials & methods: 170 HIV+ subjects (males: 47%, mean age: 24 years) with a median duration of HIV infection of 24 year and of cART of 13 years were analyzed. Currently, 67.3% of the participants receive a NRTI- based regimen, 13.6% a NNRTI- based regimen and 19% 3-class regimen. HIV viral load was tested by quantitative RT-PCR (Cobas TaqMan HIV-1 Test Roche Molecular Systems, USA) and plasma samples with HIV RNA level > 1,000 copies /mL were sequenced in the pol gene (ViroSeq HIV-1 Genotyping System,Abbott Laboratories, USA); drug resistance mutations were assessed using the Stanford HIV-Drug Resistance algorithm.

Results: 58% of the participants have achieved viral suppression and 45% showed no sign of immunosuppression (CD4 count >500 cells/ul). Lower CD4 T-cell counts (p=0.02), longer time on cART (p=0.04) and longer exposure to monotherapy regimens (p=0.03) were significantly associated with virological failure. No correlation was found between VF and current treatment regimens. Only 24% of the subjects presented HIV viral load > 1000 copies/m. The rate of antiretroviral resistance was 15%, with 6% of the subjects presenting resistance to two drug classes, 4% having triple class resistance and 1% having multiple resistance mutations to all currently available drugs. Reverse-transcriptase inhibitors mutations were predominant, followed by PIs resistance mutations. The presence of HIV-drug resistance was associated with lower nadir CD4 count (p=0.01) and longer duration of HIV-infection (p=0.04).

Conclusions: Our results show a high rate of virological suppression in the Romanian cohort of HIV-infected patients, long term survivors, parenterally infected in early childhood; a low prevalence of virologic failure and ARV drug resistance was recorded despite the fact that these patients were exposed to suboptimal treatment and sequential monotherapies in the past.

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Baseline resistance to Doravirine and genetic barrier of Doravirine containing regimens in Spain, 2018.

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Background: The Spanish cohort of naïve HIV infected individuals (CoRIS) offers relevant information about the current epidemiological profile of HIV infection, and is an excellent scenario to characterise the prevalence of TDR over time in Spain. We have previously characterized TDR in RT/Pro/Integrase in CoRIS throughout the period 2007-2017. Here we report the results on the prevalence of Doravirine associated mutations, clinical resistance to this drug and other NNRTIs, and the genetic barrier of Doravirine containing regimens in Spain during 2018.

Patients & Methods: To investigate the prevalence of NNRTI transmitted drug resistance, we used the WHO-2009 list with the following additional mutations E138A/G/K/Q/R, V108I, V179L, G190Q, H221Y, F227C/L/V, M230IDR, L234I, P236L, Y318F. The prevalence of Doravirine associated mutations, as described by Soulie et al. 2018 (doi:10.1093/jac/dky464) was evaluated. Clinically relevant TDR were investigated using the latest versions of ANRS and RIS Algorithms. Resistance to tenofovir (TDF) and 3TC, the drugs that will be included in a Doravirine-based STR were also investigated.

Results: Our cohort included 617 patients. Overall, NNRTI mutations were detected in 61 patients (9.9%). The prevalence of NNRTI associated mutations was: K101E (n=4, 0.6%), K101P (n=1, 0.2%), K103N (n=20, 3.2%), K103S (n=2, 0.3%), V106M (n=1, 0.2%), V108I (n=3, 0.5%), E138A (n=23, 3.7%), E138G (n=5, 0.8%), E138K (n=3, 0.5%), Y188C (n=2, 0.3%), Y188L (n=1, 0.2%), G190A (n=3, 0.5%), H221Y (n=1, 0.2%), F227L (n=1, 0.2%) and Y318F (n=1, 0.2%). Doravirine associated mutations are highlighted in bold and were detected in 5 patients, making a prevalence of 0.8%. Clinically relevant resistance to Doravirine was 0.3%, while it was 5.3% to Efavirenz, and 8.4% to Rilpivirine. In the cohort, for the first line NNRTIs TDF and 3TC clinically relevant resistance was in both cases 0.3%. No patient shared resistance to TDF, 3TC and/or DOR while one patient shared resistance to DOR and 3TC (K70G, M184V, K103N, V106M, V179T, Y318F).

Conclusions: Transmitted Drug Resistance to Doravirine, in contrast to Efavirenz and Rilpivirine, is very low in 2018 in Spain. Less than 0.5% of the newly diagnosed patients in Spain will be resistant to at least one of the drugs in first line Doravirine containing regimens.

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Lack of effect of pretreatment low frequency HIV-1 NNRTI resistance on treatment outcome in Uganda

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Background: Prevalence of pretreatment drug resistance (PDR) to widely used nonnucleoside reverse transcriptase inhibitors (NNRTIs) has risen considerably in recent years in low- and middle-income countries such as Uganda, where the majority of individuals on first-line antiretroviral therapy are prescribed NNRTI-containing regimens. Although integrase inhibitor dolutegravir is now a recommended component of first-line regimens by the World Health Organization (WHO), this option excludes women of reproductive age due to the potential risk of neural tube defects in infants. Standard genotypic resistance tests detect drug resistance-associated mutations (DRMs) present at ≥15-20% of the viral quasispecies population and are unable to detect potentially rare clinically-relevant DRMs present at lower frequencies. In this study, we examine the prevalence of pretreatment low frequency DRMs in Uganda using next-generation sequencing, and assess its impact on viral suppression.

Materials & Methods: Participants were treatment-naive individuals ≥18 years of age, initiating NNRTI-based regimens as part of the Uganda AIDS Rural Treatment Outcomes cohort. The 90-234 amino acid region of the reverse transcriptase gene was amplified, and consensus-based sequences were obtained on an Illumina MiSeq. Low frequency DRMs were defined as resistance-associated substitutions detected at a threshold of 2%, 5%, and 10% of viral population. Resistance was defined as ≥1 DRM(s) which resulted in cumulative low-, intermediate-, or high-level resistance to NNRTIs, as defined by a score ≥15 based on the Stanford University HIV Genotypic Resistance Interpretation Algorithm v8.5. Individuals contributed to the prevalence count of PDR if they had study-defined NNRTI resistance detected in their pretreatment genotypic drug resistance test. We assessed the impact of low frequency DRMs at different viral frequencies on viral suppression at one-year post-therapy initiation using univariate and multivariate binomial logistic regression models with age at enrollment, baseline CD4 count and HIV-1 RNA viral load, and sex treated as covariates.

Results: In total, 234 unique individuals (68% female; 60% subtype A1) contributed data from 2005-2013. Prevalence of PDR was 12%, 14%, 16%, and 18% at 20%, 10%, 5%, and 2% viral frequency, respectively. The most prevalent NNRTI DRMs were E138A and K103N. Individuals harbouring DRMs at higher viral frequencies had higher odds of having detectable viral load (≥400 copies/mL) at one-year post-therapy initiation (20% viral frequency: adjusted odds ratio [aOR] 1.20; 95% confidence interval [CI] 0.5-2.9; p=0.64) compared to lower viral frequencies (10% viral frequency: aOR 1.10; CI 0.4-2.4; p=0.87). Age was a statistically significant protective variable (OR 0.7; CI 0.5-0.9; p=0.03). There were five instances of DRMs detected at higher viral frequencies not being detected at lower viral frequencies in individuals, an artefactual result of mixtures interpreted using standard interpretation algorithms.

Conclusions: The prevalence of NNRTI PDR detected at 20% is similar to those previously reported in Uganda by the WHO. Overall, pretreatment low frequency DRMs did not have a statistically significant impact on viral suppression, potentially due to small sample size. Analysis of low frequency DRMs using consensus-based sequences present potentially significant complications using standard genotypic interpretation algorithms due to the ambiguous interpretations of nucleotide mixtures detected at low viral frequencies.

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Four Amino Acid changes near the p17/p24 cleavage site of HIV-1 CRF02_AG isolates confer reduced protease inhibitor susceptibility

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Background: Protease Inhibitors (PIs) are the second- and last-line therapy for the majority of HIV-infected patients worldwide as access to third-line therapy is still limited. Only around 10%–20% of individuals who fail PI regimens develop major resistance mutations to PIs by week 48, and this proportion increases over time.
Previous studies showed gag mutations can confer resistance to PIs in the absence of PI resistance mutations inside HIV-1 protease. We studied gag-protease changes within patients who failed PI treatment in a Nigerian treatment program.

Materials & Methods: Full length gag-protease of baseline (pre-PI) and virological failure (VF) samples of six HIV-1 CRF02_AG and subtype G infected patients was amplified and cloned into a p8.9NSX+ vector. Lopinavir (LPV) susceptibility of the VSV-g pseudotyped viruses was measured using cell-based, single replication-cycle assays. Susceptibility was expressed as IC50 fold-changes between isolate and the HIV-1 subtype B reference strain (p8.9NSX+).
We performed sequence alignment of the isolates identifying gag and protease amino acid substitutions which have occurred. Using site-directed mutagenesis (SDM), roles of the different amino acid changes was studied by reverting the amino acid changes and carrying out drug assays on the mutants.

Results: No significant differences in fold change (FC) IC50 of LPV in 5 out of 6 patient sample pairs, also these did not exhibit the unique gag mutations observed in the phenotypically-significant patient pair.
One patient had a 5x FC between baseline and VF virus isolates. Sequence alignment of gag-protease of this patient revealed 19 amino acid changes in the p17, one amino acid change in each of p24, p2 and NC as well as an insertion of four amino acids (Glu, Leu, Arg and Glu) in the p6 region and two amino acid changes (positions 14 and 46) between baseline (susceptible) and VF (resistant) isolates. Fold change IC50 to LPV of baseline vs VF isolates was 5.3 vs 20.3 respectively.
When Ser and His residues at positions 126 and 127 respectively were deleted in the susceptible virus, there was a decrease in LPV susceptibility of the mutant virus (FC IC50 from 5.3 to 8.2). Conversely, the insertion of Ser and His residues in the resistant virus increased susceptibility of the mutant (FC IC50 from 20.3 to 13.3). A combination of S126del, H127del and T122A, G123E mutations in the susceptible virus led to a 4x decrease in susceptibility (FC IC50 from 5.3 to 22.7). Conversely, S126Ins, H127Ins and A122T, E123G in the resistant virus, led to a 3x decrease in resistance (FC IC50 from 20.3 to 7.6). Western blotting of virus containing supernatants from producer cells did not reveal significant cleavage defects at the p17/p24 cleavage site.

Conclusions: In this failure case of LPV/r- based second line regimen in a Nigerian patient and with no major protease mutations, we provided evidence that the emergence of non-cleavage site gag mutations in the p17 domain which have not been previously described could be associated with PI failure in CRF02_AG viruses. The mechanism of this is currently being studied.

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Evaluation of integrase inhibitor resistance in PBMC and plasma compartments in clinical isolates from HIV-1 infected patients with low/undetectable plasma viral load.

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Background: Protease/reverse transcriptase genotypic resistance test (GRT) performed on peripheral blood mononuclear cells (PBMCs) is a useful tool to detect resistance in HIV-1 infected patients with low/undetectable plasma viral load. Despite the increasing usage of integrase inhibitors (INIs) in clinical practice, few data about integrase resistance on PBMCs are available. Thus, this study aims to explore INI-resistance detected in PBMCs in comparison to plasma compartment, as previous or contextual GRT.

Materials & Methods: Patients with low (<1000 copies/mL) or undetectable plasma HIV-RNA (<50 copies/mL) with an available integrase GRT on both PBMC and plasma compartments were included. INI major (MRM) and accessory (ARM) resistance mutations were evaluated according to the Stanford resistance list 2018. Mutations detected in PBMCs were compared to those detected in contextual and/or previous cumulative plasma GRTs. The presence of stop codons and/or APOBEC-associated substitutions was also considered.

Results: Among 150 patients included in the analysis, 51% had a plasma HIV-1 RNA <50 copies/mL at the moment of PBMC GRT, while in the remaining 49% of patients, the median (IQR) viremia was 124 (78-259) copies/mL. The majority of patients was infected with HIV-1 B subtype (73.9%) and was Italian (80.7%), showing median (IQR) viremia zenith and nadir CD4 count of 5.44 (4.95-5.75) log10 copies/mL and 163 (50-292) cells/mm^3, respectively. One-hundred and two (67.9%) patients were previously exposed to an INI before PBMC GRT, while 82 (54.7%) were under an INI-based regimen at the moment of GRT.
Regarding INI-resistance, the proportion of patients with at least one MRM detected in PBMCs was lower (n=4, 2.7%) compared to that with MRMs in plasma GRTs (n=14, 10.0%; P=0.009, by Chi Squared test). Among 15 patients harboring INI-resistance in at least one compartment, 11 (61.1%), 3 (16.6%) and 1 (5.6%), showed resistance only in plasma, in both compartments or only in PBMCs, respectively. Concerning the specific INI-resistance mutations, the unique patient harboring resistance only in PBMCs previously failed a raltegravir-based regimen, and showed the E138K MRM together with several stop codons and APOBEC-associated substitutions. All the other three patients who showed resistance in PBMCs were previously exposed to INIs and showed the same mutations in plasma (1: G140S+Q148H; 2: Y143C/H; 3: N155H). No stop codons were found in these three cases.
Considering the INI-ARMs, the proportion of patients with at least one ARM was similar in both compartments (33.3% in PBMCs vs. 28.7% in plasma, P=0.454) and no difference in the median [IQR] number of ARMs detected per patient was observed (PBMCs: 0 [0-1] vs. plasma: 0 [0-1], P=0.684).

Conclusions: Major resistance to INI in patients with low/undetectable plasma HIV-1 RNA is low. Integrase GRT performed in PBMCs might be useful for patients without any previous therapeutic and/or resistance information, revealing with good reliability polymorphisms potentially associated with resistance. Further investigation, preferably through ultra-sensitive technology, are needed to clarify the clinical impact of INI-resistance present in PBMCs.

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Prevalence of Transmissible HIV Resistance among PLHIV in Kazakhstan

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Background: The formation of HIV drug resistance to first-line antiretroviral drugs is a serious problem in achieving the effectiveness of antiretroviral treatment. HIV drug resistance to NRTIs and NNRTIs is detected every year in more than 50% of cases among PLHIV with virological failure of therapy. More often, the virus is resistant to NVP, EFV, 3TC, FTC (up to 90% among all cases of HIV resistance). The purpose of this study is to describe circulating viral subtypes and to assess the prevalence of primary HIV drug resistance among ART-naive patients diagnosed in the period from 2013-2017 years.

Materials and methods: 494 blood samples from HIV-positive patients from 10 regions of Kazakhstan were examined. RNA extraction and RT-PCR of the protease gene (1-99 codon) and parts of the reverse transcriptase gene (30-265 codon) were performed using the "AmpliSens-HIV-resist-seq" diagnostic kit (Russia). Sequencing of pro/rev genes was performed on the genetic analyzer AB3130 (Applied Biosystems). To obtain a consensus sequence and interpret the results of HIV drug resistance, Deona software (Russia) was used. The HIV-1 subtype was determined using the Comet HIV-1 program (http://comet.retrovirology.lu/) and phylogenetic analysis. Mutations were determined by using Surveillance Drug Resistance Mutation list (SDRM). The level of transmissible HIV drug resistance was determined using CPR (Calibrated Population Resistance) software (http://cpr.stanford.edu/cpr.cgi).

Results: Most of patients in the study group were male (53%); the median age of the individuals was 34.8 years (range, 30 - 37.8 years); the median number of CD4 cells - 476.2; by risk groups: people who inject drugs – 33.1 %; heterosexually infected – 63.1 %, MSM – 2.2%, and 4.4 % cases are unknown. Of the 494 patients included, 46.2% harbored the subtype A6, 49.0% - subtype AG (including Central Asian variant of HIV-1 CRF02_AG and recombinant forms between CRF02_AG and A6), 2.5% - subtype B, and other (CRF_03AB, CRF07_BC, CRF55_01B) – 2.3%. In each region, at least one case of infection with HIV strains was identified, in the genome of which there were mutations associated with drug resistance. In total, 18 people infected with drug-resistant strains of HIV-1 were identified, of which 5 patients (0.9%) had HIV drug resistance mutations to NRTIs (M41L - 2, L210W - 1, K219R- 2), 6 patients (1.4%) had mutations to NNRTIs (K103N+Y181C – 1; K103N - 3, K101E - 2), and 7 people (1.6%) had mutations to PIs (M46I - 6 and M46I+L90M - 1). Thus, the average level of transmissible HIV-1 resistance in the Republic of Kazakhstan was 4.1% (range, 1.9 % - 10%). The highest level of transmission of HIV was registered in the North Kazakhstan and Pavlodar regions (10% and 7.3%, respectively).

Conclusion: HIV-1 subtype A6 and recombinant form AG dominate in Kazakhstan. The transmission rate of HIV-1 drug resistant in Kazakhstan is low; however, its prevalence has increased in last years in the country. Due to the increase in treatment coverage, it is important to introduce National HIV drug resistance monitoring programs.

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Improved viral control outcome in response to optimized salvage therapy based on integrase inhibitors in Mexican multi-treated HIV-1 positive patients

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Background: Multi-experienced patients are considered more challenging to treat, especially in places where potent drugs as well as drug resistance testing are hardly available. In this scenario, an optimized regimen for patients failing raltegravir (RAL) is often the best and only approach considering the evidence from clinical trials. Despite this, there is limited data regarding the outcome of this approach in real life cases. The aim of the study was to measure the virologic outcome in multi-treated patients failing raltegravir following optimized salvage therapy compared to patients non-failing to integrase inhibitors.

Material & Methods: We conducted a retrospective case-control analysis of multi-treated HIV-1 positive patients failing integrase inhibitors under salvage therapy with an optimized regimen. The control group weas randomly selected from multi-treated patients failing a regimen without integrase inhibitors experience that were paired by the number of previous regimens and by the date of lost of viral control. Susceptibility was analyzed with the Stanford HIVdb Genotypic Resistance Interpretation Algorithm version 8.8. Viral control was defined as having a viral load <40 copies/mL after 6-months under salvage therapy. Time-to-event analysis, Cox regression hazard analysis model and correlation statistics were performed with SPSS. A $p<0.05$ was considered statistically significant.

Results: 22 subjects were included in total (11 in each group). Nine (82%) subjects of the RAL-failing group achieved viral control at 6 months compared to four (36.4%) in the control group. From the case group only 7 subjects were sequenced with an integrase inhibitor, whilst only 4 isolates harbored integrase mutations: The most frequent was N155H. We found a higher probability of viral control in the case group compared to the control group despite having optimized regimens in both groups (Log rank test, p=0.008), with the same number of active drugs. Not having an integrase inhibitor in the salvage regimen was associated with a lower probability of achieving viral control (hazard ratio [HR], 0.049; 95% CI 0.01-0.247; p=<0.05), whereas the viral load level was not related to the outcome. Only in the case group the probability of patients achieving viral control was higher for subjects undergoing antiretroviral combinations based on integrase inhibitors compared to protease inhibitor (p=0.009) and also for the use of DTG compared to the other regimens (p=0.06).

Conclusions: We documented higher levels of viral control in our multi-treated subjects in response to salvage therapy including an integrase inhibitor compared to regimens without integrase inhibitors. Moreover, the use of dolutegravir in these real-life cases was associated with higher levels of response. However, we emphasize the importance of assembling a salvage regimen based on the virtual susceptibility of the drugs, always taking into account the clinical context of each patient. We believe that the use of integrase inhibitors can offer an improved probability of viral outcome in multi-treated patients, despite the previous use and failure to RAL . Close surveillance of cases requires to be implemented in order to observe mid and long-term viral control.

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Frequency of mutations of genotypic resistance to antiretrovirals in patients living with HIV/AIDS in the period of 2013 to 2015, in state of Pará, Brazil

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Introduction: The Human immunodeficiency Virus is able to depress the host immune system by eliminating important defence cells like macrophages and lymphocytes resulting in an Acquired immunodeficiency syndrome (AIDS). The Antiretroviral therapy (ART) is the most commonly used method to contain viral replication. In addition, treatment adherence has particular relevance to avoid the emergence of virus resistance. Despite advances against AIDS, there is still a great HIV resistance to the drugs implemented for disease control which leads to therapeutic failure. This study aimed to characterize the epidemiological profile of HIV /AIDS patients residing in Pará, regarding their resistance mutations profile, the drugs used in the failed treatment, also identify their HIV-1 subtype most prevalent in Pará, Brazil. All obtained by genotyping test.

Materials and Methods: This study is cross-sectional, descriptive retrospective, that included patients living with HIV-1, residing in Pará. The epidemiological and clinical data were obtained through information collection in medical records, in which samples were submitted to genotyping test between 2013 to 2015 in Central Laboratory of Pará (LACEN-PA). All information acquired was edited, tabulated, quantified and presented in an excel spreadsheet.

Results: The male gender was predominant with 53%. The group aged from 36 to 45 years presented the highest rates, being the majority considered brown with elementary school as prevailing education attainment. Subtype B was the most prevalent in this study, with the M184V mutation as the most prevalent in the class NRTI with the highest mutations, followed by Protease inhibitors with mutation 41K as the most frequent and ITRNN in third place with mutation 103N as most prevalent. Among the drugs used in the therapy, those with NRTI class showed the highest resistance profile, with 3TC/FTC associated with most of treatment failure, being NNRTI class the second highest frequency of resistance with NVP, and Inhibitors of PR the class with highest susceptibility profile. Thus, mutations may or not generate resistance to drugs, factors such as drug class, as well the mutations profile will define, or not, the resistances appearance.

Conclusion: In summary, it's important to do pharmacological and genotyping monitoring of each patients submitted to ART to observe mutations appearance and the correlation with their drugs resistance profiles.

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First Reported Case of Transmitted Integrase Inhibitor Resistance discovered after Rapid Initiation of HAART, and a survey of Similar Resistance (G140, Q148) in an Urban Ambulatory Clinic in NY

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Background: Primary integrase inhibitor (INI) resistance transmission is rare. INI are recommended as first-line therapy for HIV infection on both the DHHS and IAS guidelines. INI resistance testing has not been routinely performed in ART-naïve HIV-1 infected individuals.

The CDC has reported that among newly diagnosed individuals in Florida, INI Resistance increased significantly from 2015 to 2016, with specific significant increases for raltegravir and elvitegravir.

This report describes an antiretroviral-naïve individual discovered in a Rapid Initiation of Highly Active Antiretroviral Therapy (HAART). Baseline resistance testing later revealed a G140 and Q148 mutations. A retrospective survey of resistance in an urban ambulatory clinic in The Bronx was performed to survey for the same resistance combination. The Stanford University drug resistance database reports the G140S/A/C usually occur with Q148 mutations. Alone, they have minimal effects on INSTI susceptibility. However, in combination G140 and Q148 mutations are associated with high-level resistance to RAL and EVG and intermediate reductions in DTG and BIC susceptibility

Methods: The Rapid Initiation of HAART and discovery of Primary INI resistance was at a community health center in NY, NY. Subsequently, a retrospective resistance surveillance was performed at a Bronx, NY clinic for similar cases of G140 & Q148 INI substitutions. The data mining effort identified ten individuals. Medical providers then performed retrospective review of clinical history and resistance testing through chart abstraction.

Results: A 38 year-old man tested positive for HIV on 10/11/2018. Baseline viral load (VL) was 40,140 copies/ml and CD4 count was 541. Genotypic resistance testing was sent and he was rapidly initiated on dolutegravir and tenofovir alafenamide and emtricitabine (TAF/FTC). Pre-HAART resistance testing later revealed INI mutations G140S and Q148H, the reverse transcriptase mutation K70R, the non-nucleoside reverse-transcriptase inhibitor mutation V90I, and protease mutations I62V, and I13V On 10/19/2018 hIs regimen was switched to darunavir 800 mg/cobicistat 150 mg and TAF/FTC. His VL on 10/26/2018 was 280 copies/mL. He subsequently suppressed to less than 20 copies/mL.

Our retrospective survey of resistance in a Bronx clinic revealed ten similar G140 and Q148 combination mutations. Five (50%) of these patients did not achieve durable viral suppression and continued to have high levels of viremia with concern for transmitting INI resistance.

Conclusion: While primary transmitted INI resistance has been thought to be rare, the Florida CDC report of 2015 to 2016 reports that it is increasing. We believe that Rapid Initiation of HAART needs to be followed by Rapid Follow Up. Here we report that Fifty percent of patients who engendered G140 and Q148 INI resistance mutations had viral loads at levels known to be transmissible.

The NYS Dept. of Health, AIDS Institute reported at CROI 2019 that while INI drug resistance is low, the level of clinically significant INI resistance mutations observed suggested that transmitted INI resistance is emerging. Our data is consistent with this suggestion.

As INIs are recommended first line therapy, baseline INI resistance testing should be considered in high-risk demographic settings and Rapid Initiation of HAART should be followed by Rapid Clinical Follow Up.

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HIV-1 genetic diversity assessment among treatment- naïve patients in Poland in 2012 - 2018

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Background: Widespread access to highly effective combination antiretroviral therapy (cART), despite wide range of the benefits, entails the risk of increasing emergence of HIV-1 drug resistance mutations related to the treatment failure. Transmitted drug resistance mutations (TDRM) are still severe problem because, when occur, can spread in the population intensively limiting the available therapeutic options. Long term surveillance of TDRM clinical and epidemiological aspects are crucial in planning strategy of effective initial therapy regimens to avoid spread of TDR. Public health interventions assuming reducing the spread of HIV-1 epidemy requires up-to-date knowledge about trends in TDRM prevalence over time. The aim of the study was to analyse the distribution of: HIV-1 subtypes, the prevalence of TDRM and evaluate the potential phylogenetic relationship of TDRM and its impact on the baseline susceptibility in newly diagnosed patients in Central Poland between 2012 and 2018.

Materials and Methods: One thousand seven hundred and seventy four plasma samples obtained from antiretroviral treatment naïve HIV-1 positive patients, newly diagnosed in our Hospital during the period 2012-2018, were analysed. In the studied cohort dominated men –1648/1774(92,9%). Viral RNA isolation, PR-RT coding region amplification and sequencing were performed using ViroSeq HIV-1 Genotyping_Kit (Celera) and 3130-Avant Genetic Analyzer (Life_Technologies). Surveillance drug resistance mutations (SDRMs) were examined according to the Stanford Genotypic Resistance calibrated population resistance (CPR) tool version 6.0 based on the WHO surveillance transmitted drug resistance mutation list of 2009. For HIV-1 subtype determination REGA HIV-1&2 Subtyping Tool was applied. Phylogenetic analysis was based on maximum likelihood method with aLTR.

Results: The percentage of subtype B infections is decreasing in studied period from 84,7% in 2012 to 65,22% in 2018. Opposite trend in prevalence of subtype A (with dominating A-1FSU) was determined: increase from 6,55% in 2012 to 24,4% in 2018. Prevalence of TDRM fluctuated in the years 2012-2018: 10,16% in 2012, decreased to 3,5% in 2015 and finally reached 5,5% in 2018. According to drug classes, mean resistance prevalence was: NRTI–4,07%, PI–1,4% and NNRTI–1,14%. The most frequent mutations were: NRTI–T215* (56,18%), NNRTI–K103N (47,83%) and PI–L90M (40%). Single cases of infection with variants resistant to two (NRTI and NNRTI) and three (NRTI, NNRTI, PI) drug classes were detected. Phylogenetic analysis revealed transmission clusters in the analysed populations.

Conclusions: Presented data shows dynamic, epidemiologically interesting and clinically important, growth in prevalence of infections with non-B subtypes in particular with predominance of A-1FSU strains. Intense economical migration, mainly from Former Soviet Union Republics, enriches genetic heterogeneity of HIV-1 variants in Poland. Phylogenetic analysis revealed multiple, independent introductions of HIV-1 A-1FSU, followed by local transmissions. Detected prevalence of TDRM among Polish naïve patients stabilized on level 5,50% in 2018. Determined prevalence is low when compared to this observed during the first studies performed in the years 2000-2007, when TDR reached 20-15%. These data probably are a result good cooperation between clinics and Laboratory as well as documents high adherence of the patients and high antiviral potency of the new drugs.

*This work was in part sponsored by Foundation_for_Research_Development in Hospital_for_Infectious_Diseases,_Warsaw

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Prevalence of transmitted HIV-1 drug resistance in Tel-Aviv, Israel, and antiretroviral strategy in a potential transmitter population from 2010-2018

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Background: Until recently the rate of transmitted drug-resistance mutations (TDR) was relatively high mainly to NNRTIs. The prevalence of TDRs is regularly evaluated in treatment-naïve patients in Tel-Aviv. This study evaluated the rate and pattern of TDR among HIV-1 treatment-naïve patients in Tel Aviv from 2010 to 2018.

Material and methods: We analyzed TDR mutations prevalence in blood samples of treatment-naïve patients in Tel-Aviv (2010-2018). Integrase region was sequenced in samples from 2016. Transmission dynamics were analyzed by reconstructing viral phylogenies from pol sequences of HIV-1 subtype A, B and C viruses the most common subtypes in Tel Aviv.

Results: 862 Viral sequences revealed 12.9% TDR. Over three-fourths (76%) of men who have sex with men (MSM) were born in Israel, and 81.6% harbored subtype B viruses. Other groups include intravenous drug users (IVU), 78% of whom were born in the former Soviet Union countries and 88% of whom harbored subtypes A viruses. The heterosexual group was very heterogeneous in origin, including patients born in Israel, Ethiopian immigrants, immigrants from the former Soviet Union, and worker immigrants mainly from Africa. NNRTIs TDR was the major class resistance (40%) followed by PIs (30%), NRTIs (23%) and 7% more than one class. Integrase inhibitors resistance mutations were represented only by minor mutations: L74m (n=1), T97A (n=4), E138K (n=1), E157Q (n=4) and G163K (n=1) .
Phylogenetic analysis of subtype A and B viruses supported clustered transmission of TDR among men who have sex with men. These clusters were represented by the mutation K103N in RT and L90M in PR. No major integrase inhibitors TDR were found in 2016-2018.

Conclusion: TDRs among patients followed in Tel-Aviv were represented by clusters in MSM. These clusters contained resistance associated mutations to drugs less frequently prescribed in recent years, so their effect on treatment strategy is not straightforward.
Characterization of evolving clusters and transmission networks is useful to concentrate prevention and control efforts where they are most needed and to assess the impact of these interventions.

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Extensive Pre-treatment Drug Resistance Compromising Current First-line Regimens among HIV-infected Immigrants from LMIC Presenting in the Netherlands

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Background: In Europe pre-treatment drug resistance has been stable around 10% since 2002, as shown by the SPREAD surveillance program. Most patients with resistance at baseline are MSM infected by subtype B virus with a single RT mutation, which has limited impact on the susceptibility to currently used regimens. With increasing use of integrase inhibitors as first-line cART, the role of baseline resistance testing has been under debate. However, recently we observed unexpected cases of extensive resistance in patients from low and middle income countries (LMIC) newly presenting in care in the Netherlands.

Methods: We identified 11 HIV-infected patients originating from LMIC and presenting in 4 HIV care centers in the Netherlands (Groningen, Tilburg, Zwolle and Utrecht) in the past 5 years with unexpected extensive pre-treatment resistance profiles. Clinical data were obtained from the ATHENA cohort. Sanger sequencing was performed and results interpreted with Stanford HIVdb v8.6.1.

Results: The majority was male (n=8) and diagnosed with HIV-1 at a mean age of 34 years (range 20-49). Seven patients originated from Sub-Saharan Africa and reported to be infected via heterosexual contact. Four patients were MSM and originated from the Caribbean (n=3) and Latin America (n=1). They presented in the Netherlands in 2012 (n=1), 2014 (n=1), 2016 (n=3) and 2018 (n=6). At presentation, seven patients had a CD4 count below 350 cells/mm3 (median:212, range:27-489). They were infected with subtype A (n=2), B (n=2), C, D, G and various CRFs (n=4). Genotypic testing revealed a median of 7 mutations in RT, most frequently M184V (n=7), T215Y/F (n=5), and Y181C (n=6). This resulted in predicted high-level or intermediate resistance to all NRTIs available (n=5), high-level resistance to emtricitabine and lamivudine (n=2), or susceptibility to zidovudine only (n=1). In ten patients high-level or intermediate resistance to all available NNRTIs (n=9) or nevirapine and efavirenz only (n=1) was observed. In one patient the major protease mutation 54V was detected predicting low-level resistance to atazanavir and lopinavir. The majority did not report a prior treatment history before presentation in the Netherlands. Based on resistance testing, most patients were switched to a 3-class regimen including a protease inhibitor, integrase inhibitor and either an optimized NRTI backbone or maraviroc.

Conclusion: We have observed an increasing number of patients from LMIC who present in the Netherlands with extensive pre-treatment drug resistance, compromising the efficacy of the NRTI backbone used as part of the current recommended first-line regimens in our setting. Physicians should be aware that with the roll-out of cART in LMIC, patients originating from these settings are at risk of extensive pre-treatment drug resistance due to either (undisclosed) prior treatment in their country of origin or transmitted resistance. Baseline resistance testing should be highly recommended in these patients.

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Virological suppression and delay in clinical management in response to viral rebound in South African treatment programme: A multicentre cohort study.

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Introduction: Uptake of antiretroviral therapy (ART) is expanding rapidly in low-income and middle-income countries. Monitoring of virological suppression is recommended at six months of treatment and annually thereafter. In case of confirmed viral rebound a switch to second-line ART is indicated. We report the first multicentre assessment of suppression over time and clinical response to viral rebound under programmatic conditions.

Methods: 104719 patients on first-line ART at 52 South African centres were studied. Virological suppression, switch to second-line ART, death, and loss to follow-up were analysed. Multistate models and Cox proportional hazard models were used to assess suppression over time and predictors of treatment outcomes.

Findings: In on-treatment analysis, suppression below 1000 copies/mL was 89·0% at month 12 and 90·4% at month 72. Suppression below 50 copies/mL was 73·1% at month 12 and 77·5% at month 72. Intention-to-treat suppression was 75·0% and 64·3% below 1000 and 50 copies/mL at month 72 respectively. Viral rebound occurred in 19·8% (20766/104719) of patients during an average follow-up of 152 [61-265] weeks. Male, young, and late presenting patients were at highest risk. After rebound, confirmatory testing took 29 weeks [IQR: 16-54]. Viral resuppression without switch of ART occurred frequently (45·6%; 6030/13210) but was associated with renewed viral rebound and switch. Of patients with confirmed failure who remained in care, only 41·5% (1872/4510) were switched. The median time to switch was 68 weeks [35-127], resulting in 12325 person-years spent with a VL above 1000 copies/mL.

Interpretation: 90% virological suppression was achieved using the threshold of 1000 copies/mL in on-treatment analysis. However, this target was not met at the 50 copies/ml threshold, nor in intention-to-treat analysis. Clinical management in response to rebound was profoundly delayed prolonging the duration of viraemia and potential for transmission. Diagnostic tools to establish the cause of rebound are urgently needed to accelerate clinical decision-making.

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Evidence of HIV-1 genital compartmentalization before and after antiretroviral therapy initiation in females recently diagnosed in Bamako, Mali

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Background: To achieve the 90-90-90 targets assigned by UNAIDS, it is crucial to monitor the antiretroviral therapy (ART) of HIV-1 infected patients, especially in resource-limited countries (RLCs). In addition, little is known about the dynamic of HIV-1 shedding and resistance profiles in the genital reservoir after ART initiation in RLCs, which is critical for evaluating the residual risk of HIV-1 transmission by sexual route in ART-experienced people.

Objectives: To evaluate the immune and viral responses in blood and genital secretions after 12 months of ART in newly HIV-1 diagnosed females in Bamako, Mali; to determine primary and acquired resistance rates to antiretroviral drugs.

Patients and methods. Seventy-four consenting females were enrolled between January and June 2016 at the time of their HIV-1 infection diagnosis. HIV-1 RNA loads (Abbott RealTime HIV-1 assay) were tested in blood and cervicovaginal fluids (CVF) before and at month 3, 6 and 12 after initiation of ART. Primary and acquired resistances to ART were evaluated by Viroseq™ HIV-1 genotyping assay. The vaginal microbiota was analysed by using the IonTorrent™ NGS technology (Thermo Fisher Scientific).

Results: During the study, 9.5% of people deceased and 31.5% were lost to follow-up. Rates of primary drug resistance mutations in blood and CVF were 13.3% and 25%, respectively. Among blood/CVF paired samples tested by genotyping assay and exhibiting resistance mutations, discrepant profiles were observed in 75% of cases. The acquired resistance rate was estimated at 3.1% in blood samples. In the 44 patients tested at month 12 after ART start, undetectable HIV-1 RNA was reached in 84.1% and 77.3% of blood and CVF, respectively. In seven females (15.9%), HIV RNA was detected in CVF but not in the corresponding blood sample. A vaginal dysbiosis was associated with HIV RNA shedding.

Conclusions: Our study evidenced a huge proportion of non-adherent people to ART program but a reassuring high percentage of virological success as well as a low level of acquired mutations in adherent patients after one year of therapy. These findings emphasize the need of reinforcing education to improve retention in care system. We also observed a worrying high primary resistance level to ARV drugs underlying the necessity of regular virological monitoring to optimize the use of therapeutical options. Lastly, the high percentage of persistent HIV-1 RNA in female genital tract despite ART pleads in favor of a systematic screening and treatment of STI in order to decrease the risk of HIV-1 transmission.

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The changing landscape of HIV infection among women in Israel 2010-2018

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Background: According to the Israeli ministry of health (MOH), women comprise 37% of the reported HIV-1 positive individuals. However, in depth analysis of the characteristics of this population has not been reported. Here we studied women diagnosed with HIV-1 in 2010-2018.

Materials & Methods: The data-base of the National HIV reference center, which covers all HIV-1 reported cases in the country, was screened for records of all newly diagnosed HIV patients identified between January 2010 and December 2018. Men, women <16 years and women diagnosed in years other than 2010-2018 were excluded. Women who are foreign citizens were also excluded due to lack of reliable data. The final cohort included 727 women. Sequencing of early samples collected from treatment naïve carriers was performed using 41.4% (301) of the samples selected by a stratified random selection design. Demographic (age, country of origin and risk factor for HIV acquisition)) and viral (time of HIV diagnosis, HIV-1 subtype and TDRM including RT-A138) characteristics were recorded and examined by Chi-Square test. Logistic regression was used to assess the associations between these variables. Yearly trends of TDRM rates were examined by segmented Poisson regression.

Results: Median age was 38 at diagnosis. 17.1% (124) were older than 50. The prevalence of women from Africa (OGE-IL, n=227) decreased while that of women from former Soviet Union (FSU, n=307) increased significantly (p<0.001) over the years. Statistically significant yearly decrease in the rate of subtype C (Africa origin) was also observed (p=0.004). 23.6% (71 of 301 patients) had TDRM; 3.8%, 9.6% and 16.2% had PI, NRTI and NNRTI TDRM, respectively. Significant yearly increase in TDRM rate (1.6% per year, mainly NNRTI) was observed (p<0.001). The NRTI A62 (6.0%), NNRTI E138 and K103 (5.3% and 4.3%, respectively) were the most prominent mutations. Age of diagnosis (>50) was associated with a higher TDRM rate (p=0.04; 95% CI of OR 2.00).

Conclusions: The epidemiology of HIV infected women in Israel is changing, shifting to women from FSU infected through heterosexual contact with persistent escalation in the rate of TDRM. Delayed age of diagnosis contributes to the higher TDRM rate. These results re-enforce the national policy of resistance testing at baseline and call to apply appropriate preventive measures to women at risk.

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A touchdown PCR assay of HIV-1 pol for a comprehensive genotypic drug resistance analysis of patients infected with group M HIV-1 strains

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Background: PCR assays for the genotypic drug resistance analysis of all antiretroviral agents (reverse transcriptase, protease and integrase inhibitors) are increasingly in demand. This study was focused on the development of an assay for the PCR amplification of the entire HIV-1 pol region of major circulating group M HIV-1 strains in Europe. Nucleic acid amplification was followed by DNA sequencing, to enable the potential discovery of mutations associated with drug-resistance. Furthermore, phylogenetic analysis was performed on the sequenced samples, for the identification of active transmission clusters to facilitate a potential prompt public health response to those clusters.

Materials and Methods: The touchdown RT-PCR protocol used in this study was developed to process viral RNA, extracted from the plasma of blood samples of consenting HIV-1-infected patients in Cyprus (2017-2018). For the amplification of the pol region, touchdown PCR was utilized for both RT-PCR and the nested PCR that followed, with primers designed to have a broad coverage of major group M HIV-1 subtypes and recombinant strains. The temperature of the annealing phase in each PCR, was selected to be about 10°C higher than the calculated optimal primer melting temperature, with a ΔT=-1 °C in each subsequent cycle until the optimal temperature is reached followed by a final amplification step at the optimal temperature. Successful PCR amplicons were then sequenced by the Sanger method, followed by the inference of genotypic drug resistance through the Stanford drug resistance tool. Phylogenetic analyses were then performed through MEGAX and maximum likelihood trees were generated. Transmission clusters were then identified through Cluster Picker.

Results: A PCR assay that successfully amplifies the entire HIV-1 pol region (3,258 nucleotides long) of major group M HIV-1 subtypes and recombinant strains was developed and evaluated by group M strains isolated from HIV-1-infected patients in Cyprus. Crucial to the design of the assay was the higher than optimal starting temperature of the initial amplification of touchdown PCR, which conferred increased specificity by ensuring that only perfectly matched primers bound to the templates. Since the assay was created to be indiscriminate of subtype, it had to accommodate variability, which was achieved by designing primers that cover a broad range of HIV-1 subtypes. Through this assay, a number of subtypes were identified such as A1, A2 and B, but also common circulating recombinant forms such as CRF02_AG and CRF04_CPX. Furthermore, through phylogenetic analysis, the identification of transmission clusters growing in near real-time was achieved, and another cluster consisting of an uncommon recombinant (Rec_B, A1, G) was also revealed.

Conclusion: The developed touchdown PCR assay reliably amplifies the entire pol region of a broad array of group M HIV-1 subtypes and recombinant strains; and the drug resistance analysis of protease, reverse transcriptase and integrase inhibitors is achieved as a result of a singular PCR assay and sequencing. Furthermore, the follow up phylogenetic analysis facilitates the identification of transmission clusters among the sequenced data, which in turn enables the option for a prompt public health intervention.

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Impact of point-of-care pharmacist counseling at late refills of antiretroviral therapy: A Study Following the Early Warning Indicators of World Health Organization Recommendations

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Background: With increasing global use of antiretroviral therapy (ART), World Health Organization (WHO) has developed HIV drug resistance (HIVDR) Early Warning Indicators (EWIs) to optimize prevention of HIVDR. Recent studies have reported on time pharmacy refills, the fourth EWI, to be the strongest predictor of clinic-level viral load suppression. The primary objective of this study was to assess the impact of pharmacist counseling at the point of late ART refill. We also sought to determine the percentage of patients who picked up prescribed antiretroviral drugs on time as described by WHO, common reasons and predictors for late refills.

Method: A cross-sectional study was conducted among 751 Malaysian HIV-infected individuals receiving ART from November 2017 until February 2018. Patients with late refills were actively absorbed for a comprehensive counseling session. Follow-up pharmacy refills after the counselling was then evaluated using medication possession ratio (MPR) for a duration of 6 months. MPR of more than 90% was categorized as optimal refill adherence according to published conventions. Paired T-test was used to test the effectiveness of counselling at late refills whilst multivariate regression models were used to examine predictors of late refills.

Results: Of 751 HIV-infected patients, 91% had on time refills. Patients with late refills (n=65) were predominantly male (85%), of Malay ethnicity (45%) and age 35 years old and above (65%). Mean duration on ART was 4 years. Being outstation accounted for the highest reasons for late refills (32%) followed by 23% due to work commitments. Identifying patients with late refills and providing concurrent counseling increases MPR or refill adherence significantly in patients who had previously poor MPR scores (MD=14.76; SD= 18.04; p=0.001). Multivariate binary logistic regression analysis found history of self-reported non-adherence (AOR= 4.506; 95% CI [1.822-11.143]; P=0.001) and travelling more than 20km to the hospital (AOR= 4.749; 95% CI [1.966-11.474]; P=0.001) were significant predictors of late refills.

Conclusion: Although the proportion of patients with on time pill pick up was desirable, our study further suggests integration of identification and counseling for patients with late refills as it significantly increases pharmacy refill adherence. This targeted intervention could serve as an early proxy of retention in care especially in resource-limited settings.

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INSTIs DO NOT PREVENT FOLATE BINDING TO FOLATE RECEPTOR ALPHA

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Background: A rare safety finding of neural tube defect (NTD) was reported among babies born to women living with HIV in Botswana, who were exposed to the antiretroviral dolutegravir (DTG) at the time of conception (Zash et al; NEJM 2018). As folate deficiency has been linked to NTD, we investigated the potential inhibition of folate binding to folate receptor by HIV integrase strand transfer inhibitors (INSTIs), including DTG, as a possible mechanism for folate deficiency and the formation of NTDs.

Methods: KB cells were chosen for their selective expression of folate receptor alpha (FOLR1) and for their lack of expression of folate receptor beta (FOLR2), reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT), as determined by FACS analysis. The in vitro folate receptor binding assays were performed using 3H-folic acid in folate free-media, in the presence and absence of INSTIs at supra-therapeutic concentration. The INSTIs tested were bictegravir (BIC), cabotegravir (CAB), dolutegravir (DTG), elvitegravir (EVG), and raltegravir (RAL). Following incubation with drugs, cells were washed with PBS and lysed with 1% SDS. Lysates were transferred to scintillation vials; levels of 3H- folic acid were measured. After Kd and Bmax determination for 3H-folic acid binding, IC50 and Ki values were determined for all INSTIs. The anti-folate methotrexate was used as positive control.

Results: Bmax and Kd values were established by titration with 3H-folic acid. A Kd value of 5.0 nM was determined, with a Bmax corresponding to 1.4 x 10^7 receptors/cell. Folic acid and methotrexate yielded Ki values of 2.9 nM, and 4.0 µM, respectively, all values being consistent with previously published data in the literature. For all INSTIs (BIC, CAB, DTG, EVG, and RAL), the maximum concentration used (10 µM) was supra-therapeutic. None of the five INSTIs showed inhibition of 3H-folic acid binding to FOLR1; the Ki values for five INSTIs were >3.3µM.

Conclusions: In this in vitro study, none of the INSTIs evaluated showed inhibition of folate binding at supra-therapeutic concentrations. This is in agreement with the recent pharmacovigilance review, showing no evidence of increased risk of NTDs with the use of EVG- or BIC-containing products during pregnancy (Farrow et al; HIV Drug Therapy 2018, Glasgow UK), and in agreement with the lack of NTD among prospective cases for 3 INSTI evaluated (EVG, DTG, RAL) (Albano et al; CROI 2019, Seattle WA, USA). These data are highly relevant to inform reproductive age women living with HIV and their treating physicians.

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Transdisciplinary Systems Map of Causes Leading to HIV Drug Resistance

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We hypothesized that HIV drug resistance (HIVDR) is a wicked problem with several causes which find roots in different fields of science. Molecular medicine, unraveling the molecular basis of HIVDR, pharmacy and the development of antiretroviral therapy (ART) with a higher genetic barrier and anthropology and psychology aiming to improve adherence and reduce stigmatization are all crucial fields of science in the quest to prevent HIVDR, next to several other disciplines. To our knowledge no transdisciplinary overview of all causes leading to HIVDR has been made before. A well-documented approach to clarify complex causes of wicked problems is the development of systems maps. Such maps can be used to identify key causes to the problem that can and should be addressed, but also to develop guidelines specifically for certain populations. We therefore aimed to develop the first transdisciplinary systems map of causes leading to HIVDR.

The systems map was gradually developed by literature study combined with a series of interviews with experts from different disciplines (epidemiology, pharmacy, psychology, public health, medicine, bioinformatics, virology, anthropology, visual methodology) all working in the field of HIVDR in Sub Saharan Africa. The interviews were transcribed and factors leading to HIVDR and their links with each other were extracted and entered into a systems map with the KUMU software. The map was adapted throughout a series of discussions with experts from several disciplines.

73 drivers of HIVDR and 130 connections between those elements were identified and visualized in one systems map. To structure the map, the elements were divided in four categories according to their relation to: 1) access to treatment, 2) adherence, 3) healthcare system 4) biology and pharmaceutics. When organizing the elements according to discipline, we found that disciplines are strongly interconnected and disciplinary boundaries are vague with regards to causes leading to HIVDR. For example, adherence is not only influenced by psychological factors but also pharmaceutical aspects (pill design), comorbidities, religion, gender equality, culture, education and the financial status of the person living with HIV (PLHIV).

We conclude that HIVDR is indeed a wicked problem with causes related to different disciplines which are interconnected with each other. Rather than through a multidisciplinary approach, HIVDR should thus be approached from a transdisciplinary point of view. The map will be further optimized with insights from people living with HIV and experts from several other disciplines and can be used as a tool for researchers to orient their research question and discover new connections between their results and other elements which might at first seem unrelated.

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Late presenters among persons with a new HIV diagnosis in Kyiv, Ukraine

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Background: In Ukraine, among 2892 new diagnosed cases of HIV-infection reported in January and February, 2019, 1498 (48%) were persons diagnosed having AIDS-defining condition. In general, late presentation is an important issue for healthcare and is associated with increased HIV-related morbidity and mortality, shorter survival, poor response to treatment, increased healthcare costs and increased rates of HIV transmission. The aim of the study was to analyze the characteristics of patients who were diagnosed late among newly diagnosed HIV-positive persons in Kyiv, Ukraine, in January-February, 2019.

Materials & Methods: We analysed data from records of newly diagnosed HIV-positive individuals who presented with with CD4 ≤ 200 cells/μL or AIDS (regardless of the CD4 cell count) defined as patients with advanced HIV disease (AHD) in Kyiv City HIV Centre in January-February, 2019. Descriptive analysis was performed to assess the prevalence and charasteristics of late presenters.

Results: The study included 139 patients (53 women - 38.1%, and 86 men - 61.9%) diagnosed with HIV infection at the time of AHD. The median age was 43,2 (IQR 24-78). 38 patients (27.3%) acqired HIV by injection drug use, 12 (8.6%) by homosexual and 89 (64.0%) by heterosexual contact. The most common AIDS defining conditions included: Pneumocystis pneumonia (in 60 patients - 43.2%), tuberculosis (in 79 - 56.8%), CNS toxoplasmosis (in 41 - 29.5%), oesophageal candidiasis (20 - 14.4%), chronic herpes simplex infection (in 58 - 41.7%), cytomegalovirus retinitis or meningoencephalitis (26 - 18.7%) and progressive multifocal leukoencephalopathy (4 - 2.9%). The median CD4 count was 45.4 (IQR 1-196) cells/μL. It was noted that 101 (72.7%) sought medical care during last 5 years, 88 (63.3%) were not offered an HIV test and 13 (9.4%) did not agree to do it before their condition became critical. 8 patients (5.7%) died within 2 months after being diagnosed with HIV. 128 patients (92.1%) started ART.

Conclusion: The results shows the rates of AIDS-defining conditions reported in newly diagnosed HIV-infected individuals in Kyiv in January-February, 2019. Pneumocystis pneumonia, tuberculosis, CNS toxoplasmosis, oesophageal candidiasis and cytomegalovirus retinitis or meningoencephalitis are reported among the most common opportunistic infections. The study also highlights the need of intensification of HIV testing strategy, showing that 63.3% of patients were not offered HIV test while seeking medical care within 5 years before being diagnosed with advanced HIV disease.

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INSTI-based triple regimen in treatment-naïve HIV-infected patients is associated with HIV-RNA viral load suppression at ultrasensitive levels

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Background: During antiretroviral therapy (ART), HIV-1-infected patients may present with ultrasensitive (US) HIV-RNA viral loads (VL) below quantification levels of current assays. Reasons for US-VL detection and its relation to virological rebound (VR) are unclear. The purpose of this study was to determine the virological, immunological and therapeutic correlates of US -VL detection in a large group of ART-naïve patients from outpatient clinics initiating more recent ART combinations. Furthermore, we aimed to use long-term repeated sampling of US-VLs during treatment in order to more concretely establish the effect of persistent residual viremia on VR.

Methods: HIV-1-infected, ART-naïve patients followed at two university hospitals were included. HIV-RNA had to be >200 copies/mL at ART-initiation and VL<50 copies/mL achieved during ART. US-VL was defined as an undetectable PCR signal from a commercially-available assay (COBAS® TaqMan, Roche). Random-effect Poisson regression was used for assessing determinants of US-VL<1 copy/mL over time and conditional risk-set analysis for VR (one VL>200 copies/mL or two VL>50 copies/mL), while accounting for frequency of VL measurements.

Results: Between 2009-2013, 717 patients initiated ART containing 2 nucleos(-t)ide reverse transcriptase inhibitors (NRTI) plus a non-NRTI (29.4%), protease inhibitor (58.4%) or integrase-strand transfer inhibitor (INSTI) (12.1%). During a median 3.4 years (IQR=2.3-4.6), 676 (94.3%) patients achieved US-VL<1 copy/mL. In multivariable analysis, US-VL<1 copy/mL over time was associated with decreased age (p<0.001), female gender (p=0.04), lower baseline VL (p<0.001), baseline CD4+ >500 versus <350/mm3 (p<0.001), and INSTI-containing ART (p=0.009). 131 (18.3%) patients had VR during follow-up, which was independently associated with CD4/CD8 ratio <0.8 during follow-up (p=0.01) and time spent <1 copy/mL (p<0.001). When US-VL<1 copy/mL occurred ≥50% of follow-up duration (n=290), faster time to US-VL<1 copy/mL (p<0.001), faster CD4+ T-cell count increase (p=0.03), and faster CD4/CD8 ratio increase (p=0.001) were observed.

VR occurred 237 times during follow-up and was defined by two consecutive HIV-RNA VL >50 copies/mL for 102 (43.0%) VRs, one HIV-RNA VL >200 copies/mL for 124 (52.3%) VRs, or both criteria for 11 (4.6%) VRs. In multivariable analysis accounting for conditional risk-sets (Model 1), VR was associated with having a CD4/CD8 <0.8 during follow-up (p=0.01) and higher number of HIV-RNA VL tests/year (p=0.001). When replacing cumulative duration under <1 copy/mL with ART regimen in the final multivariable model (given the collinearity between these two variables), longer periods of US-VL suppression were significantly and inversely associated with VR (Model 2). Patients with VR during follow-up had a significantly longer time until achieving HIV-RNA <1 copy/mL (p<0.001), slower increase in CD4+ T-cell count (p=0.04), and slower increase in CD4/CD8 ratio (p<0.001) than those without VR.

Conclusion: This study demonstrated the effect of ART regimen and immune status not only on residual viremia but also subsequent VR during a median follow-up of more than 3 years. Indeed VL-suppression at ultrasensitive level is associated with INSTI-class ART initiation. Moreover, given the association between residual viremia and immune activation, INSTI class antiretrovirals, a preferred third agent for first-line ART according to current recommendations, could also lead to improvement in immunological parameters and possibly reduced inflammation.

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HIV-1 Resistance Through Week 360 in ART-Experienced, Integrase Inhibitor-Naïve Participants Receiving Dolutegravir (DTG) in the SAILING Study

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Introduction: SAILING was a Phase3 clinical trial evaluating DTG 50mg once daily vs. raltegravir (RAL) 400mg twice daily in ART-experienced, integrase inhibitor (INSTI)-naïve, HIV-1 infected participants. Previous Week(WK) 48 results reported fewer participants meeting protocol defined virologic failure (PDVF) in the DTG arm: 21 PDVFs versus 45 in the RAL arm. 4/354 (1%) failed with integrase(INI)-genotypic or phenotypic resistance on DTG versus 17/361 (5%) on RAL (p=0.003) through WK48. Three additional participants receiving DTG in the open-label phase had emergent INSTI resistance through WK132. Here we present post hoc, longitudinal assessment of PDVF and viral resistance in patients remaining on DTG up to 360 weeks.

Methods: PDVF required confirmed HIV-1 RNA >400 c/mL (Abbott RealTime assay). PDVF non-response was <1 log10 c/mL decrease by WK16, unless <400 c/mL, OR ≥400 c/mL on or after WK24. PDVF rebound was ≥400 c/mL after confirmed <400 c/mL, OR >1 log10 c/mL above nadir of ≥400 c/mL. Data was collected through 08/28/2018 for this instream work. Results were generated from observed data. Population-based genotypic and phenotypic testing was performed on baseline and PDVF timepoint samples by Monogram BioSciences.

Results: Low rate of PDVF was maintained for 295 participants entering the open-label continuation phase and remaining on DTG arm following WK48 through WK360, with 25 additional participants meeting PDVF criteria: 2(0.7%) at WK60, 2(0.7%) at WK70, 8(2.9%) at WK84, 4(1.6%) at WK96, 2(0.9%) at WK108, 2(0.9%) at WK120, 1(0.5%) at WK132, 2(1.2%) at WK156, 1(2%) at WK264 and 1(3%) at WK324. Overall INSTI emergent resistance through 360 weeks showed that one participant added E138T/A+T97A to baseline Q148H+E138A+G140S, one with polymorphic V151V/I, three with R263K, and two with N155H. For the R263K cases, DTG fold-change (FC) ranged from 1.12 (R263R/K) and 1.93 (R263K) to 3.82 (A49G+S230R+R263K); for V151V/I, DTG FC was 0.92; for the two participants who developed N155H, DTG FCs were 1.8 (N155H) and 2.4 (I60L+T97A+N155H). An assessment of post-baseline emergent resistance to the background regimen (BR) was performed for DTG participants experiencing PDVF. No additional treatment-emergent NRTI resistance was observed in participants receiving DTG+2 NRTIs, even without full backbone activity. 3/7 PDVF participants with emergent INSTI resistance showed treatment emergent resistance to the BR: 2 had NNRTI resistance and one with pre-existing RAL resistance Q148+>2 (G140S+E138A) pathway developed PI resistance mutations V32V/I+I50L. For those 39/46 PDVF participants without emergent INSTI resistance, only one exhibited treatment emergent PI resistance mutation G48G/I/R/V to the BR.

Conclusions: The post-WK48 open-label phase evaluation of participants remaining on DTG continues to demonstrate low PDVF rate and low frequency of treatment emergent resistance to DTG and BR. Observed INSTI resistance had overall low impact on DTG FC. DTG+2 NRTIs was shown to be associated with few cases of emergent resistance to the BR. The cumulative 8+ years of SAILING resistance data provides additional evidence to support the DTG barrier to resistance and supports WHO interim guidelines with updated recommendations for including DTG+2 NRTIs as a recommended second-line treatment option for patients failing an NNRTI or PI first-line ARV regimen.

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DTG vs LPV/r (DAWNING): Efficacy by Baseline NRTI Resistance and Second-Line NRTI Use

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Background: DAWNING is a non-inferiority study comparing dolutegravir (DTG) + 2 nucleoside reverse transcriptase inhibitors (NRTIs) with lopinavir/ritonavir (LPV/r) + 2 NRTIs in HIV-1 infected adults failing first-line therapy (HIV-1 RNA ≥400 c/mL) of a non-nucleoside reverse transcriptase inhibitor + 2 NRTIs.

Methods: Participants were randomized (1:1, stratified by Screening HIV-1 RNA and number of fully active NRTIs) to 52 weeks of open-label treatment with DTG or LPV/r + 2 investigator-selected NRTIs, including ≥1 fully active NRTI based on Screening resistance testing. The primary endpoint was the proportion of participants with HIV−1 RNA <50 c/mL at Week 48 (Snapshot algorithm). Post-hoc efficacy analyses were performed based on baseline NRTI resistance profile and NRTI use in the second-line background regimen (BR).

Results: Of 624 participants randomized and treated, 499 (80%) received <2 active NRTIs at baseline. Overall, 84% (261/312) of participants on DTG versus 70% (219/312) on LPV/r achieved HIV-1 RNA <50 c/mL at Week 48 (adjusted difference 13.8%, 95% confidence interval [CI]: 7.3-20.3; P<0.001 for superiority). This difference was consistent regardless of the use of <2 or 2 fully active NRTIs in the BR. NRTI resistance was present in 561 participants (90%) at baseline, M184V/I (alone or plus additional NRTI resistance-associated mutations [RAMs]) in 513 (82%), K65R in 187 (30%), and ≥1 thymidine-analogue mutations (TAMs) in 152 participants (24%). Of participants with M184V/I alone or plus ≥1 NRTI RAMs, 430 participants (84%) took lamivudine (3TC) or emtricitabine (FTC) as part of their BR. Tenofovir disoproxil fumarate (TDF) was included in BR in the presence of K65R in 15 participants, whereas 86 participants with ≥1 TAMs took zidovudine (AZT). Among participants receiving 3TC or FTC in the presence of M184V/I, 85% (187/220) of participants on DTG versus 72% (152/210) on LPV/r had HIV-1 RNA <50 c/mL at Week 48 (difference 12.6%, 95% CI: 4.9-20.3). High responses were also observed in the DTG arm, when AZT or TDF were included in the BR in the presence of TAMs or K65R, respectively; however, participants numbers in these subgroups were small.

Conclusions: In DAWNING, response rates were high in participants receiving DTG + 2 NRTIs regardless of pre-existing resistance to one of the NRTIs in the BR, including in participants using 3TC or FTC in the presence of M184V/I. In World Health Organization interim guidance on HIV treatment, DTG + 2 NRTIs is now a recommended second-line treatment option for patients failing a non-NRTI-based regimen.

Data included in this abstract have been previously presented at the Conference on Retroviruses and Opportunistic Infections; March 4-7, 2019; Seattle, WA, USA.

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Comparison of Viral Replication for 2-Drug (DTG+RPV) vs 3-Drug Current Antiretroviral Regimen (CAR) in the SWORD-1 and SWORD-2 Studies

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Background: The overall goal of HIV therapy is to maintain virologic suppression over the entire course of a patient's treatment. Despite that the clinical significance and subject management of transient "blips" remains controversial, their appearance may lead to concerns about the durability of an antiretroviral therapy regimen. Within the SWORD trial, we assessed elevated viral load (VL), including blips during 2 years of study conduct, with the 2-drug regimen (2DR) of dolutegravir (DTG) + rilpivirine (RPV).

Materials & Methods: SWORD-1 and SWORD-2 are identical open-label, multicenter, global, phase III, non-inferiority studies evaluating the efficacy and safety of switching from current antiretroviral regimen (CAR) to DTG+RPV once daily in HIV-1-infected adults with HIV-1 RNA <50 c/mL (VL<50 c/mL) and no history of virologic failure. Participants switched to either DTG+RPV on Day 1 (Early Switch [ES] DTG+RPV group) or remained on CAR (CAR group) and switched to DTG+RPV at Week 52 (Late Switch [LS] DTG+RPV group) if still on the study and experienced viral suppression. US FDA Snapshot algorithm uses HIV-1 RNA 50 c/mL as the viral suppression cut-off. Patients with ≥1 on-treatment VL ≥50 c/mL were categorized as either: (1) participants with VL between 50 and 200 c/mL and no VL ≥200 c/mL or (2) participants with ≥1 VL ≥200 c/mL. Blips were defined as any VL between 50 and 200 c/mL preceded and followed by VL <50 c/mL.

Results: 1024 participants were randomized and exposed (DTG+RPV, 513; CAR, 511) across both studies. At Week 100 in the ES DTG+RPV group, 456 (89%) participants had Snapshot VL <50 c/mL and 6 (1.2%) met confirmed virologic withdrawal (CVW) criterion. In the LS DTG+RPV group, 444 (93%) had Snapshot VL <50 c/mL and 2 (<1%) met CVW criterion. During the first year of exposure to DTG+RPV, 34 (6.6%) and 20 (4.2%) participants had blips in the ES and LS groups, respectively. During the second year of follow-up, there were only an additional 3% of participants in the ES DTG+RPV group with blips.

Conclusions: The incidence of blips in the first year after switching to DTG+RPV 2DR was low in both the ES and LS DTG+RPV groups, and it was comparable with the 3-drug regimen (3DR) comparator and remained low in the second year. All other categories of VL>50 c/mL occurred infrequently in all groups. These results suggest no difference exists in blip rates or in any clinical consequences from VL elevations ≥200 c/mL, because efficacy rates were high and equal between arms (95% each) and CVW numbers were low DTG+RPV and traditional 3DRs of therapy.

Data included in this abstract have been previously presented at HIV Drug Therapy Glasgow; October 28-31, 2018; Glasgow, UK.

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Initial Viral Load Decline and Response Rates by Baseline Viral Load Strata With Dolutegravir Plus Lamivudine vs Dolutegravir Plus Tenofovir Disoproxil Fumarate/Emtricitabine: Pooled Results From the GEMINI Studies

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Background: At 48 weeks in the GEMINI-1 and GEMINI-2 studies (NCT02831673 and NCT02831764), the 2-drug regimen (2DR) dolutegravir (DTG) + lamivudine (3TC) was non-inferior to the 3-drug regimen (3DR) DTG + tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC) in achieving plasma HIV-1 RNA <50 c/mL in treatment-naive adults with baseline HIV-1 RNA ≤500,000 c/mL. To better understand the potency of DTG+3TC compared with the 3DR, we explored the rapidity of initial viral load (VL) decline and efficacy response rates in those with baseline VL >100,000 c/mL.

Materials & Methods: Participants were randomized 1:1 to receive DTG 50 mg + 3TC 300 mg once daily or DTG 50 mg + TDF 300 mg/FTC 200 mg once daily (stratified by baseline HIV-1 RNA and CD4+ cell count). The primary endpoint was the proportion of participants with HIV-1 RNA <50 c/mL at Week 48 (using snapshot algorithm, intention-to-treat–exposed population), with a 10% non-inferiority margin. As a post hoc analysis, mean change log10-transformed HIV-1 RNA from baseline and 95% confidence intervals (CIs) were calculated at Weeks 4, 8, 12, 16, 24, 36, and 48. Proportions of participants with plasma HIV-1 RNA <50 c/mL at Week 48 (using snapshot) for the 2DR vs 3DR therapy by baseline HIV-1 RNA strata ≤100,000 c/mL, >100,000 c/mL, >250,000 c/mL, and >400,000 c/mL were also analyzed.

Results: In the pooled analysis at Week 48, 91% (655/716) of participants in the 2DR vs 93% (669/717) in the 3DR group achieved HIV-1 RNA <50 c/mL (adjusted treatment difference, −1.7%; 95% CI, −4.4 to 1.1). 20% (140/716) in the 2DR and 21% (153/717) in the 3DR group had baseline HIV-1 RNA >100,000 c/mL (including 2% with baseline VL >500,000 c/mL). Similar rapid VL log decline was observed in both treatment groups overall (median change from Baseline at Week 4: −2.77 log10 c/mL in the 2DR and −2.80 log10 c/mL in the 3DR group) and in participants with baseline VL >100,000 c/mL. (median change from Baseline at Week 4: −3.38 log10 c/mL in the 2DR and −3.40 log10 c/mL in the 3DR group). High and similar response rates were seen in participants across baseline VL strata < and >100,000 c/mL. For participants with baseline VL ≤100,000 c/mL, 91% (526/576) in the 2DR vs 94% (531/564) in the 3DR group achieved HIV-1 RNA <50 c/mL (adjusted treatment difference, −2.8%; 95% CI, −5.8 to 0.2). For participants with baseline VL >100,000 c/mL, 92% (129/140) in the 2DR vs 90% (138/153) in the 3DR group achieved HIV-1 RNA <50 c/mL (adjusted treatment difference, 1.9%; 95% CI, −4.5 to 8.4). A consistent response pattern was also observed in the HIV-RNA strata >250,000 c/mL and >400,000 c/mL.

Conclusions: Viral load decline with the 2DR DTG+3TC was rapid and comparable with that of the 3DR DTG+TDF/FTC. Response rates in participants with baseline HIV-1 RNA >100,000 c/mL were high with DTG+3TC, consistent across strata, including participants with HIV-1 RNA >400,000 c/mL, and similar to the 3DR group. These data demonstrate a high potency of DTG+3TC, similar to that of standard-of-care 3DR.

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HIV Replication at <40 c/mL for DTG+3TC vs DTG+TDF/FTC in the GEMINI 1&2 Studies

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Background: The GEMINI-1/-2 studies in treatment-naive adults with screening HIV-1 RNA ≤500,000 c/mL showed the 2-drug regimen (2DR) dolutegravir (DTG) + lamivudine (3TC) was non-inferior to the 3-drug regimen (3DR) DTG+TDF/FTC at Week 48 by FDA snapshot algorithm; 91% (655/716) in the 2DR group vs 93% (669/717) in the 3DR group achieved HIV-1 RNA <50 c/mL. Abbott RealTime HIV-1 assay used in the studies measures viral load (VL) from 40 to 10,000,000c/mL and provided qualitative target detected (TD) or target not detected (TND) for VL <40 c/mL. Clinical and subject management implications of more stringent low-level VL data needs clarification. We assessed the proportion of participants with TND over time and by baseline (BL) VL for 2DR vs 3DR.

Methods: Participants were randomized 1:1 to treatment with 2DR or 3DR. The proportion of participants with HIV-1 RNA <40 c/mL and TND status at Week 48 was analyzed using a Cochran-Mantel-Haenszel test stratified by plasma HIV-1 RNA (≤100,000 vs >100,000 c/mL) and CD4+ cell count (≤200 vs >200 cells/mm3) at BL. Proportion of participants with TND status were summarized by visit and at Week 48 by BL HIV-1 RNA subgroup. Time to plasma HIV-1 RNA <40 c/mL and TND status overall and by BL HIV-1 RNA subgroup were estimated using the non-parametric Kaplan-Meier method.

Results: At Week 48, a similar proportion of participants had snapshot TND in the 2DR and 3DR arms (77% [553/716] vs 73% [525/717]; adjusted difference 3.8%; 95% confidence interval: −0.6 to 8.2), and proportions were also similar at earlier visits: Weeks 4 (34% vs 32%), 8 (52% vs 49%), 12 (60% vs 57%), 16 (59% vs 56%), 24 (65% vs 63%), and 36 (65% vs 68%). While similar response rates were seen in participants with BL VL ≤100,000 c/mL, response rates were higher in 2DR vs 3DR participants with BL VL >100,000 c/mL. Median time for 2DR vs 3DR to TND was 57 days for both overall, 57 days for both in ≤100,000 c/mL at BL strata, and 113 vs 169 days for BL >100,000 c/mL subgroup.

Conclusions: DTG/3TC and DTG+TDF/FTC had similar proportions of TND by snapshot at all weeks. Snapshot response rates based on TND status at Week 48 were similar between arms at ≤100,000 c/mL BL subgroup and higher for DTG/3TC in >100,000 c/mL BL category. Median time to TND was similar overall and in BL VL ≤100,000 c/mL subgroup, and less for DTG/3TC vs DTG+TDF/FTC if >100,000 c/mL at BL. These data, utilizing a more stringent snapshot criteria, continue to demonstrate the effectiveness and potency of DTG+3TC in treatment-naive patients.

Data included in this abstract have been previously presented at the Conference on Retroviruses and Opportunistic Infections; March 4-7, 2019; Seattle, WA, USA.

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Comparison of Viral Replication <50 c/mL for 2-Drug (DTG+RPV) vs 3-Drug Current Antiretroviral Regimen (CAR) Therapy in the SWORD-1 and SWORD-2 Studies

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Background: Abbott Realtime assay measures quantitative HIV-1 RNA viral load (VL) from 40 to 10,000,000 c/mL and generates qualitative target detected (TD) or target not detected (TND) for VL<40 c/mL. The US FDA snapshot algorithm uses 50 c/mL as the cut-off. Clinical significance and subject management implications of low-level quantitative and qualitative VL data remain controversial. We assessed the number of participants having 40 c/mL ≤ VL <50 c/mL, and TD/TND over 48 weeks for DTG+RPV 2-drug regimen vs CAR (PI-, NNRTI-, or INSTI-based 3-drug current antiretroviral regimen).

Materials & Methods: SWORD-1 and SWORD-2 are identical open-label, multicenter, global, phase III, non-inferiority studies evaluating the efficacy and safety of switching from CAR to DTG+RPV once daily in HIV-1-infected adults with HIV-1 RNA <50 c/mL (VL <50 c/mL) for ≥6 months and no history of virologic failure. We explored VL shifts from baseline, cumulative, and per visit classification of participants into >50 c/mL, 40 c/mL ≤ VL< 50 c/mL, or TD/TND when <40 c/mL across arms throughout 48 weeks.

Results: 1024 participants were randomized and exposed (DTG+RPV, 513; CAR, 511) across both studies. At Week 48, 95% of participants in both arms had Snapshot VL <50 c/mL in the intention-to-treat–exposed population. Confirmed virologic withdrawal (CVW) rates were <1% in both arms. Similar proportion of participants at Week 48 had Snapshot VL <40 c/mL and TND in the 2 arms (84% vs 80%, adjusted difference 3.1%, 95% confidence interval, −2.2 to 8.3). Participants with Baseline TD had similar and low occurrence of ≥1 VL ≥50 c/mL (DTG+RPV 14%; CAR 17%) or at ≥1 VL between 40 c/mL and 50 c/mL (DTG+RPV 4%; CAR 8%). Those participants with Baseline TND had similar and low occurrence of ≥1 VL ≥50 c/mL (DTG+RPV 5%; CAR 5%), or a similar percentage of ≥1 VL between 40 and 50 c/mL (DTG+RPV 3%, CAR 1%), or ≥1 VL<40 c/mL and TD (DTG+RPV 44%; CAR 41%) through Week 48.

Conclusions: DTG+RPV was non-inferior to CAR at Week 48 by Snapshot <50 c/mL. The two groups were similar with Snapshot <40 c/mL and TND as endpoint. Proportions of TD at Baseline and over time were similar between arms with higher rates of TD post-Baseline in those with TD at Baseline. Incident viremia (≥40 and ≥50 c/mL) was similar between arms by Baseline TD vs TND, but it was more common with TD. However, this had limited clinical consequence, because efficacy rates were high (95%) and equal between arms and CVW numbers were low and equal between arms.

Data included in this abstract have been previously presented at HIV Drug Therapy Glasgow; October 28-31, 2018; Glasgow, UK.

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Efficacy of DAA in a alternative model of HCV care for individuals attending in harm reduction units

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Background and Aims: The World Health Organization recently called for the elimination of hepatitis C virus (HCV) and has identified people who inject drugs (PWID) as a key target population. Clinical trials analyzing currently available all-oral regimens have demonstrated a high degree of efficacy in this population. There is an urgent need to confirm these data in a harm reduction and active consumption setting.
The aim of this study was to assess the efficacy of HCV therapy among drug users followed at low-threshold mobile harm reduction units (LTMHRUs) included in a program of coordination between a mobile harm reduction unit and a Hospital.

Method: We included active drug users (persons who had smoked or injected heroin/cocaine in the previous 6 months) who received HCV treatment and were attended at 2 LTMHRUs. Participants received HCV treatment while in active consumption or recent abstinence in a low-threshold setting. Recent abstinence was defined as cessation of drug consumption for at least 15 days and no more than 6 months. The program implemented through a patient navigator consisted of: Take the patient to the healthcare Centre, remind the patient about their next appointment, accompany the patient to Fast Track Clinic (2 dedicated vehicles available), contact hospital by phone to receive notification of appointments, collect medications from pharmacy and deliver them like supervised treatment.
Sociodemographic variables and characteristics related to drug use were collected at initiatiation of HCV treatment and during follow-up. End-of-treatment response (ETR) was defined as an undetectable HCV viral load at the end of treatment. SVR was defined as undetectable viral load at the first available HCV RNA measurement obtained a minimum of 12 weeks after the end of treatment. Virological failure was defined as detectable plasma HCV RNA before the date of SVR.

Results: A total of 165 individuals initiated interferon-free therapy for HCV infection with DAA at the 2 LTMHRUs between January 2016 and July 2018. Notably, 120 patients (72.7%) were homeless, and 122 (73.9%) and 88 (53.3%) reported IDU in the 6 months and 30 days prior to HCV treatment, respectively. In addition, 142(86.1%) were receiving OST, 59(36%) had HIV coinfection, 19(13.6%) had cirrhosis, 31(18.9%) had mental disorders, and 71(43%) started therapy in recent abstinence. Of those who started therapy in recent abstinence, 39(55%) relapsed during follow-up.
159 patients had achieved ETR, 1 had discontinued treatment and was lost to follow up, and 5 were still on treatment. Virological failure was recorded in 3 participants. Eight participants were lost to follow-up after achieving ETR. Thirty participants with ETR were waiting for an SVR analysis.The overall SVR rate was 80% (108/135) in the ITT analysis (reinfection=failure) and 97.5% (118/121) in the mITT analysis. SVR rates showed no differences according to HIV status, IDU status, or OST status.

Conclusions: Given the high efficacy of treatment, active drug users attended at LTMHRUs should be treated for HCV.Models of care must be adapted to the circumstances and the needs of the target population. Low-threshold access and a flexible model are essential for PWIDs.

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Sofosbuvir, Velpratasvir, Veloxpravir Efficacy in 12 week treatment in triple infected (Chronic Hepatitis C, Chronic Hepatitis B and HIV} Geno 3 naive population: An open level prospective clinical trial - SOLVVE - C

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Objectives: Chronic Hepatitis C treatment is no longer challenging in the era of DAAs with an SVR of up to 97%. Triple infection treatment with HCV, HIV and Hepatitis B has not been explored in real life situations. HCV Genotype 3 is still the most challenging clinical state in Hepatitis C treatment. Regardless of concomitant triple infection, shorter duration of therapy revealed favorable outcome with the highest retention, fewer side events, and cost containment. This study evaluates the efficacy and safety of Sofosbuvir, Velpratasvir, and Veloxpravir in the treatment of triple infection with HBV, HIV, and HCV (Genotype 3).

Methods: Twenty-two (n = 22) HCV treatment-naive patients with Triple Infection (HIV HBV HCV Genotype 3) were recruited for the study.

Patients with HIV were on Atripla for over three years with HIV with Undetectable Viral load and HBV Viral load Undetectable. HCV infected patients had a Median Viral load of 3 million IU and Genotype 3 prior to treatment.

Demographics:

HCV Genotype	3	3a	3c	3b
No of people	22	10	9	3

Patient Characteristics

	No of Patients		Mode of transmission		
Race	Males	Females	IVDU	MSM	Blood transfusion
African-American	1	0	1	0	0
Caucasian	1	0	0	1	0
Haitian	2	0	0	0	2
Asian	0	18	1	0	17
India	0	4	1	0	3
Pakistan	0	12	0	0	12
Bangladesh	0	2	0	0	2

Mean Age 56 (44 – 68)
Mean BMI 27 (21 – 29.6)
Mean Fibrosis F3

Patient HBV characteristics

	No of Patients		Genotype					
Race	Males	Females	A	B	C	D	G	H
Asian	0	18	0	1	5	12	0	0
India	0	4	0	1	0	3	0	0
Pakistan	0	12	0	0	3	9	0	0
Bangladesh	0	2	0	0	2	0	0	0
Caucasian	1	0	1	0	0	0	1	0
African-American	1	0	1	0	0	0	1	0
Haitian	2	0	0	0	0	0	2	2

Infection	Mean years of acquisition
HIV	20
HBV	15
HCV	7

HBV Characteristics

HBeAg Negative	19
HBeAg Positive	3
HBsAg Positive	22
HBcAb Positive	22

Exclusion Criteria:
Active Drug Abuse or excess Alcohol intake, CHF NY heart Type IV, Cardiomyopathy, Arrhythmia, COPD, Renal Failure with creatinine clearance less than 30 %, Decompensated Cirrhotic HCC, Transplant recipients,

Results:

Duration of treatment	HCV Viral load	
	Viral load - Undetectable	Viral load detectable
A .Fourth week	18/21	3/21 detectable, 200 copies mean
B .Eighth week	18/21	3/21 detectable
c. Twelfth week	18/21	
d. Twenty fourth week	18/21	

Resistance-associated substitution	Pre-therapy	Post-therapy
RAS 31	1	3
RAS 36	0	1
RAS 93	1	1

Conclusion: The study demonstrates the efficacy of DAAs in 12-week treatment with an SVR of 87% in a very challenging triple infected cohort, with significant efficacy, tolerability, and safety. A larger trial is needed to validate the results.

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Hepatitis HIV co-infected clinic: Is a specialised service worth implementing in medium sized HIV cohorts?

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Introduction: 8% of our HIV cohort is co-infected with hepatitis C. Chronic infection with both HIV and Hepatitis C (HCV) has a potential adverse bidirectional impact. Widespread use of antiretroviral therapy (ART) has resulted in a dramatic decline in AIDS related mortality. With patients living longer, the complications associated with long term HCV infection has emerged as one of the most important clinical issues for people living with HIV (PLWH). Treatment barriers, polypharmacy, drug-drug interactions and liver toxicity are few of the common challenges encountered in this cohort. By integrating Hepatitis and HIV care pathways, patients are offered a more streamlined service with fewer clinic appointments, and real-time decisions can be made on drug switches and complications to improve the quality of care that co-infected patients receive. For these reasons, a specialist bimonthly co-infection clinic, managed jointly by a HIV specialist and a hepatologist was set up in November 2014.

Method: We analysed the data of the co infection clinic lists done between 2016 and 2018 for the reasons of referrals, treatment administered if any and outcome of the attendance.

Results: Out of the 95 co-infected cohort, 65 referrals were made between 2016 and 2018. 30 of these were for hepatitis C (46.1%).14 patients were seen in view of hepatitis B infection (21.5%), 10 for suspected non alcoholic fatty liver disease (NAFLD) (15.3%), 7 because of alcohol related liver disease (10.7%), 2 in view of portal hypertension secondary to drugs or portal vein thrombosis (3%) and 1 in view of autoimmune hepatitis (1.5%). 6% had more than 1 pathology identified.
83% of our Hepatitis C positive cohort successfully completed direct acting antiviral treatment (DAAs) and have reached SVR (12 weeks). The treatment regimens used were various. The most common prescribed DAA regimens were Sofosbuvir/ ledipasvir, elbasvir/grazoprevir and Ombitasvir/paritaprevir+ritonavir. 2 patients (3%) failed first line DAA and have been retreated with second line therapy. They have both successfully eradicated Hepatitis C.
The remaining untreated co-infected patients have either been declining treatment, not engaging with our services or are being investigated for other complex medical issues, hence putting the hepatitis C treatment on hold.

Conclusion: Now that hepatitis C is curable with a relatively short course of DAA with minimal to none side effects, getting our co-infected patients engaged in our services is more important than ever before. A Joint HIV-Hepatitis clinic allows patients to receive a comprehensive and consistent approach to evaluation for treatment, support during treatment and careful monitoring and management of treatment response and complications in a timely and efficient manner. This type of streamlined service not only improves the quality of care but also the patient experience.

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Hepatitis B virus infections in HIV infected PMTCT mothers on ART and their exposed infants in a tertiary hospital in Kenya

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Hepatitis B virus (HBV) infection is a major public health problem affecting approximately 360 million people globally. Mother-to-child transmission (MTCT) is responsible for more than one third of chronic HBV infections worldwide. Mothers who are co- infected with HBV/ Human Immunodeficiency virus (HIV) and are antiretroviral therapy (ART) naïve have a high tendency of transmitting the two viruses during pregnancy, delivery or postnatally. This study aimed to determine the prevalence and associated risk factors of HBV infections among Highly Active antiretroviral therapy (HAART) receiving HIV-infected mothers and their exposed infants at the Kenyatta National Hospital (KNH) in Kenya. Eligible mothers and their exposed infants were recruited from a cohort enrolled in a Prevention of mother to child transmission of HIV (PMTCT) program in KNH.

A structured questionnaire was used to capture the socio-demographic data of the participants and information on associated factors to HBV infections. Four milliliters (ml) sample of paired whole blood were obtained from HIV positive mothers and their exposed infants. Whole blood was separated into plasma and stored at -80oC. HBV infection was determined using Euromedi Equipp (EME) rapid kit for Hepatitis B surface antigen (HBsAg) test and confirmed by a HBsAg Enzyme linked immune sorbent assay (ELISA). The HBsAg sero reactive samples were further screened for HBV envelope antigen (HBeAg) using ELISA (Accubiotech co.ltd). Samples which turned positive with ELISA and rapid tests were subjected to Polymerase-chain reaction (PCR) targeting the preS1 region using nested primers. HBV infection was presented as a proportion with 95% confidence interval and the associations tested using chi-square tests. A total of 534 HIV-infected mothers and their highly exposed infants were recruited. The mean age of the mothers was 31.2 years (SD 5.4 years) and the infants had a median of 6 months (IQR 3-10 months). Four hundred and thirty-three (81.1%) of the mothers were married, 272 (50.9%) having tertiary education and 113 (59.5%) were employed. One hundred and thirteen (21.2%) of the mothers were aware of HBV infection and HBV vaccination. Most of the mothers were currently receiving HAART with 502 (94%) of the mothers taking TDF/3TC/NVP and 32 (6%) on AZT/3TC/ NVP or AZT/3TC/EFV. Out of 534 mothers, 19(3.6%) were positive for HBV. All the 19 samples that gave positive HBsAg results tested negative for HBeAg. Out of the 19 samples that tested positive with ELISA, also gave positive results with PCR targeting the preS1 gene. All exposed infants tested negative for HBV with the HBsAg rapid, ELISA and PCR tests. History of dental surgery was associated with increased rate of HBV infection among the HIV-infected mothers (OR 3.3 (95% CI 1.1-9.6). In conclusion, the results of this study suggest that the HAART regimen received by the HIV infected pregnant mothers may have prevented vertical transmission of HBV infections to exposed infants.

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HEV infection in Portuguese HIV-infected patients

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Introduction: Despite rare cases of chronic hepatitis E described in the literature, the seroprevalence of Hepatitis E virus (HEV) and its chronicity rate in the HIV-infected individuals has not been well established.

Material and Methods: with the aim of knowing the prevalence and chronicity rate of HEV, the authors investigated 160 HIV-infected patients attending the out-patient clinic of a central hospital in Coimbra, during a six month period. All randomly included patients were tested for anti-HEV IgM/IgG (recomLine – Mikrogenm) and RT-PCR. Levels of CD4 cell count, HIV viral load, ART, HBV and HCV co-infection, as well as demographic features were analyzed, to find out if any factor is associated with higher prevalence.

Results: One hundred and sixty HIV patients were tested, mainly male (83,1%) with an average age of 51,5 years old. All patients were under ART, and 93,1% had undetectable HIV RNA. Anti-HEV IgG was found in 29 patients (18,1%) and none of the total had detectable HEV RNA. Patients with IgG+ were older than those with IgG- (p 0.03). CD4 cell count at the time of HIV diagnosis and in the present had no statistically difference between anti-HEV IgG+ /– [453/mm3 vs 410/mm3 initially (p 0.470) and 594/mm3 vs 640/mm3 in the present (p 0.244)], as also when analyzing the level < 200/mm3 at diagnosis (p 0.72). There were no differences in IgG+ or IgG- in initial HIV viral load (165 659 cp/ml vs 97 179 cp/ml, p 0.654), in the HBV coinfection (3,4% vs 3,1%), HCV coinfection (37,9% vs 36,5%) or HAV prevalence (82,8% vs 83,2%). There was only one case of cirrhosis (3,4%) in IgG+ group, also infected with HCV and treated with SVR, contrasting with 7 cases (5,3%) in IgG- group. No statistically differences in AST or ALT levels were found in both groups.

Conclusions: The HEV seroprevalence in this sample was 18,1%. No chronic hepatitis was found of the exclusive responsibility of HEV. Higher prevalence was not associated with lower level of CD4, higher viremia or co-infections with other virus; age was the only independent factor associated with anti-HEV IgG+. Although HEV prevalence is high in this population, chronic HEV infection may be considered uncommon chronic liver disease in HIV-infected individuals.

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Polymorphisms of HIV-1 subtype-B gp41 coding region in a large dataset of drug-naïve and ARV-treated infected individuals

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Ongoing combinations of antiretroviral drugs for the treatment of Human Immunodeficiency Virus (HIV) infection can successfully maintain long-term suppression of HIV-1 replication in plasma. but an effective vaccine against this virus has not still found. It is desirable to develop multifunctional strategies that improve coverage of epitope diversity and allow for understand conformational changes that occur during attachment and membrane fusion. For this purpose, in relation to the pivotal role played by gp41 in these polyvalent vaccine approaches, the conservation of the gp41 protein was evaluated, using a large dataset of sequences retrieved from person to drug-naïve or ART-treated.

To genetically characterize gp41 in terms of amino acid variability, the Los Alamos databases were used and 24.505 full-length Env sequences derived from HIV-1 subtype-B infected individuals at all stages of infection were analyzed. To select the B-subtype strains and to analyze gp41 mutations, multiple alignments were obtained using ClustalW2 manually edited with Bioedit software. The final resulting dataset was composed by 546 drug-naïve infected individuals and 2.746 antiretroviral drugs (ARVs)-treated patients, respectively: these 3.292 sequences were then used for the entire study (Table 1).
To calculate the average hydropathy of sequences Grand average of hydropathy (GRAVY) calculator was used.

In drug-naïve patients, among the 231 gp41 variable residues (variability >1%), 141 were mutated in >5% of patients and 48 of them (13.9%) were highly variable (substituted in >25%). Therefore, 114 out of 345 gp41 amino acid residues (33.0%) were highly conserved (≤1% variability), and 13 never muted (11.4%) (Table 1). In ARV-treated patients, among the 224 variable residues (variability >1%), 143 were mutated in >5% of patients and 53 of them were highly variable (substituted in >25%). Consequently, 121 out of 345 gp41 amino acid residues (35.1%) were highly conserved (≤1% variability) and just 1 never muted (0.9%) (Table 1).

The study of gp41 variability shows similar sequence variability between drug-naïve and ARV-treated infected individuals thus strengthening the growing appreciation for the identification of specific single sensitizing mutations in the control of HIV infection. Overall, these results shed light on the specific mechanism related to host cell antiviral control and provide important implications for the therapy of HIV infection.

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Early virological response in patients with HCV genotype 1 in treatment with Elbasvir / Grazoprevir

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Introduction: In Romania, with a population of 19.6 million inhabitants and a prevalence of 3.23%, are estimated approximately 600,000 patients with HCV infection. Romania holds the 1st place in Europe as a total number of HCV cases, the fourth place as a mortality rate caused by liver disease - 44.5 deaths per 100,000 inhabitants.
Approximately 10% of HCV patients in Europe are Romanians (OMS). Under these circumstances, HCV is a serious public health problem. Currently, there is a national registry of hepatitis, a national screening and treatment program.
The purpose of study is to evaluate the efficacy and safety of a short-term therapeutic regimen, allowing the treatment for more patients with the same financial resources.

Material and methods: According to the national criteria for inclusion in therapy: detectable viremia and fibrosis > 1 (determined by Fibromax), between November 2018 and February 2019, a number of 32 patients - naive and experienced, aged 33-80 years, were included in the study.
Evaluation of patient viremia was performed at baseline, after 3 weeks of therapy and after 12 weeks (EOT).

Results:
-Of the 32 patients, 23 were evaluated after 3 weeks and after 12 weeks of treatment;

After 3 weeks:
• ND viremia = 13 patients;
• <15UI (detection limit) = 6 patients;
15UI (15-95UI) = 4 patients

After 12 weeks (EOT) - all this patients had undetectable viremia;
-A patient interrupted therapy after 2 weeks of treatment (cardiac decompensation)
-8 patients were evaluated only after 12 weeks of treatment (EOT); all were undetectable.

Adverse effects:
- 1 case- cardiac decompensation hospitalized - discontinuation of therapy
- 1 case -ALT ↑ 10 * N (without discontinuation)

Other side effects: fatigue, headache, muscle pain (intermitent and low intensity).

Conclusions and discussions:
The Elbasvir / Grazoprevir treatment regimen proved to be effective and safe: all patients were undetectable at EOT, few side effects.
Along with other studies, this presentation shows the possibility of shortening the duration of therapy (8 weeks), thus allowing:
- Increasing adherence;
- Reduction of the possibility of adverse effects;
- Faster return to previous medication that has been changed due to possible drug interactions;
- Increasing the number of patients treated with the same financial resources.

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Comorbidity in patients of the AIDS center in Krasnodar region of Russia

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Background: The development of the diagnostic and treatment process has increased an average age of people living with HIV (PLHIV). Therefore, an assessment of comorbidity is necessary for planning and organizing medical care for them.

Materials & Methods: 537 outpatient cards have been analyzed by descriptive and comparative statistics of Statistica 10.0 software, Microsoft Excel 2013.

Results: The study group consisted of 537 people including 324 men (272 – 83.9% with co-morbidity) and 213 women (162 – 76.1% with co-morbidity). Distribution by age: up to 34 years 102 (19.0%), 35-44 – 236 (43.9%), 45-54 – 82 (15.3%), 55-64 – 73 (13.6%), 65 years and older – 44 (8.2%). It is noteworthy that the general morbidity in study group is 1.5 times higher than that of the adult population of the Krasnodar region (1918.1 and 1272.4 per 1000 people, respectively). A comparative analysis of the structure of these indicators demonstrates the following differences: diseases of the hepato-biliary system in the study group were more common by 8.9 times, skin by 3.4 times, the urogenital system 1.6 times, respiratory organs in 1.5 times, neuropsychiatric diseases 1.5 times more often.

The structure of overall incidence of liver diseases was represented by viral hepatitis C - 78%, Toxic hepatitis - 13%, viral hepatitis B - 4%, viral hepatitis D - 2%, liver cirrhosis - 3%. The overall incidence of the listed groups progressively decreased in the range of 35-65 years.

Assessment of the general morbidity in age groups brought us some interesting results for practical work. The general morbidity in most of the listed groups progressively decreased in the range of 35-65 years. At the same time, the incidence of CVDs increased in the age groups of 35–65 years from 3.7 to 89.4 per 1000 people.

The problem of polypragmagia in PLHIV is associated with the need for a combination of ART and treatment for concomitant diseases. Most often ART included lamivudine (99.2%), tenofovir (56.4%), efavirenz (57.7%). The ART regimen contained 4.7 ± 1.7 tab., The treatment regimen for comorbidity treatment - 4.4 ± 0.8 tab.

Conclusions: 1. The overall incidence of PLHIV in comorbidities is 1.5 times higher than that of the adult population of the Krasnodar region in 2017. The difference in the structure of comorbidity among PLHIV is in the prevalence of diseases of hepato-biliary system (8.9 times higher than the regional level). 2. Dynamics of the incidence of CVD in the study group by age (steady growth with maximum rates in the group of 65+ years). 3. The predominance of tenofovir in the ART regimen is the basis for the dynamic monitoring of renal function and bone density, especially in older age groups. 4. The combination of ART drugs and therapy for comorbidities should be under the obligatory control of drug-drug interactions.

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Investigation of factors predicting being in a national transmission cluster and of late presentation with HIV-1 in Denmark, 2009-2017

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Introduction/Background: In Denmark, around 200 people are diagnosed with HIV-1 every year despite access to free highly efficient antiretroviral therapy (ART). Continued HIV-1 transmission in local networks as well as late presentation of HIV-1 are two major public health and clinical challenges. Sequence and epidemiological data from the Danish HIV-1 surveillance project (SERO) are used to monitor the spread of drug resistance and to identify risk groups and transmission clusters. Here we report on statistical investigations of correlates to being in a transmission cluster and being a late presenter.

Material & Methods: HIV-1 pol sequences from 1225 newly diagnosed (2009 to 2017) were aligned in MAFFT and phylogenetically analyzed in Mega 6.0 using the ML GTR method with 1000 bootstrap replicates. Clusters were identified using Cluster Picker (thresholds: bootstrap≥90, Genetic Distance≤4.5). Late presenters (LP) were patients with a CD4+ count<350 and/or AIDS defining illness. Statistical analyses were performed in R statistical software. The relation between risk factors and the odds of being in a cluster ("no-cluster", "cluster size of 2", and "cluster size of 3+) was investigated using partial ordinal logistic regression with infection method (MSM or heterosexual) as nominal effects. Risk factors for presentation status (LP versus non-LP) were investigated using standard logistic regression as well multi-level regression with cluster identity as a random effect.

Results: HIV-1 pol sequences from 1032 of the 1225 (84%) patients were eligible for analysis. Of these, 499 (48.4%) belonged to clusters. Odds ratio (OR) for being in a cluster were as follows: being of Danish ethnicity 2.97 (95% confidence interval (CI): 2.21–4.00); younger age (continuous variable): 1.03 (CI: 1.02–1.04); subtype B: 1.43 (CI: 1.05–0.96); non-LP: 1.46 (CI: 1.13–1.89); and MSM: 1.47 (CI: 1.05–2.04) for no-cluster vs a cluster size of 2, and 2.52 (CI: 1.73–3.66) for a cluster size of 2 versus 3+. No difference was found between active (new infection within the last 3 years) and non-active clusters, OR of 1.09 (CI: 0.83–1.42). Of the eligible patients, 499 (48.4%) were classified as LP. There was increased odds of being a LP among heterosexuals compared to MSM for individuals of non-Danish decent, but not for those of Danish ethnicity, with OR of 2.90 (CI: 1.79–4.71) and 1.42 (CI: 0.95–2.12) respectively. Also odds ratios increased more with age for those of Danish ethnicity compared to non-Danish ethnicity (OR: 1.03, CI: 1.00–1.06). The odds of being a LP was increased for non-clustered individuals (OR: 1.59, CI: 1.07-2.37), whereas cluster-activity played no role. Subtype was not associated with presentation status (OR: 0.88, CI: 0.62–1.23). A multi-level analysis did not significantly change the results.

Conclusions: Endemic HIV-1 transmission within clusters was primarily associated to subtype B infections among younger MSM of Danish ethnicity, who were diagnosed early after infection. LP were more commonly non-MSM, of non-Danish origin and not in a cluster. This knowledge can help to inform and design intervention strategies, such as groups targeted for PrEP and more frequent HIV-1 testing.

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Key HBsAg C-terminus mutations correlate with lower HBsAg levels in vivo, hinder HBsAg release in vitro and hamper HBsAg structure in HBeAg-negative chronic HBV genotype D infection

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Background & aims: This study is aimed at i)evaluating HBsAg levels in different HBV genotypes (D, A and E) in HBeAg-negative individuals with chronic HBV infection, and ii)the correlation of specific mutations in HBsAg C-terminus (critical for a proper HBsAg-release) with HBsAg levels in vivo, iii)their impact on HBsAg-secretion in vitro and on structural stability in silico.

Methods: HBsAg levels were investigated in 323 drug-naïve HBeAg-negative patients chronically infected with HBV genotype D (N=228), A (N=65) and E(N=30).
In 228 genotype-D infected patients, association of mutations in HBsAg C-terminus with HBsAg<1000 IU/mL (N=130) is assessed by Fisher's Exact test. Association among mutations in C-terminus is assessed by binomial correlation coefficient (phi). Impact of mutations on HBsAg-secretion is analyzed by transfecting HepG2 cells with plasmids encoding WT- and mutated-HBsAg, linked to a streptavidin-tag (StrepTag). The StrepTagged-HBsAg amount in supernatants is quantified by an ELISA targeting StrepTag, not affected by HBsAg-antigenicity and thus, capable to identify defects in HBsAg-secretion. HBsAg-structures and their stability are predicted by I-Tasser ($\Delta\Delta G$[WT-mutated]<0 indicating reduced stability in presence of mutations).

Results: HBV genotype D is characterized by HBsAg levels lower than genotypes A and E (2,016[520-6,173]IU/ml, 6,416[3,140-14,587]IU/ml, 9,937[4,566-16,032]IU/ml, respectively P<0.001 for all comparisons). Results confirmed by ANOVA multivariable analysis (P<0.0001 for genotype D vs A, P=0.02 for genotype D vs E). In order to unravel factors contributing to lower HBsAg levels in genotype D, we focused on HBsAg C-terminus known to play an important role in HBsAg-secretion. In particular, we found that specific C-terminus mutations (V190A, S204N, Y206C, Y206F and S210N) significantly correlated with HBsAg<1,000IU/ml (P from <0.001 to 0.04).
These mutations lie on divergent pathways involving other mutations in HBsAg C-terminus: V190A with F220L (Phi=0.41, P=0.003), S204N with L205P (Phi=0.36, P=0.005), Y206F with S210R (Phi=0.47, P<0.001) and S210N with F220L (Phi=0.40, P=0.006). Notably, patients with these pairs of mutations are characterized by HBsAg levels 1log lower than patients without them (P=0.003-0.02). Some pairs of mutations also decrease serum HBV-DNA levels (V190A+F220L:2.2[1.6-2.7]logIU/ml; S204N+L205P:2.3[1.9-2.9]logIU/ml, WT: 3.4[2.7-4.1]logIU/ml for wt, P=0.01-0.04), suggesting a detrimental impact also on the release of viral particles.
Similarly, in vitro, all the above-mentioned pairs of mutations determine a significant decrease (up to 90%) in the amount of extracellular HBsAg compared to wt (P values ranging from 0.022 to <0.001). Notably, for the pairs of mutations S204N+L205P, Y206F+S210R, Y206F+S204T and Y206F+M197T, the decrease in HBsAg-release is significant also compared to the single mutation S204N (P<0.001) or Y206F (P=0.007, P=0.001 and <0.0001, respectively).
Finally, by structural analysis, these pairs of mutations determine a relevant reduction in the stability of HBsAg C-terminus and a profound rearrangement of this domain.

Conclusions: HBsAg levels in HBV genotype D are significantly lower than in genotype A and E in different phases of HBeAg-negative chronic infection. In genotype D infected patients, specific clusters of mutations in HBsAg C-terminus correlate with lower HBsAg levels in vivo, hamper HBsAg-release in vitro and affect HBsAg structural stability, supporting their detrimental role on HBsAg-secretion. Knowledge of these mutations can help in optimizing the clinical interpretation of HBsAg levels in HBV genotype D.

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Distribution of high risk HPV genotypes among HIV positive patients in Outpatient Clinic, Warsaw, Poland

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Introduction/Background: Human papillomavirus (HPV) infection is a common sexually transmitted disease worldwide. HIV positive patients are exposed for persisted HPV infection because of their immunological impairment. Detection and genotyping of high risk (HR) HPV strains are part of integrated gynaecological care (IGC) established at the HIV Outpatient Clinic in Hospital for Infectious Diseases in Warsaw. The aim of this study was to determine prevalence and genetic distribution of HR-HPV among HIV positive patients.

Materials/Methods: Two hundred ninety cervical swabs samples obtained from 290 (93,85%) women and 19 tissue biopsies obtained from 19 men (6,15%), treated in our Clinic since 2016 to 2018, were analysed. Cervical swabs were taken during a routine, once per year, visit; tissue biopsies were collected only when clinical symptoms of HPV infection were observed . The median age of study participants was 40 years (IQR:36–46) and median CD4 count was 584,5 cells/µL (IQR:393-782,5). Eighty four percent patients had undetectable or <40 copies/mL HIV viral load, 16% had HIV VL ≥40 copies/mL and median VL was 5205 copies/mL (IQR:147-34514). HPV DNA detection and genotyping was performed using HPV Genotypes 14 Real-TM Quant kit (Sacace, Italy). The test enables following genotypes detection: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. PCR reactions were carried on CFX96 cycler (BioRad, USA). Obtained results were analyzed using software provided by manufacturer.

Results: The HR HPV DNA was found in 42,75% (124/290; 95% CI:37,05-48,46%) cervical swabs and in 89% (17/19) tissue biopsy samples. The most frequent genotypes among women were: 16 - 20%(25/124) and 68 - 18,5%(23/124); among men: 16 - 65%(11/17) and 59 - 29,5%(5/17). Genotype 18 was detected in 5,6%(7/124) women and in 17,6%(3/17) men. In cervical swabs samples mixed HPV genotypes were detected: 2 genotypes (22,7%-28/124), 3 genotypes (9,7%-12-124), 4 (4,8%-6/124) and 5 (1,6%-2/124). In tissue biopsies mixed infections were more frequent than monoinfections - 58,9%-10/17 (2 to 7 genotypes) versus 41,1%-7/10. The most complex infections contained following genotypes: 39, 52, 56, 66, 68 (female, CD4 628 cells/µL; HIV RNA 5208 copies/mL), 16, 33, 39, 51, 66 (female, CD4 626 cells/µL; undetectable HIV RNA) and 18, 31, 39, 45, 52, 59, 68 (male, CD4 698 cells/µL, HIV RNA <40 copies/mL). In the tested group the longest duration of HPV infection was determined as at least for 3 years (genotype 56).

Conclusions: Our work documents HPV prevalence and genotype distribution in poorly characterized, for that pathogen, group of HIV infected patients in Central Poland. Analysis revealed that HPV-16 infection was more frequent than HPV-18 in both, women and men, groups of patients. Additionally, mixed infections (≥2 genotypes) were common in analysed cohort; in some cases 6-7 genotypes were detected simultaneously. Furthermore, we document that HPV infection could be established for at least 3 years. However, common anti-HPV vaccination probably will change the prevalence and genotypes distribution of HR-HPV in the future, also in the group of HIV positive patients. Therefore long-time surveillance is necessary to recognize upcoming trends and changes.

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Performance of the Abbott RealTime HCV Genotype II assay in a real-life setting

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Background: HCV genotyping before treatment remains crucial for optimal selection of direct acting antiviral therapy. Due to high genetic variability of HCV, assays can struggle to provide clear genotyping and subtyping results. Our aim was to evaluate the performance of the Abbott RealTime HCV Genotype II assay (Abbott Molecular, Illinois, USA) in a real-life setting.

Materials & Methods: In total 539 samples were genotyped using the Abbott assay between September 2016 and November 2018 at the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia. Samples yielding undetermined results (no genotype result, no 1a/1b subtype result or reactivity with another genotype) were further evaluated by using either universal core PCR or subtype-specific PCRs, followed by sequencing.

Results: The Abbott assay provided a clear genotype or subtype in 95.2% of samples (513/539), namely subtype 1a (n=190), subtype 1b (n=70), genotype 2 (n=13), genotype 3 (n=232), and genotype 4 (n=8). In 12 samples (2.2%), subtype of genotype 1 could not be determined by the Abbott assay; eight of these samples were resolved by sequencing as subtype 1a, three as subtype 1b and one as subtype 6i. Co-infection of subtype 1a and genotype 2 was detected by the Abbott assay in one sample and later confirmed by subtype-specific PCRs and sequencing. In nine samples, reactivity with another genotype was observed. In two of the nine samples co-infection with two HCV genotypes was confirmed (1a+3 and 1b+4) by sequencing, while the remaining seven showed only one genotype in the genotype-specific PCRs, indicating a probe cross-reactivity or contamination issue. In addition, two samples yielded an HCV indeterminate result and two HCV inhibition by the Abbott assay; all four were further resolved by sequencing as genotype 3.

Conclusions: Our study shows that the Abbott RealTime HCV genotype II assay provides unambiguous genotype/subtype information in a vast majority of samples. However, the laboratory should still have back-up assay(s) prepared as well as sufficient knowledge to resolve unclear genotyping results. Since the Abbott RealTime HCV genotype II assay erroneously identified the subtype 6i sample as genotype 1, resolving samples with initial indeterminate 1a/1b subtyping results is crucial.

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Phylogenetic Clusters of HIV-1 Subtype B Demonstrate Development of Local Outbreaks in the Vulnerable Groups in Bulgaria (Preliminary Analysis)

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Background: Phylogenetic clusters show the evolutionary history of constantly changing viral strains. By means of phylogenetic clusters we analyzed the development of HIV-1 subtype B epidemic in major transmission groups - men who have sex with men (MSM), heterosexuals (HET) and people who inject drugs (PWIDs).

Materials & Methods: In this national representative study we analyzed 534 HIV-1 pol subtype B sequences from individuals infected with HIV-1 and diagnosed between 1988 and 2017. HIV-1 pol gene was sequenced using the Applied Biosystems 3130xl or an OpenGene DNA sequencing systems. HIV-1 subtype B was determined using the automated tool COMET v2.2. The phylogenetic tree was reconstructed with IQ-Tree v1.6.8 and visualized in FigTree v1.4.3. HIV-1 transmission clusters were defined with the ClusterPicker v1.2.3 program with a genetic distance parameter of 1.5% and a bootscan support greater than or equal to 90%. Clusters with three or more sequences were assumed as phylogenetic clusters.

Results: From the analyzed 534 sequences, 475 (89.0%) were from men and 59 (11.0%) from women. The average age at diagnosis was 31.9 years. According to self-reported patients data, 296 (55.4%) were MSM, 218 (40.8%) HET, 16 (3.0%) PWIDs and 4 (0.7%) infected by blood products in the early years of the epidemic. 10 (1.9%) from the individuals were sex workers, of which 8 (80.0 %) men and 2 (20.0%) women. 65 (12.2%) of them reported co-infection with other sexually transmitted infections. Our phylogenetic analysis identified 18 phylogenetic clusters and 31 pairs of closely related sequences. 73 (13.7 %) of the analyzed sequences fall into phylogenetic clusters. The largest cluster was composed of 10 sequences - 7 from MSM and 3 from HET. 12 (66.7%) of the clusters were formed from viral strains isolated only from men and 6 (33.3%) were formed of sequences from both sexes. There weren't any clusters composed of sequences isolated solely from women. 7 (38.9%) of the transmission clusters contain sequences only from one transmission group (6 from MSM and 1 from HET), 11 (61.1%) of the clusters contained sequences from two or more transmission groups. The largest number of mixed clusters n=9 (81.8%) were formed from MSM and HET, 1 cluster from MSM and PWIDs and 1 cluster from individuals from all of the three transmission groups - MSM, HET and PWIDs.

Conclusions: The phylogenetic clusters in our study represent microepidemic outbreaks showing a rapid dissemination of HIV-1 among a limited number of contact individuals. Strains isolated from MSM most often fall into phylogenetic clusters. The presence of clusters from mixed transmission groups indicates that there is a bridge through which viruses were transmitted from one group to another. There is a possibility that some individuals have not self-reported their actual HIV risk-related behavior. Our study contributes to the clarification of the epidemiological characteristics of HIV-1 subtype B transmission in Bulgaria and highlights the importance of detailed molecular-epidemiological surveillance of the HIV-1 infection among vulnerable groups.

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High Genetic Diversity and Unequal Distribution of HIV-1 Subtypes among Vulnerable Groups in Bulgaria (Preliminary Analysis)

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Background: In Bulgaria 1242 cases with HIV/AIDS were diagnosed from 2012 to 2017. The number of HIV-infected men who have sex with men (MSM) has increased in recent years, while the populations of people who inject drugs (PWIDs) with HIV have been decreasing. Epidemiological data indicated great heterogeneity of HIV-1 positive populations, including 493 (39.7%) heterosexuals (HET), 560 (45.1%) MSM, 151 (12.2%) PWIDs, 28 (2.3%) MSM who inject drugs and 10 (0.8%) infants infected by vertical transmission. The aim in this national representative study was to analyze the diversity among newly diagnosed HIV-1 infected individuals in Bulgaria for the period 2012 - 2017.

Materials & Methods: HIV-1 pol sequences were generated with TruGene and/or ViroSeq Genotyping Systems. HIV-1 subtypes were defined using COMET v2.2. The sequence alignment contained Bulgarian sequences and reference sequences from the Los Alamos database. Manual phylogenetic analysis and possible transmission clusters were inferred by ML analysis using IQ-Tree program.

Results: 642 (51.7%) HIV-1 pol sequences were obtained and analyzed. Men dominated significantly with 536 (83.5%) while women were 106 (16.5%). The main transmission groups were HET with n=241 (37.5%), 311 (48.4%) MSM, 70 (10.9%) PWIDs, 13 (2.0%) were MSM who inject drugs and 7 (1.1%) infants infected by vertical transmission. The main HIV-1 clade was defined as subtype B n=388 (60.4%) followed by 11 different HIV-1 subtypes including: 85 (13,2%) CRF 01_AE, 40 (6.2%) CRF 02_AG, 38 (5.9%) A1, 33 (5.1%) F1, 31 (4.8%) unclassified and 7 other subtypes and CRFs representing 4.2% of all individuals in the study. The most prevalent subtype among HET was B with 53.5%, followed by CRF 01_AE 10.8%, and CRF 02_AG 7.9%. The most prevalent subtype in MSM was B with 80.4%, followed by subtypes A1 and F1 respectively with 7.5% and 4.5%. Among PWIDs most widespread were CRF 01_AE, CRF 02_AG and subtype B respectively with 64.3%, 14.3% and 7.1%. Among MSM who inject drugs the prevailing subtypes were CRF 01_AE 46.2%, CRF 02_AG 38.5% and subtype B with 15.4%. In the infected infants the most widespread HIV-1 subtypes were CRF 01_AE 42.9%, subtype B 28.6% and CRF 02_AG 14.3%.

Conclusions: Our analysis identified a significant proportion of diagnosed MSM with HIV-1 in recent years. The most prevalent subtypes were B and the recombinants: CRFs 01_AE and 02_AG. High genetic diversity and unequal distribution of HIV-1 subtypes in the vulnerable groups were found. The biggest subtype diversity was introduced and disseminated in HET transmission group, while among MSM subtype B dominated with higher proportion. Our study highlights the importance of sustained molecular-epidemiological surveillance of the dynamics of HIV-1 infection among the most vulnerable groups.

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Time trends in HIV-1 diversity in Croatia: a follow up on HIV-1 subtype distribution

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Background: The geographical distribution and prevalence of HIV-1 subtypes in Europe are highly heterogeneous. Subtype B is predominant in Western and Central Europe but non-B subtypes, introduced mainly via migration, are also present in the region. Croatia is a small Central European country with distribution of HIV-1 subtypes similar to that of other European countries dominated by subtype B. Several studies on HIV-1 subtype distribution have been published in Croatia so far that show that the most common HIV-1 subtype is subtype B. Molecular analysis of HIV subtypes in period 2001-2003 showed a high prevalence of subtype B (>74%) with non-B subtypes found only in heterosexuals. A study on transmitted-drug resistance (TDR) in newly diagnosed HIV-infected patients conducted in the period 2006-2008 showed a high prevalence of subtype B among MSM population with only 11 % of patients infected with non-B subtypes. Subtype B was also confirmed as predominant in respond-driven sampling (RDS) study among MSM conducted in 2006 and 2010. The aim of this study is to show a more recent data on of HIV-1 subtype distribution in newly diagnosed patients in Croatia and compare it with previous years.

Materials and methods: The study included all newly diagnosed HIV-1 patients during 2014-2017. The mean age was 36.3 (0-67) years and males accounted for 95.3% of the study population (385/404). The most common route of transmission was MSM (87.9%, 355/404), followed by heterosexual transmission (10.4%, 42/404), IDU (0.5%, 2/404) and mother-to-child transmission (MTCT) (0.5%, 2/404). Partial pol gene sequences were generated from 404 samples and analysed by using REGA HIV-1 subtyping tool version 3.0.

Results: Subtype B was detected in 368 samples (91.2%), followed by sub-subtype A1 (4.2%, n= 19), subtype C (1.73% n=7), CRF02_AG (0.7%, n= 3), CRF06_CPX (0.5%, n=2), recombinant forms A1-C (0.5%, n=2), A1-G (0.25%, n=1), A1-B (0.25%, n=1) and (CRF) 01_AE (0.25%, n=1). Within the subtype B group there was a predominance of males belonging to MSM group (82.4%, 333/404). Males accounted for 7.2% (29/404) of non-B subtype infections compared to 1.7% of females (7/404). According to other risk groups, 8.7% (n=35) of subtype B infections were due to heterosexual, IDU or MTCT transmission compared to 3.5 % (n=14) of non-B subtype infections.

Conclusions: Croatia belongs in the group of Balkan countries with the highest prevalence of subtype B but significant number of non-B subtypes was introduced into the population from different sources. The present study confirmed that the epidemic in Croatia was predominantly affecting MSM infected with subtype B but the appearance of different non-B subtypes demonstrate molecular heterogeneity of HIV-infections in Croatia. Heterosexual and IDU risk group are still linked to infection with non-B subtypes but we observed a recent rise of non-B subtypes, (sub-subtype A1 being most prevalent) in MSM risk group, most probably caused by traveling abroad. Despite the increased spread of various non-B subtypes, the origin and risk group to which the patient is linked still remain a good subtype prediction.

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Lamivudine-based maintenance 2-drugs regimens: an algorithm for the estimation of 2-years risk of virological failure in clinical practice

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Background: Dual therapies (DT) with lamivudine (3TC) plus either a boosted protease inhibitor (PI) or dolutegravir (DTG) have shown good efficacy as maintenance antiretroviral therapies (ARVs) but some patients (pts) could be at risk of virological rebound.

Material and methods: We retrospectively evaluated the predictors of virological failure (VF, i.e. a single HIV-RNA≥1000 cp/mL or 2 consecutive HIV-RNA≥50 cp/mL) in virologically-suppressed pts (i.e., with undetectable HIV-RNA according to the centre-specific threshold) from 3 clinical centres switching to 3TC plus either boosted darunavir/r (bDRV), atazanavir/r (bATV), lopinavir (bLPV) or DTG from any other ARV. Multivariate Cox regression was used to identify predictors of VF at 2 yrs. The Cox model was then transformed into a point-based rule, by approximating the contribution of specific risk factors in determining VF and by estimating the overall risk of VF at 2 yrs for different scores (as described by Sullivan et al., Statist. Med. 2004; 23:1631–1660). The discriminatory power of the prediction rule was expressed as the area under the receiver-operator characteristic curve (ROC AUC).

Results: Overall, 703 pts were eligible for the study: 17.2% of them started bATV, 37.6% bDRV, 4.1% bLPV, 41.1% DTG. They were mostly men (70.1%), reporting heterosexual (40.8%) intercourses as risk factor for HIV, with 50 yrs of median age, 11 yrs since HIV diagnosis and 8 yrs of cumulative ARV exposure. Characteristics of study population are summarized in table 1.
Over 2 years of median follow-up time, 58 VFs occurred (4.1 VFs per 100 pt-years of follow-up). Non-B viral subtype (vs B, aHR 3.06, p=0.002), CD4 count nadir (per 100 cells/mm3 more, aHR 0.83, p=0.063), residual HIV-RNA (per 1 cp/mL more, aHR 1.03, p=0.002), yrs since HIV diagnosis (per 1 yr more, aHR 1.08, p<0.001) and time with HIV-RNA<50 cp/mL before switching to the DT (per 1 month more, aHR 0.99, p=0.011) independently predicted VF. Considering the risk associated with an increase of 10 cp/mL of baseline residual HIV-RNA as the reference point, 4 points were attributed to non-B subtype, -2 to 1 point to different nadir CD4 categories, 0 to 3 points to different residual HIV-RNA categories, -1 to 4 points to different HIV duration categories, -4 to 2 points to different months of virological suppression at the DT start. A point total<0 and ≥11 reflected a risk of VF at 2-yrs <0% and ≥20%, respectively (table 2). The ROC AUC was 0.70 (IQR, 0.63-0.76; see fig. 1). The cut-point to maximise Youden's J was 5, corresponding to a sensitivity, specificity, positive and negative predictive values for predicting VF of 65.5%, 65.7%, 14.7% and 95.5%, respectively.

Conclusions: Some viro-immunological characteristics of pts switching to a 3TC-based maintenance DT can predict the risk of VF and could be incorporated in a clinically-useful algorithm. Validation of the derived score in a different cohort is necessary to confirm these results.

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HIV drug resistance and distribution of genotypes among ART-naïve patients in Uzbekistan.

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Background: In 2018, to study the prevalence of pretreatment HIV drug resistance, 180 blood samples were collected among adult naïve-patients in Uzbekistan for genotyping HIV and evaluating HIV drug resistance. The results were compared with the results of a similar study conducted in 2015. Patients of both studies are proportionally collected from 15 clinics located throughout the country;

Materials & Methods: In total, 367 sequences HIV-naïve patients were obtained (158 of them are from samples of 2018, 209 from 2015). Then a phylogenetic analysis of the HIV subtypes was performed. SDRMs were determined using the Calibrated Population Resistance Tool.

Results: The overall prevalence of SDRM was the same in 2015 and in 2018 and was 3.8%. The structure of the SDRM also has not changed: the non-polymorfic K103N mutation is most common for NNRT and reduced susceptibility to NVP and EFV for 2.5% patients; M41L (TAMs), K65R (It reduces TDF, ABC and ddI susceptibility), M184I (are selected by 3TC/FTC and reduce susceptibility to these drugs >100-fold) mutations observed for NRTI in single cases; M46L mutation detected for PI(M46I/L are associated with reduced susceptibility to ATV, FPV, IDV, LPV and NFV) in 1% cases. But the distribution of genotypes has changed. In 2015, the HIV-1 subtypes frequency found in the studied population were 55.0% of CRF_02AG, 37.3% subtype A6, 1.4% subtype B, 0.5% subtype C and 5.8% of recombinant forms. In 2018 the subtype frequency were 48.7% of A6, 38.6% CRF_02AG, 1.3% subtype C, 0.6% subtype G, 0.6% subtype B and 10.2% of recombinant forms.

Conclusions: Genotype CRF_02AG for a long time was the most common in Uzbekistan. The increase in the occurrence of A6 genotype compared to CRF_02AG can be associated with an increase of population mobility, since A6 genotype is the most common in neighboring countries. The prevalence of resistance mutations found in this research is considered to be low; therefore, performing genotyping tests before initiating antiretroviral therapy cannot be yet recommended.

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Molecular-genetic characteristics of occult hepatitis B in Uzbekistan

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Background: The aim of the study was to evaluate the characteristics of isolates causing the occult form of hepatitis B. We have previously studied the occult form of hepatitis B among patients with viral hepatitis C and cryptogenic liver cirrhosis by identifying ccc DNA of HBV in samples;

Materials & Methods: 51 liver biopsies and blood plasma was collected from patients admitted to the intensive care department in a serious condition, including 6 patients with hepatitis B + C (control group), 20 biopsies from patients with hepatitis C, 25 biopsies from patients with cryptogenic cirrhosis were collected. All patients were from different regions of the country. The nucleotide sequences of Pre-S1 / Pre-S2 / S regions for 32 isolates were obtained (6 from patients with hepatitis B + C, 26 from patients with occult form of hepatitis B). The primary analysis of the obtained fragment was performed using the NCBI Blast program in comparison with the nucleotide sequences presented to the GenBank. To align the nucleotide sequences and phylogenetic analysis used the Mega 6 software.

Results: The phylogenetic analysis of all 32 isolates showed the prevalence of D genotype, which is the most common in Central Asia. At the same time, the D1 subtype prevailed - 84.38% compared with the D2 subtype - 3.12% and the D3 subtype - 12.5%. The nucleotide identity in the group was 97.65±0.4%. There was no correlation between genotype of virus and the geographic region. Thus, patients with the D3 subtype, whose intragroup percent nucleotide identity was more than 99%, came from different regions of the country. The prevalence of genotypes and subtypes in different groups was associated with the paths of transmission.

Conclusions: The prevalence of subtype D1 was detected among patients in serios condition. Genotype D of HBV than other genotypes can cause a more severe disease and a higher level of drug resistance. Subtype D1 is characterized by low viral load and early HBeAg seroconversion, which can create problems for the well-timed detection of the virus and lead to the development of a more serious condition in patients.

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The Risk of Hepatitis B and C Virus Infection in HIV Positive Individuals Attending General Hospitals in Southwestern Nigeria

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Background: Triple infection of Hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) can results in increased hepatic complications. This study aimed to evaluate the prevalence of HBV and HCV in HIV infected individuals on HAART attending general hospitals in southwestern Nigeria.

Materials and Methods: Ethical approval was obtained from Ministry of Health, Nigeria and data was fetched through an informed consent questionnaire. A total of 891 HIV infected individuals participated in the study. Samples were tested for hepatitis B surface antigen (HBsAg) and anti-HCV antibodies by rapid assay and later confirmed with enzyme linked Immunosorbent assay (ELISA). Hepatitis B e antigen (HBeAg) and anti-HBe antibodies were tested on HBsAg positive samples. Quantification of HBVDNA was performed with quantitative real-time PCR. HBV-DNA and HCV-RNA were extracted from each sample and subjected to polymerase Chain reaction (PCR) using specific primers and PCR conditions. Each PCR products was then electrophoresed on 1.5% agarose gel. Data was analyzed using packages within SPSS software and p-values less than 0.05 was considered significant.

Results: Triple infection of HBV, HCV and HIV was seen in 27 (3.03%). Co-infection of HIV with of HBeAg and anti-HBe antibodies was seen in 108 (12.1%) and 297 (33.3%) respectively. Serum concentration of ALT and AST were higher in those with triple infection than those with co-infection. Average CD4 count in triple infection was 136cell/mm3 compare to 201cell/mm3 of those with co-infection. Averagely, the HBV viral load in triple infection was 59copies/ml compares to 70copies/ml in co-infection in the OBI samples. The mean average age is 27 years. Sexual promiscuity, blood transfusion history and multiple sex partners were significantly associated with triple infection (p=0.04; p=0.05 and p=0.049) respectively.

Conclusion: This study found high prevalence of triple infection and co-infection of HIV, HBV and HCV among study population. This is alarming; therefore HBV and HCV screening must be compulsory included in routine screening of HIV positive individuals in Nigeria. Also liver enzymes must be closely monitored in those with triple and co-infection

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