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Abstracts

1

Molecular surveillance reveals a recent outbreak of HIV-1 subtype C infections among people who inject drugs (PWID) in Munich, Germany

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Background: Needle sharing among PWID can result in a rapid regional spread of an individual HIV-1 variant that forms a cluster in phylogenetic analyses. Notable HIV-1 outbreaks have been identified recently in several countries and have raised significant public health attention. In order to monitor the HIV-variants circulating in Germany and their phylogenies, we have established a national molecular surveillance that enables the identification of recent regional sub-epidemics.

Methods: Dried serum spots from newly diagnosed HIV cases were routinely received together with statutory notification data. Samples were genotyped in the HIV-pol-region and the subtype determined. The cases were classified as "recent infections" (<155 days) by a `Recent Infection Test Algorithm´. Phylogenies were analyzed using IQ-TREE and BEAST software. HCV coinfections were determined using an ELISA (Monolisa HCV Ag-Ab ULTRA V2, Bio-Rad) and an in-house real-time RT-PCR targeting a sequence located in the 5'UTR.

Results: In total 1,879 newly diagnosed HIV-1 infections from 2013-2016 were sequenced. Subtyping revealed a >75% increase in the proportion of subtype C infections in 2016. In both, maximum likelihood (ML) phylogenetic trees and maximum clade credibility (MCC) trees, a distinct cluster of 23 subtype C sequences was identified. Three of the clustering infections were diagnosed in 2015 and the remaining in 2016. 22 cases were reported from the city of Munich and one from the nearby city of Augsburg. 18/23 of the individuals reported to be of German origin and 21/23 assumed to have acquired the infection in Germany. A large proportion (16/23) reported intra-venous drug use as the presumed mode of infection and in 21/23 individuals a resolved or acute/chronic HCV co-infection has been determined along with the HIV-1 infection.

Conclusion: We have identified a recent regional outbreak of at least 23 HIV-1 subtype C infections in Bavaria. The reported mode of transmission and the high HCV seroprevalence suggest that this is a cluster among PWID. The molecular surveillance of newly diagnosed HIV-1 infections reported from Bavaria has been intensified in 2017. The sequences from 2017 are currently being analyzed and the results will be presented at the conference. An association of the outbreak cases with frequent intra-venous application of psychoactive substances (synthetic cathinones, known as "Badesalze") is regarded as likely.

2

Is there a need for HCV resistance testing in routine diagnostics and patient treatment? - Routine HCV genotyping and resistance testing and performance of the Sentosa SQ HCV Genotyping v2.0 assay

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Background: The needfulness of HCV resistance testing in routine diagnostics is still a subject of debate. We use the Sentosa® SQ HCV Genotyping Assay v2.0 from Vela diagnostics a next generation sequencing-based test for identification of HCV genotypes 1 to 6 and the detection of resistance associated substitutions (RAS). Prevalence of RAS in samples from our cohort in Berlin is reported here.

Material/methods: We used the Sentosa® SQ HCV Genotyping Assay v2.0 for genotyping and resistance detection in the NS3, NS5A and NS5B genes of HCV for so far 220 samples amongst others within the PEPSI study(1). The further enhancement of version 2.0 is the extended length of sequence for NS5B starting at aminoacid (aa) 1 (instead of aa 339 in version 1) up to aa 565, and the inclusion of genotype 3 (version1 only 1a/1b) in resistance analysis. Beside the included sequence analysis in the Vela system we interpreted the raw data using the torrent suite version 5.6 to map the sequences. We generated sequences with a minimum of 100/20/4 reads for each base with individual minority cut-offs at 30/20/15/10/5 and 2%. Sequence interpretation was performed with the geno2pheno [hcv] 0.92 online tool (http://hcv.geno2pheno.org). Predicted genotype and resistance mutation for NS3, NS5A and NS5B were analysed.

Results: Sequences for 219/220 samples could be generated with declining quality in low viral loads (<1000 IU/mL) with drop outs for single genes (NS3 23/220, NS5A 6/220 and NS5B 3/220) mainly in non-1, non-3 genotypes. Genotype distribution was 1a (80), 3a (63), 1b (40), 4d (15), 2b (9), 4a (6), 6a (3) and each of 1g, 2c, 2k and 4o. No resistance mutations were detected in genotypes 2b/c/k, 4a/d/o and 6a. Overall NS3, NS5A and NS5B genes showed RAS in 26.4%, 16% and 8.8%, respectively. 26 of 40 1b genotype samples showed resistance, two samples were resistant to three classes. 40/80 genotype 1a samples had resistance mutations, mainly NS3 Q80K (30x) but also NS5A (6x 28VT and 4x 93HN) and NS5B (2x556G; 316Y, 553V and 556R, one each). Ten out of 63 samples with genotype 3a had NS5A mutations (6x 30K, 2x 30V and 4x 93H), except one 80K the genotype 3a samples had no NS3 or NS5B mutations. Overall 132 RAS were detected. (63, 45, 24 for NS3, NS5A and NS5B) including 24 RAS in minorities with a cut-off below 10% and greater than 2% of population.

Conclusions: The Sentosa® SQ HCV Genotyping Assay v2.0 performed excellent in the tested samples. A relative high proportion of investigated sequences showed RAS at a minority cut-off of 10%. This high percentage of resistance increased only a little lowering the cut-off range to 5 or 2%. Despite the low percentage these viral quasispecies were detected in a relevant absolute quantity. RAS were spread over all three genes and resulted in restriction for single drugs or hole drug-classes. The relatively high rates of RAS clearly indicate a resistance analyses before starting a HCV-therapy to avoid therapy failures and unnecessary given drugs avoiding side-effects and costs.

3a

The prevalence of resistance associated substitutes through Europe

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Background: Treatment of hepatitis C virus (HCV) infections has substantially improved with direct antiviral agents (DAA)introduction. Although DAAs are very effective, virological failure may occur in the presence of resistance associated substitutions (RASs). RASs can remain present for a prolonged period of time jeopardizing future treatment options. Little is known about RASs that mediate resistance, due to the small number of treatment failures in clinical studies. Therefore, we have established a European collective, Hepatitis C antiviral therapy failure registry (HepCare), to gather resistance data in a larger population. In this study we use data from HepCare to measure the prevalence of treatment emerging RASs in Europe.

Material & Methods: We extracted data of patients who previously failed DAA therapy. Geno – and subtype were provided by submitters and mostly based on in-house assays. They were reassessed using Geno2Pheno-HCV. All sequences were aligned and trimmed to equal length with their reference strain using bio-edit. We considered RASs to be relevant if they were associated with DAA failure in vivo previously reported in literature. These RASs were scored at position 36, 54, 56, 80, 155, 156, 168, and 170 for NS3 and at position 28, 30, 31, 32, 58, 92 and 93 for NS5A. Single positions that were not covered by the sequence were excluded from the analysis. Chimeric HCV genomes were also excluded from the analysis. We compared NS3 and NS5A sequences from 811 patients from six different European countries. We analysed a total of 232 genotype 1a, 266 genotype 1b, 17 genotype 2c, 124 genotype 3a, and 172 genotype 4a/d patients. For every particular gene segment we limited our analysis to those patient who received an inhibitor who targeted that gene.

Results: Our results showed that the prevalence of particular RASs strongly depend on genotype. The most frequently found NS3 RASs were the R155K 37.4(29.4-46.5), Y56F 26.1(19.8-33.6), D168V 100(34.2 - 100), Q80K 20(3.6-62.4)and, D168E 11.1 (2.0 - 43.5) in genotypes 1a, 1b, 2c, 3a, 4a/d respectively. The most surprising result was the high frequency of the Q80K RASs that are associated with resistance to protease inhibitors that are not recommended for genotype 3a. The overall prevalence of NS3 RASs varied between 20 – 66% depending on the genotype. The most frequently found NS5A RASs were the Q30R 33.6(26.5-41.5), L31M 25(4.6 – 69.9) and, T58P 64.2(50.7-75.7) in genotypes 1a, 2c and 4a/d respectively. The Y93H was the most common RAS for both genotype 1b 69.8(61.4-77.0) and 3a 63.8(54.3-72.4). Interestingly, we found the C92S mutation among genotype 2c failures. This RAS has only been described in vitro as resulting in resistance against second generation NS5A inhibitors. The overall prevalence NS5A RAS varied between 70-100% depending on the genotype.

Conclusions: RASs can occur with a high overall prevalence among DAA treatment failures varying between 20-66% and 70-100% for NS3 and NS5A, respectively. The position of RASs depends of the HCV geno- and subtype. Assessment of the presence of RAS after DAA failure is important before starting retreatment in order to avoid virological failure.

3b

The European prevalence of hepatitis C virus NS5A polymorphisms

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Background: Direct antiviral agents (DAA) against hepatitis C virus (HCV) are potent and highly efficient. The NS5A protein is a very attractive target for therapeutic intervention and several NS5A protein inhibitors are widely available. However, the effectiveness of NS5A protein inhibitors can be jeopardized by the presence of naturally occurring mutations. Due to the high genetic variability of the HCV the distribution of the polymorphisms differ between geographic region and genotype. In addition, the impact on the DAAs differ between viral geno- and subtype. In order to assess the prevalence of NS5A polymorphisms throughout Europe, we used data from the European Hepatitis C antiviral treatment failure registry (Hepcare).

Materials & Methods: We have established a European HCV cohort in which sequences were combined with clinical and virological data of adult HCV infected patients from who a baseline sequence is available. Geno – and subtype which were provided by the submitter and mostly based in house assays were reassessed using Geno2Pheno-HCV. All sequences were aligned and trimmed to equal length with their reference strain using bio-edit. We considered polymorphisms to be relevant if they were associated with DAA failure in vivo, previously reported in literature. These polymorphisms were located on position 28, 30, 31 and 93. Single positions that were not covered by the sequence were excluded from the analysis. Chimeric HCV genomes were also excluded from the analysis. The prevalence of naturally occurring RASs at baseline was evaluated among genotype 1a, 1b, 3a, and 4a/d.

Results: We analysed sequences of 810 patients from five different European countries (Italy, the Netherlands, Spain, Slovenia and, Poland). We analysed a total of 360 genotype 1a, 210 genotype 1b, 12 genotype 2c , 159 genotype 3a, and 81 genotype 4a/d DAA- naïve patients. Our results show that the most common polymorphism in genotype 1a was the M28V 6.9%(95% CI 4.7 – 9.9) which is associated with resistance against both first and second generation NS5A inhibitors. The L31M was the most common polymorphism for both genotype 1b and 2c, 6.2% (3.7 – 10.3) and 16.7% (4.7 – 44.8), respectively. The L31M reduces the effect of all first and second generation NS5A inhibitors besides pibrentasvir. The Y93H has the highest prevalence in genotype 3a 8.2% (4.9 – 13.6) and confers in a high level of resistance to daclatasvir and velpatasvir. The L30R was most common in genotype 4a/d 70.4% (59.7 – 79.2) and reduces the effect of ombitasvir and ledipasvir. The overall prevalence of at least one NS5A polymorphism ranges between 25 – 80% depending on the genotype.

Conclusions: Naturally occurring RASs are common in Europe and can vary over HCV genotype. Fortunately, due to the high potency of the second generation DAAs, these RASs become less clinically relevant. However, if the prevalence of a resistance polymorphism is too high, baseline testing may be recommended.

4

A 5 amino-acid insertion in the C-terminal region of HIV-2 integrase impacts phenotypic susceptibility to the five integrase inhibitors

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Background: Integrase strand-transfer inhibitors (INSTI) represent an important therapeutic option in HIV-2-infection, as the number of active ARV is limited. We recently observed that a 5 amino-acid insertion in the integrase region of HIV-2 was selected in patients experiencing virological failure (VF) on raltegravir (RAL)-based regimens. We aimed to assess phenotypic susceptibility of those mutated-viruses to 5 INSTI and to explore the dynamics of acquisition of this mutation profile.

Methods: Six HOIV-2-infected patients harbouring insertion in integrase were identified in our HIV-2 sequences database. Phenotypic susceptibility assays were performed on 4 HIV-2 isolates obtained from 3 patients with integrase insertion, as well as on HIV-2 ROD reference strain, using the ANRS peripheral blood mononuclear cells method. Two isolates were obtained from 2 patients receiving a RAL-based regimen and two longitudinal isolates were retrieved from one patient at VF of RAL- and of dolutegravir (DTG)-based regimen, respectively. Susceptibility was determined for bictegravir (BIC), cabotegravir (CAB), DTG, elvitegravir (EVG) and RAL. Viruses were cultured without ARV and with five 10-fold dilutions of drug, ranging from 1000 to 0.1 nM. At days 3 or 4, supernatant was withdrawn to assess viral replication (Biocentric HIV-2 RNA®). Phenotypic susceptibility was expressed as IC50 fold-changes between isolate and HIV-2 ROD reference strain. In order to determine the dynamics of acquisition of this insertion, we measured plasma drug concentrations (C24h) using UPLC-MS/MS on samples collected 6 months before detection of insertion. We also calculated the Phenotypic Inhibitory Quotients (PIQ), by dividing INSTI concentration at time of isolation by IC50; and the Genotypic Susceptibility Score (GSS).

Results: All 6 HIV-2 integrase-mutated isolates presented similar 5 amino-acids insertion in the integrase C-terminal region (S/Y-R-E-G-R/K). The 4 isolates phenotypically tested presented high fold-changes to RAL and EVG ranging from 20 to >300-fold, and intermediate fold-changes to DTG and CAB ranging from 3 to 17-fold and 11 to 58-fold, respectively. Fold-changes to BIC remain unchanged (0.4 and 1.1-fold) or moderately increased (3 and 5-fold). PIQ ranged from 0.72 to 2.08 for RAL and from 278 to 382 for DTG.
Retrospective longitudinal genotypic analyses of viruses from these 6 patients showed a selection of these insertion always occurring in the 15 months after RAL initiation (median = 12 months), despite INSTI plasma concentrations in the expected ranges. Median viral load at VF was 7,650 copies/mL (IQR=1,010-23,500) and median GSS was 2, including RAL. For one patient, an intermediate integrase genotypic profile showing a 2 amino-acids insertion (G-K) at codon 232 was detected before selecting the 5 amino-acids insertion as in other patients.

Conclusions: We describe a new INSTI-resistance pathway in HIV-2-infected patients with an insertion of 5 amino-acids in the integrase. This insertion is selected at VF of RAL-based regimen. It severely impacts RAL and EVG in vitro phenotypic susceptibility but might also compromises CAB, DTG and, in a lesser extent, BIC next generation INSTI susceptibility.

5

Dynamics of therapy options for HIV-1 infected patients with historical multi-drug resistance (MDR), based on deep-sequencing of proviral DNA – First Results: from the LOWER Study

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Background: Data on patients with multidrug resistance (MDR) and with long-standing viral suppression (VS) is scarce, particularly when focusing on changes in drug resistance mutation patterns.
The LOWER study analyzes clinical and virological characteristics of HIV-1 infected patients with MDR to antiretroviral therapy (ART).
In this sub-analysis, deep-sequencing (DS) was performed on proviral DNA in patients with VS (<50 cop/ml in plasma) and was compared with cumulative resistance patterns available from all historical resistance reports.

Material & methods: This nation-wide study has enrolled patients with extended resistance in 13 large HIV centres in Germany. For inclusion, signed informed consent and documented evidence of major resistance-associated mutations (RAMs) to ≥3 ART classes of NRTIs, NNRTIs, PIs or INSTIs were mandated.
Proviral DS was performed for the protease, reverse transcriptase, and integrase genes.
Historical drug resistance tests were analyzed in a cumulative manner using the HIV-GRADE interpretation tool. Genotypic susceptibility (active drug scores, ADS) was calculated for both, cumulative viral- and current proviral drug resistance tests using different minority cutoffs. For ADS calculation a "susceptible" result was scored as 1, whereas "limited susceptibility" or "intermediate resistance" was scored with 0.5.

Results: Of 243 patients (218 males, 25 females) with documented extended resistance, 208 (85.6%) had RAMs affecting NRTIs, NNRTIs and PIs, while 12 (4.9%) had RAMs affecting NRTIs, INSTIs and NNRTIs or PIs. The remaining 23 (9.5%) patients had RAMs affecting all four classes. For this sub-analysis, DS data was available for 193 patients with VS, and another 30 patients with non-virological suppression.
DS found 64.7% of historically reported mutations with a DS cutoff of 2%, whereas a Sanger-like cutoff of 15% found only 47.2%.
Using historical drug resistance tests, the median ADS was 2.5 with regard to the current treatment. The same result was achieved when applying DS cutoffs <10%. For Sanger-comparable cutoffs ≥10%, it increased to 3.0.
Genotypic susceptibility to all currently approved drugs increased from 6.7 (historical) to 11.5 (2% cutoff), and further to 14.1 (15% cutoff).
This potential shift to more drug options is mainly due to the non-detectability of RAMs like M184V (204 in historical reports, 133 for 2% cutoff, 99 for 15% cutoff), K103N (135, 70, 49), L90M (148, 99, 75).

Conclusions: In this sub-analysis of the LOWER study, we analyzed a putative regain in therapy options in patients with MDR but long-standing VS by comparing proviral DS with cumulative resistance patterns available from all historical resistance reports. Cumulative analyses from historical RNA resistance tests provided a more complete picture on ever emerged RAMs than DS from proviral DNA. DS detected between half and two-thirds of cumulative detected RAMs depending on the used cutoff. However, whether this "loss" of RAMs may also be explained by a true turnover of the proviral archive leading to a regain in therapeutic options or an incomplete determination of the viral archive, remains unclear.

6

Resistance mutations to different classes of antiretroviral agents predict virological failure in HIV+ patients with low level viremia: a retrospective study from the ARCA cohort

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Background: More than 90% of HIV-infected patients on combination antiretroviral therapy (cART) achieve viral control. However, a minority of patients on cART presents episodes of persistent or transient detectable low level viremia (LLV). The LLV does not always lead to negative clinical outcomes, albeit it may occasionally anticipates virological failure (VF). Here we aimed to evaluate the drug resistance mutations and other factors associated with VF in successfully treated HIV-infected patients with transient episodes of detectable LLV.

Material and methods: A retrospective study was conducted on HIV-positive patients extracted from ARCA database. We selected patients with virological suppression (<50copies/mL) on stable cART in a period ranging from 2009 to 2017 with a minimum follow-up of 6 months, starting from the first of two consecutive LLV (as defined by HIV-RNA between 50-200copies/mL) within 6 months, up to VF (first determination of HIV-RNA>200 copies/mL). Data were censored at last available follow-up, at regimen discontinuation for any cause, at loss to follow-up or at the time of death. Potential explanatory variables associated with VF were explored by Cox regression.

Results: A total of 449 patients were considered for the analysis: 73% were males, 80% carried a HIV-1 B subtype. At baseline median age was 49 years (interquartile range, IQR, 43-53), median time from HIV diagnosis was 14 years (IQR 7-20), median time on ART was 10 years (IQR 3-15), median follow-up was 3 years (IQR 2-6), mean CD4 count 478 cells/µl (95%CI 446-509), nadir CD4 count 165 (95%CI 152-177) cells/µl, median HIV-RNA (VL) was 79 copies/ml (IQR 62-115). Most of patients was on a three-drug regimen (42% PIs-based, 19% NNRTIs-based and 4% INIs-based); 17% was on a two-drug regimen. Data about HCV were available for 319 patients and 126 of them (40%) resulted co-infected. Historical genotypes were provided for 336 (74.8%) patients. Ninety-six subjects out of 188 (52.1%) with regimen containing PI carried the major PI-resistance mutations. NRTI-associated resistance mutations were present in 177/276 (64.1%) subjects with regimen containing one or two NRTIs among ABC, 3TC, TDF or FTC. 12/57 (21.1%) subjects on therapy with NNRTIs carried mutations for this class of agents. Finally only one INI-resistance mutation, N155H, was detected in 66 subjects with INI-based regimen. Twenty-two patients (4.9%) experienced VF: cumulative incidence of VF was 1.3 per 100 patients-year of follow-up. By Cox regression analysis only the presence of resistance mutations I50V for PIs, K65R for NRTIs and N155H for INIs were related to the risk of VF at univariate analysis after Bonferroni correction test (p≤0.012). No other clinical, demographical and viro-immunological variables, nor different calendar periods were predictors of VF.

Conclusions: In HIV-infected patients experiencing LLV during cART, previous genotypic resistance test could have a prognostic value in predicting VF and represents a valuable tool to guide treatment switch or intensification, even in the era of new generation antiretroviral drugs.

7

Dynamics of HIV DNA populations before and after transplantation with CCR5Δ32 stem cells

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Background: Cure of HIV infection was observed in the Berlin patient following stem cell transplantation (SCT) with homozygous CCR5Δ32 donor cells. In contrast, in the Boston patients, transplanted with cells from regular CCR5WT donors, HIV rebound occurred after treatment interruption despite loss of detectable HIV-DNA in PBMCs. It is unknown which reservoir fueled HIV rebound.

Methods: IciStem is an International consortium which guides clinicians of HIV infected patients who require an SCT and investigates the potential for HIV cure following stem cell transplantation. To date 34 patients have been registered to the consortium. Here we study HIV persistence in detail in patient #5 in whom SCT was performed using homozygous CCR5Δ32 cord blood combined with a third party donor. Before SCT we performed: 1) Phenotypic and genotypic coreceptor tropism analysis, 2) HIV reservoir quantification using ddPCR and viral characterization using deep-sequencing of PBMCs, CD4 -T-cell subsets (Tn, Tcm, Ttm, Teff) and bonemarrow, 3) Single copy assay (SCA) on plasma. Post-SCT viral dynamics were analyzed using ddPCR and SCA. The post-mortem viral reservoir was quantified using ddPCR and characterized using deep-sequencing.

Results: Patient #5 was on effective cART for 5 years harboring subtype B CCR5-tropic HIV-1 (FPR:68.8-96.2%). Before SCT, HIV-RNA could be detected in plasma (15 c/mL). HIV-DNA LTR copies were detected in PBMCs (1967 c/10^6), Tn cells (1270 c/10^6), all memory T-cells (Tcm, Ttm and Teff, 3074, 5564 and 6924 c/10^6) and bonemarrow (1130 c/10^6). Deep-sequencing revealed that two viral variants dominate all T-cell populations and bonemarrow (variant 1: FPR 87.2%; variant 2: 89.7%). Four weeks post-SCT, complete donor chimerism was observed in PBMC, HIV-DNA diminished to undetectable levels (< 1 c/10^6) and no HIV-RNA could be detected in plasma. Ten weeks post-SCT patient #5 deceased. Post-mortem analysis revealed presence of HIV-DNA LTR copies in ileum (549 c/10^6), liver (54 c/10^6), spleen (44 c/10^6) and lung (62 c/10^6), whereas no HIV-DNA LTR copies could be detected in PBMCs (< 7 c/10^6). HIV-sequences obtained from ileum and lung revealed the dominance of sequence variant 2 in both tissues.

Conclusions: In the neutropenic phase early post-SCT, HIV-DNA could no longer be detected in PBMCs. In contrast, dominant HIV-DNA populations as present in different T-cell subsets before SCT persisted in tissues indicating that tissue reservoirs may play an important role as long-standing viral reservoirs.

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Factors associated with virological response and resistance profile in HIV-1 infected patients starting first-line integrase inhibitors based regimen in clinical settings

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Background: We evaluated the virological response and the resistance profile in patients starting a first-line combined antiretroviral therapy (cART) containing integrase inhibitors (INIs) in clinical settings.

Materials & Methods: Survival analyses were used to assess probability and predictors of virological success (VS: viremia <50 copies/mL) and virological rebound (VR: two consecutive viremia >50 copies/mL or one viremia >1000 copies/mL after VS) after cART start. The presence of major resistance mutations (MRMs) to PIs, NRTIs, NNRTIs and INIs was evaluated at baseline and at virological failure (viremia >50 copies/mL under INI-treatment if VS was never achieved or after VR).

Results: Overall, 536 cART-naive patients were analyzed. At baseline, median [IQR] viremia and CD4 cell count were 5.1 [4.5-5.7] log_{10} copies/mL and 373 [179-565] cells/mm³, respectively. The proportion of patients receiving raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) was 51.1%, 25.8% and 23.1%, respectively. Concerning companion drugs, 68.1% of patients received 2 NRTIs, 28.4% PI+2NRTIs, and 3.5% other combinations.

At baseline, the proportion of patients with transmitted drug resistance (TDR) was 15.9%. Among patients with an available pre-cART integrase genotypic resistance test (GRT, N=270), no INI MRMs were detected, while 5.4% of patients had at least one accessory resistance mutation.

By one year from INI start, the probability of achieving VS was 93%. By increasing pre-cART viremia levels, patients showed an increased median time [95% CI, Confidence interval] of achieving VS (<100,000 vs. 100,000-500,000 vs. >500,000 copies/mL: 1.4 [1.1-1.7] vs. 3.0 [2.6-3.5] vs. 4.9 [3.6-6.2] months, P<0.001), with a similar probability of VS by 1 year (≥91%).

By 24 months after achieving VS, the overall probability of VR was 14%. Patients with pre-cART viremia levels >100,000 copies/mL showed a higher probability of experiencing VR compared to the others (<100,000 vs. 100,000-500,000 vs. >500,000 copies/mL: 6.5% vs. 21.2% vs. 20.7%, P=0.018).

By Cox multivariable analyses, adjusting for demographic, viro-immological and therapeutic factors, high viremia levels (compared to <100,000 copies/mL) were associated with both a lower adjusted hazard (AHR) of VS (pre-cART viremia, copies/mL: 100,000-500,000: 0.52 [0.41-0.66]; >500,000: 0.35 [0.26-0.47], P<0.001) and a higher AHR of VR (pre-cART viremia, copies/mL: 100,000-500,000: 4.19 [1.67-10.51], P=0.002; >500,000: 2.60 [0.89-7.60], P=0.080). Patients starting INI-treatment with TDR showed a lower AHR of achieving VS with a trend toward significance (0.76 [0.58-1.00], P=0.051).

Increased pre-cART cell CD4 count levels were positively associated to VS (AHR per 100 cells higher: 1.13 [1.10-1.17], P<0.001) and negatively associated to VR (AHR per 100 cells higher: 0.81 [0.66-0.99], P=0.044).

Twenty patients (3.8%) had an available GRT at virological failure (treated with: RAL=16; EVG=3; DTG=1). Five patients (25%) harbored at least one INI MRM (under RAL: N155H=1, N155H+Y143C/H/R+G163K=1, Y143R=1, G140G/R/S=1; under EVG: Q148R+G140A=1) and 3 patients (15%) showed M184V and 1 (5%) K70E. No resistance was observed in the unique DTG failing patient.

Conclusions: In clinical practice, patients receiving an INI-based first-line cART achieve and maintain very high rates of virological suppression. High pre-cART viremia (>100,000 copies/mL) and a low CD4 cell count are negative factors associated with virological response that deserve particular attention.

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Characterization of virogical failure in HIV-1 infected patients switching to dual therapy with DTG plus one RTI (NRTI or NNRTI)

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Background: Dolutegravir (DTG) plus a second agent is a feasible dual regimen for virogically suppressed patients but few data on drug resistance associated mutations (DRAMs) selected at virological failure (VF) are currently available. Aim of this study is to describe DRAMs selected in patients who failed dual regimens of DTG plus one nucleoside reverse transcriptase inhibitors (NRTI) or one non-nucleoside reverse transcriptase inhibitors (NNRTI).

Methods: Single-center, observational, retrospective study on HIV-1 infected patients, followed at the Infectious Disease Department of the San Raffaele Scientific Institute, from November 2014 to January 2018, who switched to a dual therapy (DT) based on DTG plus one RTI with HIV-RNA<50 copies/mL and experienced virological failure (VF, defined as HIV-RNA >50 copies/mL in 2 consecutive determinations or a single determination >50 copies/mL followed by ART modification or a single determination >1000 copies/mL). Genotypic resistance tests (GRTs) performed either on RNA or on DNA, either before starting DT or at DT failure were analyzed. Patients were followed until VF or treatment modification.

Results: 350 patients were switched to DTG plus one RTI: 282 to DTG+3TC, 2 to DTG+TDF, 66 to DTG+RPV. DT was discontinued in 65/350 (19%) patients with HIV-RNA<50 copies/mL; VF occurred in 20 (6%) patients: 4.9% on DTG+3TC, 7.6% on DTG+RPV. The overall follow up was 1.21 years (IQR 0.64-1.68); VF occurred after a median of 1.1 years (IQR 0.49-1.41) in patients receiving DTG+3TC and after a median of 0.58 years (IQR 0.39-0.61) in those receiving DTG+RPV.
Among patients with VF, GRT was not available neither before nor at DT failure in four. Three had GRT data both before and at DT failure: in one patient with a previous documented NNRTI resistance (K103N/Y181C), we found integrase (INSTI) resistance (G140GA/Q148QR) at VF of RPV+DTG. In one receiving 3TC+DTG the GRT performed at failure confirmed DRAM found in historical GRTs (M184MV/T215CNSY). In a third not adherent patients treated with RPV+DTG there was no emergent RT or INSTI resistance mutations at failure.
Eight patients had GRT available before starting DT: one, failed on DTG+TDF, had a documented M184V DRAM and in one, failed on DTG+3TC, historical GRTs showed the M41L, L210W, T215Y and M184V DRAMs; in the other six, historical GRTs did not show any DRAM that could jeopardize the efficacy of the failing DT.
In five patients the GRT was performed only at failure: it showed only the M184V DRAM in one patient failing on 3TC+DTG dual regimen, while no DRAM for RTIs or INSTI was found in the others.

Conclusions: VF of a DT based on DTG plus one RTI was infrequent; it occurred also in patients harboring WT HIV. DRAMs emerging at failure of these DTs were those predictable on the basis of failing drugs. Historical GRTs should always be carefully evaluated before switching to DTs based on DTG.

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Mutations in the 3'-polypurine tract of HIV-1 point to a new integrase strand transfer inhibitor (INSTI) resistance mechanism in vivo.

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Background: It was recently shown in vitro that mutations in the 3'-polypurine tract (3'-PPT) of HIV-1 result in high level resistance to INSTIs (Malet, Mbio, 2017). We investigated whether mutations in the 3'-PPT also emerged in patients with virologic failure (VF) during treatment with INSTIs. To this end, we sequenced the 3'-PPT in patients who experienced VF during the randomized controlled DOMONO study on dolutegravir (DTG) maintenance monotherapy.

Materials & Methods: Patients who experienced VF, defined as a confirmed plasma HIV-RNA ≥ 200 cop/mL, during the DOMONO main study (n=8) and its pilot study (n=2) were included. Inclusion criteria for the main study were: CD4 T-cell nadir ≥ 200 cells/mm3, HIV-RNA zenith < 100.000 cop/mL, no previous VF and/or documented resistance-associated-mutations (RAMs). Pilot study inclusion criteria were as in the main study except that CD4 T-cell nadir was < 200 cells/mm3. Sanger sequences of the 3'-PPT were generated using plasma samples during VF and pre-cART plasma samples (baseline).

Results: In 9/10 patients, no mutations were detected in the 3'-PPT at baseline nor during VF. In one patient, who experienced confirmed VF (plasma HIV-RNA 313 cop/mL and 798 cop/mL) 24 weeks after start of DTG maintenance monotherapy, mutations were detected in the 3'-PPT during VF (AAAAGAAAAGGGAGC) which were not present at baseline (AAAAGAAAAGGGGGG). No INSTI-RAMs were detected in integrase during VF and the DTG plasma level during VF was adequate (5.31 mg/L, 19 hours after last intake).

Conclusions: Mutations in the highly conserved 3'-PPT point to a new INSTI resistance mechanism in vivo. We hypothesize that mutations in the 3'-PPT result in mutated terminal bases of the 5'-LTR of HIV-DNA which is substrate for integrase. Alternatively, mutations in the 3'-PPT may result in replication of unintegrated HIV-1 (Malet, Mbio, 2017).

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Tenofovir versus Tenofovir plus Entecavir in the Treatment of Chronic Hepatitis B in Patients with Poor Efficacy of Nucleoside/Nucleotide Analogs

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Purpose: We aimed to compare the viral suppressive efficacy of tenofovir disoproxil fumarate (TDF)
mono-rescue therapy (TDF group) and TDF plus entecavir (ETV) combination-rescue
therapy (TDF + ETV group) in chronic hepatitis B (CHB) patients with a poor response to nucleoside/nucleotide analogs.

Methods: We retrospectively analyzed 266 chronic hepatitis B patients with a primary non-response, partial response, or virologic breakthrough on their current nucleoside/nucleotide analogs therapy and who were switched to TDF monotherapy (245 mg) or TDF (245 mg) plus ETV (1 mg) combination therapy and treated at least 48 weeks. Viral response and viral breakthrough during therapy were analyzed.

Results: At baseline, median age was 37.0 years, 87.1% were HBeAg(+), and median HBV DNA was 4.24 (range, 2.11–6.73) log10 IU/ml. 192 patients were treated with TDF and 74 patients were treated with TDF+ETV for at least 48 weeks. There were no significant differences between the two groups in demographic characteristics. We estimated the virologic response rate (HBV DNA level <60 IU/mL) between the two groups and defined the predictive factors of treatment outcome. Up to 48 weeks [median: 144 (range 48-192) weeks], 87.6% and 91.4% of the TDF group and TDF + ETV group, respectively, achieved a virologic response (P=.062). Only the HBV DNA level at baseline was significantly associated with a virologic response in the multivariate analysis. In a subanalysis of patients with HBV DNA levels ≥4 log (IU/mL) at baseline, a higher proportion of patients in the TDF + ETV group than the TDF group attained a virologic response (95.1% vs 70.5%; P<.001), while 94% of patients with HBV DNA levels <4 log(IU/mL) in all both TDF and TDF + ETV groups attained a virologic response. No significant differences were found in alanine aminotransferase normalization, hepatitis B e antigen loss, hepatitis B e antigen seroconversion, virologic breakthrough, and tolerability between the two groups at weeks 24 and 48.

Conclusion: We conclude that TDF/ETV combination therapy was not associated with higher rate of virologic response compared with TDF therapy in nucleoside/nucleotide analogs experienced patients who have failed prior nucleoside/nucleotide analogs therapy. On the other hand, the combination therapy should be thinked of in patients with high baseline HBV DNA levels.

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Selection of fitness-associated substitutions in patients failing NS5A inhibitors based therapy : analysis of HCV full-length genome deep sequencing by means of shotgun metagenomics

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Background and Aims: When treatment fails, HCV NS5A inhibitors select Resistance Associated substitutions (RASs) located in domain I of the NS5A protein. NS5A RASs persist for many years after DAA-containing treatment failure. By analogy to HIV or HBV resistance, it is thought that compensatory or fitness-associated substitutions are also selected during treatment and could at least partly explain the long-term persistence of NS5A RASs. However, very limited information on compensatory mutations selected under antiviral therapy has been generated thus far, principally due to the sequencing methods available that characterize short genome fragments only. Here, we used an original shotgun metagenomics method based on deep sequencing of full-length HCV genomes to characterize amino acid substitutions selected by DAA therapy in regions targeted or not targeted by the NS5A inhibitors in patients who fail to achieve SVR.

Method: Using shotgun metagenomics, we generated the full-length HCV genome sequences at treatment initiation and at virological relapse in 12 patients who failed to achieve SVR on an NS5A-containing regimen (sofosbuvir+daclatasvir, sofosbuvir+ledipasvir). Briefly, universal DNA/RNA extraction was performed followed by library preparation using Total RNA and Nextera XT kit (Illumina). Deep sequencing was performed by means of NextSeq500 (Illumina). Full-length HCV sequences at baseline and at virological relapse were analyzed using our original in-house MetaMIC® software (quality, filtering, identification, genome reconstruction and comparison) and the sequences were analyzed using a 15% cutoff, according to EASL guidelines. Candidate substitutions selected in at least two GT-3 infected patients have been characterized in vitro (conferred level of resistance, effect on the replicative fitness) in an HCV Gt-3 infectious model.

Results: A significant relationship was found between the HCV RNA levels and the number of equivalent HCV genome sequences generated ($p<0.01$; $r2=0.91$). Sequential analysis of full-length genome sequences identified selection of known RASs in NS5A domain I targeted by the inhibitors, but also of a number of substitutions in other genomic regions (core, E1, E2, NS3, NS4B, NS5A domain III). The majority of failures occurred in patients infected with genotype 3 (n=5) and genotype 4 (n=4). In genotype-3 infected patients, the following amino acid substitutions were selected together with NS5A RASs: in NS3 T98S or T98A, NS4B T48A and G114S, NS5A N448S and NS5B K114R. In vitro phenotypic assays revealed that NS3 T98S, T98A and NS5A N448S increased viral fitness (134%, 549 % and 864% respectively). NS4B G114S showed conserved viral replication (contrasting with poor fitness of NS4B G114 virus).

Conclusion: Using an original shotgun metagenomics method based on deep sequencing that generates full-length HCV sequences, we could detect amino acid substitutions independently selected in the NS3, NS4B, NS5A (domain III) or NS5B regions in patients failing sofosbuvir plus an NS5A inhibitor. Most of these changes increased viral replication in vitro suggesting that they may compensate for losses of viral replication capacity conferred by selected RASs (e.g. Y93H) in vivo.

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Evaluation of pre-treatment risk factors associated with failure in HCV-infected patients naive to direct acting antivirals: particular focus on natural resistance

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Background: This study aimed to evaluate the presence of natural resistance-associated-substitutions (RASs) and other pre-treatment risk-factors for failure (as high HCV-RNA, cirrhosis, previous-interferon (IFN) treatment) in a large group of HCV-infected patients naive to direct-acting-antivirals (DAA) with an available outcome after their first-line recommended NS5A inhibitor-containing regimen in Italy.

Material & methods: RASs in NS3/NS5A/NS5B (N=1493/1172/937) were analysed in 1589 DAA-naïve patients. Of them, 303 had an available outcome after first-line of a recommended NS5A inhibitor-containing regimen and at least a baseline NS5A-test. Sanger-sequencing of NS3/NS5A/NS5B was performed by home-made protocols. Potential differences between the sustained-virological-response (SVR) and virological-failure group were evaluated by Fisher's exact test. A multivariable logistic-regression analysis, adjusted for cirrhosis, previous IFN-treatment, baseline HCV-RNA, at least one RAS regimen-related, and genotype, was performed to define risk-factors associated to treatment-response.

Results: Overall, 484/1589 (30.5%) patients showed at least one natural RASs, particularly NS5A-RAS was observed in 17.9% of patients. 303 patients (GT1a[90]-GT1b[97]-GT2c[15]-3a[85]-4a/d[16]) had an available outcome (258 with a SVR and 45 with a virological-failure) after the following recommended NS5A-containing regimen: daclatasvir/ledipasvir/velpatasvir+sofosbuvir +/- ribavirin (N=103/71/12), 3D/2D (paritaprevir/ritonavir+ombitasvir±dasabuvir)+/- ribavirin (N=81/5), grazoprevir+elbasvir+/-ribavirin (N=31). By analysing retrospectively the baseline samples, a different distribution of risk-factors was observed among patients with a SVR and patients who experienced a virologcal-failure. In particular, a higher prevalence of natural NS5A-RASs was observed before treatment in DAA-failures (17/45, 37.8%) vs SVR-patients (33/258, 12.8%; P<0.001). Notably, ≥2 risk factors for failure were more frequently observed at baseline among patients who experienced a virological-failure to a DAA treatment (33/45, 73.3%) compared to those achieving SVR (111/258, 43.0%, P<0.001).

Furthermore, by multivariable logistic-regression, baseline HCV-RNA >800.000 IU/ml and presence of at least 1 natural RAS regimen-related were both negatively associated to SVR (adjusted odd ratios [95%C.I.]: 0.47 [0.16-0.86], P=0.021; 0.25 [0.11-0.58], P=0.001; respectively). No other risk-factors were associated to SVR.
In particular, baseline HCV-RNA >800.000 IU/ml and natural RAS regimen-related were more frequently observed before treatment in GT1 3D+/-ribavirin-failures (10/13, 76.9%; 9/13, 69.2%) and in GT3 daclatasvir+sofosbuvir+/-ribavirin-failures (8/10, 80.0%; 3/10, 30.0%) vs SVR-patients (3D: 13/38, 34.2%; 13/38, 34.2%, daclatasvir+sofosbuvir: 19/71, 26.8%; 4/71, 5.6%, respectively, P<0.05). No specific associations were found with the 71 GT1/GT4 ledipasvir+sofosbuvir+/-ribavirin treated patients (54 SVR, 17 virological-failure).
Moreover, all 15 GT2 patients (12 treated for 12 weeks with daclatasvir+sofosbuvir and 3 with velpatasvir+sofosbuvir+/-ribavirin) achieved SVR, regardless of baseline risk-factors and presence of NS5A-RAS F28C. Similarly, all 30 GT1 patients treated with grazoprevir+elbasvir+/-ribavirin achieved SVR, regardless of baseline risk-factors and length duration, while the only patient with GT4a relapsed after 12 week of treatment with ribavirin, without any risk-factor at baseline. Finally, all 12 GT1-3 patients treated with sofosbuvir+velpatasvir+/-ribavirin achieved SVR. The majority were without (or only 1) risk-factor: notably none of them showed baseline RASs regimen-related.

Conclusions: Even if these data are preliminary and the collection of new information regarding the treatment outcome is still on going, the presence of specific pre-treatment risk-factor, such as RAS regimen-related and baseline HCV-RNA >800.000 IU/ml were associated with virological failure for some specific regimens and genotypes.

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Can HBV-RNA be useful as a serological marker in patients with antiviral treatment? Presence of HBV-RNA and its correlation with other serological markers.

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Background: Circulating HBV-RNA was first described in 1996 in serum of HBV patients. It has been postulated as a new serological marker of cccDNA transcriptional activity, useful for monitoring patients with nucleoside analogues (NA)-based treatment. Recently it was observed that HBV-RNA levels could predict the seroconversion of HBeAg in patients treated with NAs or interferon. In addition, HBV-RNA levels could predict viral rebound after treatment discontinuation. This study aims to quantify circulating HBV-RNA in the serum of patients with chronic hepatitis B (CHB) during NA-treatment and correlate it with HBV-DNA and quantitative HBsAg (qHBsAg).

Materials & Methods: The study included 54 serum samples of 18 CHB mono-infected patients, naïve later treated with NAs for at least 2 years. Viral nucleic acids were extracted from serum samples with DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Circulating HBV-RNA, HBV-DNA and qHBsAg were respectively quantified through RT-qPCR with RACE primers [LightCycler® 480 Instrument II, limit of detection (LOD) 2.2 logIU/mL], qPCR (COBAS 6800, LOD 1 logIU/mL) and chemiluminiscence (COBAS 8000, LOD 0.05 IU/mL) at different time points: baseline (pre-treatment), first year and second year of treatment. A Spearman test was implemented in order to study the correlation between HBV-RNA and the other serological markers studied.

Results: 15/18 patients were HBeAg(-) and 3/18 were HBeAg(+). At baseline, median (IQR) serum HBV DNA was 4.9 (1.5-8.2) logIU/mL, median (IQR) ALT was 57 (14-151) IU/L and median (IQR) of serum qHBsAg was 4 (1.9-5.1) logIU/mL. About treatment, 15/18 patients received tenofovir and 3/18 received entecavir. At baseline, HBV-DNA and qHBsAg were detected in all patients, while HBV-RNA was detected in 8 (44%) patients [5/15, 33%, HBeAg(-) patients and 3/3, 100%, HBeAg(+)]. HBV-RNA strongly and positively correlated (p=0.002, r=0.9286) with HBV-DNA at this time point. No correlation was detected between HBV-RNA and qHBsAg. During treatment, HBV-DNA sensibly decreased reaching undetectable levels (<1 log IU/ml) in 15/18 (83%) patients. On the other hand, HBV-RNA levels also decreased but persisted with values higher than HBV-DNA levels.

Conclusions: Circulating HBV-RNA was detected at baseline in 33% of HBeAg(-) patients and in 100% of HBeAg(+) patients. At this time point, HBV-RNA significantly correlated with serum HBV-DNA. During treatment with NAs, HBV-RNA levels seemed to decrease slower than HBV-DNA and a little but consistent amount was still observed after two years of treatment. In summary, HBV-RNA could be a new marker for monitoring HBV naïve and treated patients. Of note, the high limit of detection of the assay makes difficult to study the correlation with other serological markers and with liver disease progression in presence of treatment. Therefore, technical improvements of HBV-RNA quantification assay are required.

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ABX464, by binding to and stabilizing the CBC 80/20 complex, enhances pre-mRNA splicing, resulting in the generation of novel HIV-derived RNA species and in increased expression of the anti-inflammatory microRNA miR124

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ABX464 is a first-in-class, clinical stage small molecule that was shown to bind to the CBC 80/20 complex with at least three biological effects when given to HIV patients: 1) an antiviral effect, 2) a reduction in HIV-DNA in PBMCs, and 3) an increase of anti-inflammatory miR124 in rectal tissue. Our previous analysis of splicing of cellular genes in PBMCs showed that ABX464 had a negligible effect on pre-mRNA splicing of cellular genes, consistent with the observation that these are already maximally spliced. To test whether ABX464 influences the splicing of HIV-RNA in infected cells, we performed an array-based sequence capture using customized library probes targeting HIV sequences to eliminate cellular RNA. The probes were used to capture cDNAs prepared from infected PBMCs (+/-ABX464) of 6 donors. In all ABX464-treated samples, 90% of assembled contigs correspond to spliced RNA. In contrast, in untreated samples spliced RNAs represent only a minor fraction (<10%), while >90% of the viral RNA is full length and unspliced. After filtering and analysis of splicing events in treated samples, 3 novel species of spliced RNA were identified. Interestingly, one of spliced viral RNAs triggered by ABX464 was common between YU2 and Ada8 strains, and no polymorphism along its sequence was detected in B and C subtypes that were strongly inhibited by ABX464. The capacity of this novel RNA to generate immunogenic peptides is currently being tested with plasma from a cohort of HIV treatment-naive patients who were treated with ABX464.

Given that the target of ABX464, the CBC complex, is also involved in the biogenesis of small non-coding RNA including microRNAs, we performed a microarray analysis of these RNAs from 6 healthy donors (PBMCs infected or not and ABX464-treated or not). While infection leads to large variations in the expression of small non-coding RNAs, ABX464 induced a reproducible upregulation of a single microRNA (miR124) in both infected and non-infected cells. Capture of RNAs from the three loci involved in miR124 biogenesis revealed that ABX464 induced the expression of miR124 from the miR124.1 locus but not from miR124.2 and miR124.3 loci. The locus miR124.1 happens to have different long non-coding transcripts that could be responsible for the biogenesis of miR124. ABX464 enhanced the splicing of a single long non-coding RNA in this locus. The destruction of this unspliced long non-coding RNA is faster than Drosha processing, therefore the splicing is important to make the long non-coding RNA visible to microRNA biogenesis machinery to produce miR124. All effects described above were triggered by binding of ABX464 to the CBC 80/20 complex, which resulted in a stabilization of the complex.

Conclusions: Our findings explain why in HIV patients ABX464 not only blocks HIV replication by enhancing pre-mRNA splicing of HIV-RNA, but also how the molecule could potentially trigger the generation of new viral antigens, thereby making HIV-infected cells visible for attack by the immune system. In addition, these findings explain how ABX464 enhances the splicing of a long non-coding RNA to produce anti-inflammatory miR124.

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Comparative Activity of TAF vs. Major Nucleoside Reverse Transcriptase Inhibitors against HIV-1 harboring K65R or Thymidine Analog Mutations, with or without M184V

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Background: Nucleoside reverse transcriptase inhibitors (NRTIs) are an integral part of HIV-1 treatment regimens. Tenofovir alafenamide (TAF), the most recently approved HIV-1 NRTI, is a tenofovir (TFV) prodrug . Compared to tenofovir disoproxil fumarate (TDF), TAF achieves 4-fold higher intracellular levels of TFV-diphosphate (TFV-DP) in target cells in vivo. K65R is a resistance associated mutation (RAM) for many NRTIs, including TAF. Interestingly,resistant viruses harboring K65R rarely emerge after TAF or TDF treatment. Thymidine analog mutations (TAMs) do not emerge during TAF treatment, but pre-existing TAMs do confer resistance to most NRTIs, including TAF and TDF. Patients with such HIV-1 RAMs may benefit from higher levels of TFV-DP delivered by TAF compared to TDF. In addition, the presence of the M184V mutation is known to increase susceptibility to some NRTIs (such as TAF) while conferring resistance to others (such as FTC). Virologic outcome of subjects harboring HIV with combinations of TAMs and M184V are currently being studied clinically. Here, we evaluated the in vitro activity of NRTIs in a large set of K65R-containing or TAM-containing HIV-1, with or without M184V.

Methods: Site-directed mutants (SDM) containing K65R or TAMs (M41L, D67N, K70R, L210W, T215Y, and/or K219Q), +/- M184V (n=98) as well as patient-derived (PD; n=18) mutants with K65R or TAMs were generated. Drug susceptibilities (fold change [FC] EC50 relative to wild-type) were determined for all mutants (n=116) using a 5-day Multi-Cycle PR-RT HIV assay (MT-2 cells). Comparison of TAF and TDF resistance profiles were further assessed in viral breakthrough (VB) experiments utilizing physiologically relevant drug concentrations against this large set of viruses (SDM and PD).

Results: The presence of M184V in K65R- or TAM-containing HIV-1 SDMs (n=49) significantly increased sensitivity to TAF, TDF, and AZT, while significantly decreasing sensitivity to FTC and ABC compared to corresponding SDMs without M184V (n=49) (Wilcoxon signed rank test: p-value of <0.0001). Similar trends were observed with PD mutants with TAMs. The average phenotypic resistance in PD viruses with ≥3 TAMs + M184V (n=6) and K65R + M184V (n=3) was very similar for TAF (2.5-fold vs. 2.8-fold, respectively), while higher resistance was observed for ABC (8.4-fold vs. 16.6-fold, respectively). As expected, FC resistance for TAF and TDF showed a strong 1:1 correlation (r2=0.93). However, when mutants with TAMs (n=68; 54 SDM and 14 PD) were assayed in VB experiments using physiological concentrations of TAF or TDF, 15 mutants were able to break through under TDF treatment (FC ranging from 1.9 to 12.6; viruses with 3 to 6 TAMs ± M184V) and only 3 mutants broke through under TAF treatment (FC ranging from 6.6 to 12.6; viruses with 5 TAMs without M184V).

Conclusions: In the presence of M184V, the antiviral activity of TAF, TDF, and AZT increased in HIV-1 SDM harboring TAMs. TAF and TDF had a very similar resistance profile in the EC50 assay, however, in VB assay mimicking the 4-fold higher intracellular levels of TFV-DP delivered by TAF compared to TDF in vivo, TAF inhibited viral breakthrough of TAMs-containing HIV-1 that were not inhibited by TDF.

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Real world comparison of Standard of Care Triple Therapy vs Dual Therapies in treatment experienced HIV patients with a viral load under 50 copies/ml at regimen initiation, in a large Spanish Cohort - VACH

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Background: Some European guidelines for treatment of HIV have recently included some dual therapy (DT) options as switch strategies in suppressed patients, to simplify regimens or to avoid toxicities. This study assesses persistence and risk of discontinuation due to virological failure and adverse events in treatment experienced HIV patients switching to either triple therapy (TT) or DT with a viral load under 50 copies/ml.

Materials and Methods: A retrospective analysis was performed using data from the VACH cohort - a prospective multicenter Spanish cohort of adult HIV patients. All treatment experienced patients, between 01/01/2012 and 01/06/2017, switching to a new regimen with VL<50 copies/ml, limited to a TT containing an INI (EVG, RAL, DTG) combined with two NRTIs (F/TAF, F/TDF or 3TC/ABC) or a DT containing DTG and/or a PI/r, were included. Unit of analysis was patient-regimen. Time to non-persistence, was defined as the time from patient-regimen initiation to discontinuation (for any reason), loss-to-follow-up, death or censoring, whichever occurred first. Kaplan-Meier curves and Cox proportional hazard models (controlling for demographics, comorbidities, CD4, number of previous regimens, CD4 nadir and years on antiretroviral therapy – all at patient-regimen initiation) were conducted.

Results: A total of 5596 TT and 1386 DT patient-regimens were included. Among TT, the most common regimens were DTG combined with 3TC/ABC (37.0%) and EVG/c/FTC/TAF (27.7%). Among DT 36.2% were DTG containing and 73.2% were PI containing (9.4% DTG+PI). By the end of the observation period 29.3% DT regimens and 22.3% TT regimens had been discontinued. Baseline patient-regimen characteristics differed in the two groups: DT were older and more treatment experienced. When controlling for differences in patient characteristics, a hazard ratio (HR) of 1.23 (p=0.003) on DT vs TT for discontinuation due to any reason was obtained, and of 1.86 (p=0.01) for discontinuation due to virological failure. No differences in the risk for discontinuation due to adverse events was observed (HR=1.15, p=0.29).

Conclusions: In treatment experienced patients suppressed at regimen switch, persistence remains significantly higher in patients on INI-based TT compared to INI or PI based DT, and risk of virological failure in DT is predicted to be 86% higher. No difference in the risk for discontinuation due to adverse events was observed.

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Virological efficacy of antiretroviral regimens containing elvitegravir in treatment-experienced patients in clinical practice

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Background: Integrase inhibitor based-regimens are recommended by current guidelines as first-choice antiretroviral (ARV) therapy. ARV drug resistance mutations remain a major cause of treatment failure. The aim of study was to evaluate the effect of drug mutations on virological efficacy of elvitegravir-containing ARV regimens in treatment-experienced HIV-1 infected patients in a real life setting.

Material and methods: From the ARCA database we selected treatment-experienced HIV-1 infected patients starting tenofovir disoproxil fumarate or alafenamide/emtricitabine/elvitegravir/cobicistat (from July 2012 to October 2017), with baseline PR/RT resistance genotype and at least 1 HIV-1 RNA during follow up. NRTI resistance mutations were defined as the detection of at least one mutation among those included in IAS list (2017). Primary endopoints were virological failure (VF, defined as an HIV-RNA, VL, > 1,000 copies/mL or 2 consecutive values of >50 cps/mL after week 24 for treatment experienced with baseline VL >50 copies/mL and at any time for treatment experienced with baseline values of <50 copies/mL), treatment failure (TF, defined as VF or treatment change for any reason). Survival analysis was used to investigate predictors of VF.

Results: We included 221 patients: 71% were males, 37% heterosexuals, 7% non Caucasians, with median age 48 years (IQR, 41-53), 10 years of HIV (5-19), 7 years of ART (2-16), 23 months since last VL >50 cps/mL (5-62), nadir CD4+ 165 cells/µl (51-307), baseline CD4+ 577 cell/µl (322-794), 78% had viral subtype B; 25% presented at least one NRTI mutation, 21% at least one mutation among M184V/I or K65R or TAM, 70% had VL <50 cps/mL. The elvitegravir-including treatments were started for VF in 26% and overall 59% experienced at least 1 VF in previous history; GSS of current regimen was 2 (IQR 1.75-2). During a median observation time of 44 wks (21-76), 30 VF occurred; the estimated probability of VF at 48 wks was 13% (10-16) among patients with any NRTI mutation vs 10% (5-15) among those without. During a median observation time of 39 wks (17-70), TF occurred in 50 patients, with an overall estimated probability of TF at 48 wks of 20% (17-23). At multivariate analysis adjusting for VF during the last regimen, pre-BL and any NRTI mutation, only HIV risk factor (heterosexuals vs others, aHR 0.28, CI 95% 0.09-0.86, p=0.026) and longer duration of viral suppression (aHR 0.97, CI 95% 0.95-0.99, p=0.018) resulted to be associated with lower risk of VF.

Conclusions: Elvitegravir-containing ARV regimens resulted in a high virologic suppression. Similar virologic success rates were achieved irrespective of the presence of pre-existing resistance mutations.

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A reflex testing programme for diagnosing active HCV infection in Spain.

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Background and aim: Undiagnosed HCV infection and/or inadequate linkage to care are barriers to HCV elimination. Reflex testing has proven to increase linkage to care, access to treatment, and viral eradication. In our study we intend to evaluate the impact of the implementation of a reflex-testing program in Andalucía, Southern Spain.

Patients and methods: We have conducted an observational, ambispective study across diagnostic laboratories responsible for HCV diagnosis in Andalucia, Southern Spain. First, we have retrospectively studied how many patients were not linked to care during 2016 (pre-Reflex cohort), and we have evaluated the prevalence of active HCV infection; second, we have evaluated the impact of reflex testing in 13 Andalucian diagnostic centres, that was implemented through this program in September 2017.

Results: The pre-reflex cohort included information from 13 hospitals and 1053 patients, 68 % men, with a median age of 52 years (IQR=45-59). Diagnosis of HCV was screened from Primary Care (41 %), Hospital Practitioners-excluding Hepatologists & Infectious Disease doctors- (37%), and prison or addiction centres (13 %). Slightly more than half of the patients (n = 580; 55 %) visited a specialist for treatment evaluation, in a median time of 71 days (IQR = 35-134) since date of diagnosis. To estimate the prevalence of active infection, 804 patients from 16 centres were eligible and 533 (66.3%) were viraemic (HCV RNA or Core Ag). The post-reflex cohort started running in October 2017: all participating centres have already joined the program, and so far the proportion of patients that have been linked to care has increased to 79%.

Conclusions: During 2016, nearly half of HCV new diagnoses in Andalucia were not linked to care. Two thirds of the anti-HCV antibody positive patients had active infection by HCV. Reflex testing has been effectively implemented during 2017 and has improved linkage to care. This programme will help micro-elimination of hepatitis C virus in Andalucía.

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National Quality Control and Validation of Hepatitis C NS3, NS5A and NS5B Genotypic Resistance Testing in Italy

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Background: International guidelines currently recommend HCV genotypic resistance testing (GRT) to be performed in selected cases before starting treatment and/or after failure. However, GRT application is limited in many countries by the lack of a commercial assay. As an integral part of the Italian VIRONET C network, we conducted a multicentre HCV Sanger GRT quality control study, with the aim of providing a standardized GRT at National level.

Material & methods: A panel of 10 blinded clinical samples carrying HCV genotypes (GT)1a-1b-2c-3a-4a-4d, with a median (IQR) HCV-RNA of 5.8 (5.6-5.9) log IU/ml, was provided to 21 laboratories (lab) for NS3, NS5A and NS5B GRT by Sanger sequencing. Illumina next-generation sequencing (NGS) was performed at another lab and used as reference with 15% detection cut-off. The Geno2pheno tool was used for detection of resistance associated substitutions (RASs).

Results: Sixteen labs out of 21 generated all the 30 expected sequences, while the remaining 5 generated a mean ± SD of 23.4± 3.9 sequences. Fourteen labs used the same Sanger protocol while all the others used a unique system. Geno2pheno identified a total of 7 RASs in the 30 sequences generated by NGS. The overall Sanger accuracy (defined as correct calls at all the codons involved in drug resistance in the geno2pheno rules) with respect to the reference NGS data was 93.5% for NS3, 95.2% for NS5A and 89.5% for NS5B. The majority of the participating labs detected all the NS3, NS5A, NS5B RASs identified by NGS with prevalence >15%. Among the 16 labs that provided all 30 HCV sequences, with respect to NGS results, 12 labs had 0-1 RAS discordance, 3 labs had 2 discordances, and 1 lab had 4 discordances. The majority of RAS discordances were found in 4 samples: 1 GT1a with NS5A M28V missing in 11/20 labs (99% prevalence by NGS); 1 GT1b with NS5A L31M missing in 2/19 labs (100% prevalence by NGS) and NS5A Y93H/Y detected by 2 labs but not by NGS; 1 GT2c with NS3 D168E missing in 3 labs (96% prevalence by NGS); 1 GT1a with NS3 Q80K missing in 2 labs (96% prevalence by NGS). Interestingly, 7/19 labs identified a minority NS3 Q168K RAS in one GT3 sample, detected at 7.8% prevalence by NGS.

Conclusion: The majority of the 21 labs participating to the Italian VIRONET C network provided high quality HCV GRT results with Sanger sequencing for all circulating HCV GTs and all clinically relevant genes. However, 5/21 labs should still improve the success rate of sequencing for specific genotypes or specific genes. Accuracy and inter-laboratory precision were affected by the use of different methods, highlighting the challenge of HCV variability. Quality control programs for HCV GRT should be promoted to allow its fruitful use in optimizing HCV treatment.

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LTR-5' genetic variability affects HIV-DNA burden during cART

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Background: Previous in vitro studies show that an increased genetic variability in HIV-1 LTR-5' U3 (known to contain the most critical transcription factor binding sites) promotes and modulates HIV-1 transcriptional activity. Here, we investigate the correlation between U3 heterogeneity and viro-immunological parameters in HIV-1 infected patients receiving antiretroviral therapy (cART).

Materials & Methods: This longitudinal prospective study includes 32 newly diagnosed HIV-1 infected patients (diagnosis: 2015-2017), receiving a first line modern cART (NRTI+INI. N=21; NRTI+NNRTI, N=4; NRTI+PI, N=2; OTHER, N=5). For all patients, plasma viral-load, total HIV-DNA, CD4+T and CD8+T cells are evaluated at baseline (BL), at virological success (VS, defined as viral-load <50 copies/ml), and at 6 months after virological success (6mVS). Total HIV-DNA (log copies/10^6CD4+) and residual viremia (defined as viral load at VS and at 6mVS, copies/ml) are measured by ddPCR. BL LTR5' U3 is analyzed by NGS technology (Illumina). Shannon Entropy weighted for the intra-patient prevalence of viral species (Sn, ranging from 0 [single haplotype] to 1 [presence of >1 haplotype with the same intra-patient prevalence]) defines U3 heterogeneity. Correlations between LTR-5' U3 heterogeneity and HIV-DNA burden, CD4/CD8 ratio, plasma viral-load and residual viremia at BL, VS, and 6mVS are assessed by Pearson correlation and Mann-Whitney test.

Results: At BL, median (IQR) plasma HIV-1 RNA, CD4/CD8 ratio and total HIV-DNA are 4.8 (4.4-5.3) log copies/ml, 0.40 (0.20-0.62), and 4.1 (3.4-4.7) log copies/10^6CD4+, respectively. Median U3 entropy value (Sn) is 0.82 (IQR, 0.75-0.93). VS is achieved after a median time of 7.9 (4.7-19.3) weeks. Median (IQR) total HIV-DNA reduction is 0.60 (0.83-0.33) log copies/10^6CD4+ from BL to VS, and 0.35 (0.74-0.11) log copies/10^6CD4+ from VS to 6mVS, while ultrasensitive plasma HIV-1 RNA is 6.5 (1.6-26) copies/ml at VS and 1.5 (0-24) copies/ml at 6mVS. Looking at the correlations between U3 Sn and viro-immunological parameters at BL of cART, we find that higher U3 entropy values correlate with higher HIV-DNA burden (rho=+0.41, P=0.01), higher plasma viral-load (rho=+0.35, P=0.04) and lower CD4/CD8 ratio (rho=-0.37, P=0.04). Moreover, while at VS total HIV-DNA does not differ between patients characterized by a U3 Sn>0.8 and patients characterized by a U3 Sn<0.8 (median [IQR] value: 3.21 [3.01-3.70] and 3.44 [3.28-3.72] log copies/10^6CD4+, respectively; p=0.26), from VS to 6mV higher U3 entropy values significantly correlates with slighter HIV-DNA reductions (median [IQR] delta HIV-DNA: 0.15 [0.11-0.30] for Sn>0.8 vs. 0.24 [0.10-0.69] for Sn<0.8, P<0.01, rho=-0.44, P=0.02). No significant correlations are found for CD4/CD8 ratio and residual viremia at both VS and 6mVs.

Conclusions: This proof-of-concept study shows that LTR-5' U3 heterogeneity correlates with HIV-DNA burden, plasma viral-load and CD4/CD8 ratio at cART initiation, and may be a factor influencing total HIV-DNA decay during cART.

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Routine HIV-1 drug resistance testing using proviral DNA and impact of stop codons

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Objective: HIV drug resistance testing is increasingly performed by sequencing of HIV-DNA archived in cells, especially to guide clinicians for treatment switching/simplification in long-term well controlled patients or in patients with low on-treatment plasma viral load that does not allow optimal HIV RNA sequencing. The interpretation of DNA-based resistance data is frequently challenged by the presence of stop codons that usually reflect defective, replication-incompetent proviruses. We investigated stop codons in HIV DNA sequences and the impact on routine drug resistance testing.

Materials and methods: This is a monocentric study including ART treated HIV-1 patients who underwent proviral DNA resistance genotyping as routine laboratory monitoring. Total DNA was extracted from whole blood and HIV DNA was quantified using a real-time PCR assay. Sanger sequencing of the reverse transcriptase (RT), protease (PR) and integrase (IN) genes was performed using primers and protocols provided by the French National Agency for AIDS Research (ANRS). Demographics and clinical data of patients were collected from the Nadis® database. The IAS-USA list (2017) was used to identify resistance associated mutations (RAMs) in the different HIV-1 genes.

Results: A total of 226 patients with successful sequencing for RT, PR and IN genes, were included in the analysis. At least one stop codon (SC) was identified in any DNA sequence for 39 patients, including 14 RT sequences, 20 PR sequences and 9 IN sequences. The "SC group" was similar to the "non-SC group" regarding the age, the sex-ratio, the HIV-1 subtype, the time since HIV-1 diagnosis, the time since ART initiation, the CD4 cell count and nadir. In addition the HIV DNA levels were not different between both groups.
The proportion of sequences with at least one RAM was higher in the "SC group" (p=0.0004, 0.05 and 0.0011 for RT RAMs, PR major RAMs and IN RAMs respectively).
In addition, the mean number of RAMs was significantly higher in the "SC group" namely RT RAMs (p<0.0001), major PR RAMs (0.04), and IN RAMs (0.006). The RAMs most commonly associated with SC include M184I (p<0.0001), E138K (p= 0.0018) and M230I (p<0.0001) in the RT gene and G73S (p<0.0001) in the PR gene. As consequence, the presence of SC significantly affects the interpretation for NRTIs such as 3TC/FTC and for NNRTIs.

Conclusion: The presence of SC in HIV DNA sequences appeared not related to the characteristics of HIV infection and to HIV DNA levels. Sequences with SC display a higher proportion of RAMs and significantly affect drug resistance interpretation for some important drugs. No consensus rules are currently available since "replicative variants" can also harbor these mutations. Next generation sequencing associated with specific bioinformatic tools can help for cleaning of "defective variants".

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Retreatment of HCV infected patients with a previous failure to a NS5A inhibitor-containing regimen: Italian real life experience

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Background: Retreatment of HCV-infected patients who fail to achieve a sustained-viral-response (SVR) is currently a major challenge especially when resistance-associated-substitutions (RASs) are present in NS5A and/or in multiple direct-acting-antiviral (DAA) targets. Aim of this study was to analyze Italian real-life data of patients who experienced a virologic-failure to a recommended NS5A inhibitor-containing regimen and their re-treatment.

Materials & Methods: Within the Italian VIRONET-C, a total of 241 patients infected with different HCV genotype/subtype (GT1a/1b/2c/3a-h/4a-d-v=52/80/8/74/27) with a virologic-failure to a NS5A inhibitor-containing regimen recommended by the 2016 guidelines, and available resistance-test at failure, were analyzed. Up today, re-treatment of 25 failures was also investigated. Resistance-test was performed by Sanger-sequencing by home-made validated-protocols.

Results: Overall, failures following six different NS5A-containing regimens were studied: 3D/2D (paritaprevir/ombitasvir+dasabuvir)+ribavirin (N=51/2), daclatasvir/ledipasvir/velpatasvir +sofosbuvir+ribavirin (N=81/101/2), grazoprevir+elbasvir (N=4). Notably, 90.9% of NS5A-failing patients showed at least one NS5A-RAS at failure, the prevalence was particularly higher in GT1b (98.8%) and GT3a (91.7%). Complex NS5A-resistance-patterns were observed in 35.7% of failure, particularly in GT1b (66.2%) and GT4a/d (33.3/17.4%) respectively. Multiclass-resistance was observed in 42.3%. Differently, 9.1% (22/241) of patients failed without NS5A-RAS and 7.8% with also any NS3 and NS5B RASs.
To date, 25 failures (GT1a/1b/3a/4a-d=8/8/5/1/3) started a second-line regimen, 10 of them are still on treatment. The majority was cirrhotic (88.0%) and relapser (80.0%). The overall prevalence of NS5A-RAS before re-treatment was 96.0%. In particular, 1 patient (4.0%) didn't show any NS5A-RAS at failure, 64.0% had only one NS5-RAS and 32.0% showed NS5A complex-patterns. Multiclass-resistance was observed in 44.0% of failures.
Among the 15 patients with available outcome, 80% achieved SVR. In particular, 5 patients (3 GT1b; 1 GT4a; 1 GT4d), showing at least one major NS5A-RAS at failure, were re-treated with simeprevir+sofosbuvir+ribavirin for 12/24 weeks,

and all achieved SVR. None showed any major NS3-RAS at previous failure while 2/5 patients had at least one NS5B-RASs (L159F+C316N and S282T). One GT1a relapser to ledipasvir+sofosbuvir+ribavirin re-treated with grazoprevir+elbasvir+sofosbuvir+ribavirin for 12 weeks achieved SVR. Also this patient showed at failure only one NS5A-RAS (Q30K) without multiclass-resistance. Other 9 patients were retreated with a NS5A+NS5B combination for 24 weeks and 88.9% with also the ribavirin-use. Of them, 6/9 (66.7%) achieved SVR (all had only a single NS5A-RAS at previous-failure). Differently, 3/9 (33.3%) experienced a virological-failure to velpatasvir+sofosbuvir+ribavirin (2 relapser, 1 non-responder). The two relapsers (1 GT1b and 1 GT1a, both cirrhotic) showed before retreatment NS5A complex-patterns involving Y93H-RAS, and the GT1b patient showed also NS5B-resistance (L159F+C316N). The only 1 non-responder (GT1a, probably due to poor-adherence) showed before re-treatment the NS5A-RAS L31M and at re-treatment failure the RAS L31V with an increased resistance.

Conclusions: In this real-life setting, NS5A-RASs were frequently detected at failure with or without presence of multiclass-resistance. According to resistance results and Italian guidelines, the majority of these NS5A-failing patients are still waiting for re-treatment with new DAAs combination with higher genetic barrier for resistance. In the few patients already retreated the overall SVR was high. Our results show how HCV resistance-test at failure may be useful to individualize re-treatment strategies.

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A marked decrease in acquired resistance to antiretrovirals is associated with new drug combinations and latest prescription guidelines

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Background: A reduction of acquired resistance has been recently reported in Europe. However, this hypothesis remains to be evaluated in recent years. We studied resistance and its correlates in failing patients during 2009-2016.

Methods: We analyzed 2,118 HIV-1 pol sequences from 21 Centers of Italian ARCA database. Patients were included when they have viremia >200 copies/mL and received a genotypic test while on treatment for at least 24 weeks. Mutations were identified using IAS-USA 2017 tables. Class resistance was evaluated according to the antiretroviral regimens in use at failure. Four periods of study were evaluated, encompassing years 2009-2010, 2011-2012, 2013-2014 and 2015-2016. Cochran-Armitage, Chi squared and logistic regression models were used.

Results: Males were 67.4%;IDUs, HEs, MSM accounted for 39.1%,34.7%,18.2%, respectively. Subtype B was carried by 86.1% of patients. Among failing patients,23.3% and 22.2% failed first or second line regimen, while 54.5% failed later ones. Resistance to any class decreased from 66.5% to 60.1% (p<.0001) in 2009-2016, while non B subtypes increased from 11.9% to 17.7% (p=.008). Resistance to NRTIs, PIs and INIs declined from 52%, 29.5% and 32.2% to 35.4%, 25.3% and 18.1%, respectively (p<.0001, p=.040 and p<.0001). NNRTI resistance remained stable around 37%. Resistance to at least one class was greater in subjects with non B subtypes (68.8% vs. 61.4%, p=.018), while resistance to NRTIs was higher in subjects with strain B (47.8% vs. 29.8%, p=<.0001) and resistance to PIs was higher in subjects with non B subtypes (49.8% vs. 24%, p=<.0001). The multivariate analysis indicated an increased risk to acquire resistance for patients with a larger number of lines of therapy (p<.0001). Non B subtype was a predictor of resistance (p<.0001). Reduced risk of resistance across all time periods was detected for higher strata of HIV1-RNA levels (p<.0001), as well as in patients failing a regimen in the first biennium of the study (p=.002) and subjects with MSM and IDU modality of infection compared to HE (p<.0001). The risk of resistance was not influenced by gender or age.

Conclusions: A marked reduction of resistance to NRTIs, PIs and INIs was observed over the latest 8 years indicating that new antiretroviral regimens have a higher efficacy, since they also exhibit a better tolerability .As expected, previous failure to therapy still increase the risk of resistance evolution. Our study reinforce the need for surveillance for NNRTI resistance, patients with non B subtypes and experienced patients.

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geno2pheno[ngs-freq]: a web service for identifying drug resistance in HIV-1 and HCV next-generation sequencing samples

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Background: Next -generation sequencing (NGS) is being routinely used for identifying drug resistance in viruses such as HIV-1 and HCV. Current NGS web services use rules-based approaches for analyzing resistance and require the input of raw NGS data whose preprocessing is highly time-intensive. Therefore, we set out to develop geno2pheno[ngs-freq], a web server that uses well-established approaches for analyzing viral drug resistance in preprocessed NGS files.

Materials & Methods: geno2pheno[ngs-freq] is based on the input of frequency files that tabularize the counts of either nucleotides or codons along a viral genome as determined by NGS. Upon input of a frequency file, consensus sequences are constructed using a set of user-defined prevalence cutoffs such that the constructed sequences contain only those nucleotides whose corresponding amino-acid prevalence exceeds a given cutoff. The main steps involved in the analysis of a consensus sequence are described below.
First, the viral species from which the sequence originates is automatically inferred according to pairwise alignments against a set of reference sequences. Next, drug resistance is determined by analyzing the sequence using either the statistical models of geno2pheno[resistance] for HIV-1 or the rules-based approach of geno2pheno[hcv] for HCV. Once the computations have completed, viral resistance for populations at different abundances can be explored by comparing the results for two cutoffs vis-a-vis: the reference cutoff and the personal cutoff. To enable the comparison with results from Sanger sequencing, we set the default value of the reference cutoff at 10% for HIV-1 and at 15% for HCV. The default value of the personal cutoff is set at 2% in order to allow for the investigation of minority resistant populations.
Since we had reimplemented the approach of geno2pheno[hcv], we validated the concordance between the predictions from geno2pheno[ngs-freq] and geno2pheno[hcv] using 2,918 frequency files and their corresponding consensus sequences at the 15% cutoff. Since we had not changed the implementation of geno2pheno[resistance], the 926 frequency files from HIV-1 samples were analyzed only for testing the stability of the web server.

Results: All 3,844 frequency files passed the analysis without errors. Resistance interpretations were obtained for 922 of 926 HIV-1 samples (99.6%) and 2,898 of 2,918 HCV samples (99.3%). The remaining 24 samples (0.6%) could not be analyzed due to low quality. We found an almost perfect concordance (99.7%) between the predictions of geno2pheno[ngs-freq] and geno2pheno[hcv].

Conclusions: We have developed geno2pheno[ngs-freq], a publicly available web server for identifying drug resistance in HIV-1- and HCV samples that were processed with NGS. The new interface, which is based on preprocessed NGS data, does allow not only for the rapid analysis of drug resistance but also for the application of custom NGS preprocessing pipelines. The web service is a useful resource for routine diagnostics as it enables clinicians to make treatment decisions taking into account drug resistance in both minor and dominant viral populations. In the future, clinical studies investigating the impact of minority resistant variants on treatment outcomes could rely on geno2pheno[ngs-freq]. The geno2pheno[ngs-freq] web service is freely available online at http://ngs.geno2pheno.org.

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In vitro susceptibility of CRF02_AG to fostemsavir

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Background: Fostemsavir (FMV) is a prodrug of the HIV-1 attachment inhibitor Temsavir (TMV), currently under investigation for the treatment of highly experienced HIV-1 infected patients with limited treatment options. Data from previous studies indicated a wide range of susceptibility to TMV in a panel of representative clones from different HIV-1 subtypes, where subtype CRF01_AE and group O strains showed natural resistance to TMV. However, most of the data came from subtype B strains and TMV susceptibility of non-B subtypes remains to be clarified. In this work we analyzed the prevalence of TMV resistance associated mutations (RAMs) and the phenotypic susceptibility to TMV in the CRF02_AG, which is the most frequent non-B subtype circulating in Italy.

Materials & Methods: Subtype CRF02_AG env sequences were retrieved from HIV LANL database to determine the frequency of amino acid variants found at RAM codons 116, 204, 375, 426, 434, 475, and 506. The full env gene was amplified from 15 plasma samples and sequenced. The env region was fused with the CMV promoter and used to create pseudotyped viruses together with NL4-3.Luc.R-E- plasmid, an env-defective vector expressing the luciferase reporter gene. We firstly determined the phenotypic viral tropism of pseudotyped viruses by infecting U87-CXCR4 or U87-CCR5 cells +/- coreceptor antagonists AMD3100 and maraviroc, respectively. According to the viral tropism, pseudotyped viruses were used to infect U87-CXCR4 and/or U87-CCR5 cells in presence of serial dilutions of TMV (range 10 µM – 5.12 nM) and after 72 hours luciferase activity was measured to determine IC50 values. Fold change (FC) values were calculated using IC50 values obtained with NL4-3 wild-type virus. To analyze genotype to phenotype correlation, mutants of interest were introduced into the HIV-1 NL4-3 strain through site directed mutagenesis.

Results: In the LANL CRF02_AG dataset, the TMV RAMs M426L, M434I and M475I were found in 4.8%, 16.6% and 1.1% of CRF02_AG circulating strains, respectively, while L116 and V506 were fully conserved. Among 14/15 env sequences used for TMV phenotypic susceptibility assessment, only one harbored a TMV RAM (M434I) but the relative pseudotyped virus was fully susceptible to TMV (FC 0.2). Overall, the median (IQR) fold change value was 6.4 (1.4-8.9). The highest FC was measured for a dual-tropic virus, 51.5 for the X4 and 156.3 for the R5 population, characterized by the uncommon M426P variant. When introduced into the subtype B NL4-3 backbone, the previously characterized TMV resistance M426L mutation and the new M426P showed 21.6 and 6.4 FC values, respectively. By contrast, the rare polymorphic mutations M426K and M426R did not alter TMV susceptibility (FC 1.1 and 0.7, respectively).

Conclusions: The CRF02_AG is generally susceptible to TMV, however occasional variants may display reduced susceptibility. The impact of specific TMV RAMs in the context of high env genetic variability remains to be fully elucidated through phenotypic analysis and in vivo observation.

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Prevalence of resistance to integrase inhibitors in newly diagnosed HIV cases: Results: from the national molecular surveillance, Germany 2014-2017

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Background: Integrase strand transfer inhibitors (INSTI) are a class of antiretroviral drugs designed to block the viral Integrase (INT). Raltegravir was approved in Europe in 2007, followed by elvitegravir (2012) and dolutegravir (2014). The good general tolerance and the low likelihood of resistance selection led to their widespread use and recommendation as first-line option in European guidelines. We aimed to analyze resistance against INSTIs in newly diagnosed HIV-cases between 2014 and 2017 including the prevalence within HIV-subtypes.

Materials & Methods: Within the statutory surveillance system in Germany, we receive dried serum spots (DSS) of about 60% of all HIV new diagnoses. Subsets are processed for HIV-genotyping in INT genomic region and resulting sequences are subtyped, analyzed for the presence of resistance associated mutations according to Stanford HIVdb [v.8.4] and IAS-USA 2017 charts and finally applied to different resistance prediction tools (HIVdb, HIV-GRADE [v.7/17], ANRS [v.9/17], REGA [v 9.1.0]) to calculate prevalence and level (low/intermediate or high) of resistance.

Results: Between January 2014 and June 2017 3,026 of totally 7,414 DSS were processed for genotyping resulting in 1,734 (23%) INT sequences (2014: 92, 2015: 481, 2016: 631, 2017: 530). Distribution of the main subtypes was A: 12.1%, B: 62.2%, C: 7.38%, CRF02_AG: 7.38% and 10.9% other. According to HIVdb six major INSTI mutations (T66I, E92Q, E138AT, G140S, S147G, Q148H) were detected in four cases (0.23%; all subtype B) and eight non-polymorphic accessory mutations (1x H51Y, 1x Q95K, 6x G163RK) in seven (other) cases (0.40%; subtypes A, B, F). Polymorphic accessory resistance-associated mutations T97A (n=18), V151A (n=1), E157Q (n=37) affected 54 cases (3.11%) across all main subtypes to similar proportions, while the highly prevalent L74M/I (n=212) was significantly more frequent in subtype A (118/209, 56.6%) and CRF02_AG (22/128, 17.2%) than in subtype B (54/1081, 5.0%; both p<0.001). According to IAS-USA list the T66I, E92Q, S147G and Q148H mutations are conferring a substantial reduction of susceptibility to raltegravir and elvitegravir in four cases (0.23%) and to dolutegravir in one case (0.05%). Any resistance to raltegravir and elvitegravir was predicted according to: HIVdb for 0.74% (both 13/1,734) of cases, HIV-GRADE for 0.11% (n=2) and 3.34% (n=58), ANRS for 2.24% (n=39) and 3.28% (n=57) and REGA for 1.55% (n=27) and 0.28% (n=5) of cases, respectively. Resistance level differed between interpretation algorithms. For dolutegravir, high level resistance was predicted by HIVdb, GRADE and ANRS for one case in 2016 (0.05%) while according to REGA this case plus a second one (0.11%) were predicted to have intermediate level of resistance.

Conclusion: The prevalence of major or non-polymorphic accessory INSTI mutations as well as predicted resistance to dolutegravir is still very low (<1%) in HIV new diagnoses. However, proportion of predicted resistance to raltegravir and elvitegravir was ranging from 0.12% up to 3.34%, strongly depending on the interpretation tool applied. Molecular surveillance is a useful tool to monitor the emergence of transmitted resistance selected by INSTIs and might help to assess the impact of the frequent polymorphic accessory mutations for resistance in order to adjust interpretation tools.

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Hepatitis C Virus Screening Project of Patients on current anti-HCV Therapy

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Introduction: Clinical outcome of HCV therapy with direct acting antivirals (DAAs) depends on host and viral factors.

Objectives: This observational, retrospective and non-interventional study (PEPSI) collects data of resistance-associated-mutations (RAMs) in the viral NS3/protease, NS5A and NS5B genes to predict clinical outcome using the geno2pheno[HCV] tool.

Materials and methods: Baseline and DAA-Failure sequences were obtained. Subtyping and resistance against all licensed DAAs was determined by interpretation with geno2pheno[HCV].

Results: 2078 HCV-infected patients have been enrolled until Dec 22, 2017. We could obtain 1353 NS5B sequences, which were used for genotyping: GT1a= 41.2%; GT3=20.3%; GT1b=29.0%; GT4d=4.6%; GT2=3.5%; GT4a=1.3%. There were differences between HCV mono-infection and HIV/HCV co-infection. GT-1b is significantly more frequent in mono-infections (31% vs. 10.6% in mono-infections vs. HIV/HCV co-infections, respectively), while GT4d was more prevalent in HIV/HCV co-infections (6.5% vs. 14.3% in mono-infections vs. HIV/HCV co-infections, respectively).
Baseline RAM analysis was performed. Differences in the baseline RAM prevalence were detected depending on the viral GT. The most abundant RAMs in the NS3/protease region were 80K (45% in GT1a; 1% in GT1b), 56F (0% in GT1a; 39% in GT1b), 54S (5.5% in GT1a; 1% in GT1b) and 36L (3.5% in GT1a; 0% in GT1b). In the NS5A, the most frequent amino acids exchanges were 28V (8% in GT1a; 1% in GT1b; 0% in GTs 2 and 4), 30K (7% in GT3a; 0% in GTs 1b, 3 and 4), 30R (14% in GT 4a; 2% in GTs 1a and 3; 0% in GTs 1b and 4) and 93H (8% in GT1b; 4% in GT3a; 2% in GT1a; 0% in GT4). In the NS5B, the mutations 556GNR were found in 14% of the GT1b sequences and in 4% of the GT1a cases. Baseline susceptibility for Harvoni, Epclusa, Vosevi, Zepatier and Maviret was determined (Fig. 1).
14 patients failed their DAA therapy (11x Ledipasvir+sofosbuvir;2xParitaprevir+Ombitasvir+ dasabuvir;1x Asunaprevir+Daklinza). 13/14 samples displayed RAMs leading to high levels of DAA resistance (Fig. 2).

Conclusions: We found a remarkable number of RAMs in baseline sequences depending on the specific GT. Baseline sequencing prior to the HCV therapy with DAAs could support personalized therapy decisions.

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Global dolutegravir failure registry: initiative to gain more insight in virological failure on dolutegravir containing regimens

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Background: The genetic barrier to resistance for dolutegravir is reported to be higher than for first-generation integrase inhibitors (INI). In clinical trials of dolutegravir cART in therapy-naive individuals, selection of resistance in integrase was not observed. In trials in patients with prior failure of INI, a decreased virological response was observed in the presence of multiple integrase resistance mutations at baseline, in particular at position 140 and 148.
In clinical practice, virological failure, although rare, is being observed for both naïve and INI pretreated patients often without selection of obvious resistance mutations. Recent data suggest that resistance may emerge outside the integrase encoding gene in the 3'-polypurine tract (3'-PPT) region. For a better understanding of dolutegravir resistance systematic analysis of otherwise scattered information on individual patient cases of dolutegravir therapy failure is urgently needed.

Methods: The European Society for translational Antiviral Research (ESAR) has opened a global registry to which anonymised clinical cases of failure on dolutegravir containing regimens can be submitted. Submission forms can be retrieved via www.esar-society.eu. If samples are available genotypic sequencing of integrase and 3'-PPT is performed. In selected cases phenotypic integrase susceptibility analysis is performed (MTT assay, expressed as fold change (FC) in IC50 compared to HxB2). Participants receive full reports of results. Currently, 27 cases of dolutegravir failure in patients infected with various HIV-subtypes have been collected.

Results: While the registry is growing several preliminary conclusions can be drawn. Upon failure only secondary resistance associated mutations or mutations with unknown relevance are detected in integrase. In INI-naïve patients therapy failure can be observed in plasma as well as the CNS compartment with low level phenotypic resistance to dolutegravir (FC 1.3-1.6). In INI pretreated patients high level resistance to dolutegravir (FC 160-244) can be observed despite the absence of integrase mutations at position 140 and 148.

Conclusion: Selection of resistance to dolutegravir is not impossible. Considering the envisioned global uptake of dolutegravir comprehensive information on resistance is essential. ESAR invites clinicians to submit cases of dolutegravir failure to this continuous and comprehensive global registry to assist guidelines and interpretation algorithms to ensure appropriate future application of the integrase drug class.

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Failure of Dolutegravir-containing First-line regimen

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Background: The HIV-1 integrase inhibitor Dolutegravir (DTG) has a high barrier to resistance with only few reported cases of failure during first-line therapy. Here we present a case report on a patient receiving a first-line treatment including Emtricitabin (FTC), Tenofovir (TDF) and DTG with evolution of DTG resistance resulted in virological failure with the the R263K and the G118R mutations in the integrase and the M184I/V mutations in the reverse transcriptase.

Material and methods: HIV resistance analysis within the INTEGRATE project was performed before ART initiation and at the time-point of virological failure with increasing viral loads after an initial decline and retrospectively for the analysis of resistance evolution for all available plasma samples. All resistance analyses were performed by Next-Generation-Sequencing (NGS) with the Illumia MySeq technology. In addition, drug levels were determined for DTG, FTC and TDF for all available plasma samples.

Results: We report an HIV-1 subtype F infected patient who developed a virological failure while on a dolutegravir-containing first-line regimen. The patient, a 27 year old MSM, was admitted to the hospital with a disseminated tuberculosis (TB) and a classical profile of an HIV late presenter with high HIV viral loads (>1 Mio copies/ml) and low CD4 counts (22cells/µl). ART with TDF/FTC plus DTG was initiated with an excellent therapy response indicated by a 3 log decline of VL within the first 3 weeks during hospitalization and a slow but stable recovery of the CD4 cells. TB therapy with Rifabutin was initiated 1 week after ART start. Surprisingly, at the first HIV VL control DTG was not detectable and FTC and TDF were only detected in low level concentrations. After discharge from the hospital the HIV VL increased to >300000 copies/ml interpreted as a problem with adherence as VL decreased thereafter continuously down to ~500 copies/ml. Due to an increase in VL greater than 10000 copies/ml 8 months after therapy initiation a resistance analysis was performed, presenting the FTC mutation M184V in the reverse transcriptase and the DTG mutations G118R and R263K in the integrase gene. Retrospective resistance analyses of all available plasma samples presented the DTG resistant strains with the R263K mutation already at the time-point of first viral rebound, while the HIV strains presenting the G118R mutation were first selected in a later stage of resistance development. Both DTG resistance mutations were never detected in equal values by NGS analyses indicating an evolution on different HIV strains. ART drug levels during therapy were mostly sufficient for a potent activity excluding a negative interaction with the TB medication. Thus, the low drug levels at two time-points that resulted in VL increase could be traced back to an incomplete drug adherence and not to drug-drug interactions with Rifabutin.

Conclusions: Resistance to DTG, especially in first-line treatment, is rare but can occur. Risk factors like HIV-1-nonB subtype, HIV VL levels, adherence and coinfections influencing the drug levels can facilitate the selection of DTG resistant strains, in our case the R263K and the G118R.

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Use of Next Generation Sequencing to study a first line failure to a Dolutegravir containing regimen

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Background: Virological failure to Dolutegravir (DTG) containing regimens is a rare event. So far only a few cases have been reported in treatment-experienced individuals, commonly associated with the emergence of R263K in the integrase, a substitution that confers low-level resistance against DTG and diminishes HIV DNA integration and viral fitness. In first line therapy, DTG shows a higher barrier to resistance with no reported mutations at 96 or 148 weeks. Here we report a case of early virological failure in an antiretroviral naïve patient that started a DTG based first line therapy.

Methods & Results: A 49 year old woman was diagnosed of HIV-1 infection while admission in June 19 2017 for lumbar spine surgery. At diagnosis (w0) CD4 count was 39 cells/µl and plasma HIV-1 RNA was 457.000 copies/ml. ART was started one week later with TDF/FTC and DTG 50 mg BID, as she was taking rifampicin. On July 23 (w4), VL was 3461 copies/ml and CD4 increased to 113 cells/µl. On August 22 (w12), rifampicin was removed due to a cutaneous adverse reaction and DTG was given QD. On September 14 (w18), CD4 count was 42 cells/µl and VL had increased to 126.393 copies/ml. Adherence was confirmed both by hospital records and patient interview, and virological failure was confirmed in a second sample (VL 208.518 c/ml).
No resistance mutations were detected at w0. Deep sequencing in RT and Integrase showed a sequential emergence of M184I (40%, w4; 42% w12; 14% w14) and E157Q (4% w12; 8% w14; 20% w18). R263K was detected at a very high prevalence (>97%) at w12, w14 and w18. M184I was replaced by M184V at w12 (57%) and w14 (86%). Treatment was changed to TDF, darunavir/cobicistat (DRV/cob) and rilpivirine (RPV), with a VL decrease to 25 copies/ml and a CD4 increase to 302 cells/µl (w30). Deep sequencing of pol and env revealed infection by a recombinant CRF14_BG form.

Conclusions: To our knowledge, this is the first report of first-line treatment failure with DTG with the selection of mutations in the integrase. Using deep sequencing we were able to trace the development and replacement of mutations. Interestingly we have shown, for the first time in vivo, the restoration of viral fitness that E157Q exerts on R263K containing viruses.

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Hepatitis A Outbreak in Men Having Sex with Men in Israel 2017: Environmental and Clinical Surveillance

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Background: Hepatitis A virus (HAV) affecting men having sex with men (MSM) has recently been reported in European and US cities as well as in Israel. We report the clinical and environmental surveillance of this ongoing HAV outbreak in Israel during 2017.

Method: The tested cohort included all anti-HAV(IgM) positive blood samples reported during 2017 (N=81) and 147 sewage samples collected monthly from 14 facilities around the country;56/81 blood specimen and all sewage samples were analyzed for HAV-RNA by PCR (Altona diagnostics). Sequencing (VP1-2a region) and phylogenetic analysis included comparison with the current 1a European outbreak strains (RIVM_HAV16-90 (EUROPRIDE), VRD_521_2016 (UK/SPAIN) and V16-25801_VP1.

Results: Patients median age: 34y (range 3-56y); M/F 69/24 (85.2% M); 32/69 self-identified as MSM, of whom 24 (75%) were from the Tel-Aviv area as compared to 13/37(35%) of the non-MSM males. HAV vaccination available for 43/81 revealed that 40/43 were unvaccinated. Almost all HAV-positive MSM cases (96%, 27/28) were infected with either RIVM_HAV16-90 (EUROPRIDE) or VRD_521_2016 (UK/SPAIN) 1a subtypes.HAV sequences with similar clustering were also identified in 31% (45/147) of sewage samples, with a high prevalence (63%, 26/41) in facilities in the Tel-Aviv area. The rate of HAV-positive clinical and sewage samples declined towards the end of 2017 (Figure 1).

Conclusions: These results**:** describe an ongoing HAV 1a outbreak in MSM in Israel, most probably imported, at least in part, from European countries. Despite the efficient universal mass vaccination program which lead to a dramatic annual HAV incidence decline (from 33-70 cases to 2,5 cases/100,000), HAV can still be transmitted to susceptible and high-risk adult population and raise the issue of catch-up vaccination. It also demonstrates the importance of environmental sampling in disease surveillance. Finally, with the decline in the number of positive HAV samples in the last quarter of 2017, the outbreak has been halted.

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Evaluation of HCV transmission clusters in DAA naïve infected individuals in Rome

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Background: We evaluated the characteristics of HCV transmission clusters between Directly Acting Antivirals (DAA) naïve individuals in the Italian city of Rome.

Materials & Methods: An NS3±NS5A±NS5B genotypic resistance test (GRT) was performed on HCV samples from 772 DAA naïve individuals, by using a home-made protocol. Phylogenetic cluster analysis was performed on NS5A sequences by using General Time Reversible (GTR) model and 1,000 bootstrap with maximum-likelihood method using PhyML, and then confirmed by Bayesian analysis with a posterior probability=1. We defined transmission "clusters" (TC) and "pairs" (TP) as phylogenetic clades with n≥3 and n=2 sequences, respectively. Potential differences in and out TC/TP were evaluated by Chi-squared test or Fisher's exact test, as appropriate. Logistic regression was used to define factors associated with TC/TP, using as confounders age, genotype, risk factor, HIV infection and drug resistance.

Results: Overall, most individuals were males (69.8%), with a median (IQR) age of 54 (48-63) years. Drug use (DU) was the most prevalent risk factor known (N=293, 38.0%), mainly associated with genotype (GT) 1a (45.4%), and GT3a (32.8%), and the unknown risk factor reached the 51.0%. GT1a was the most prevalent (35.6%), followed by 1b (27.0%), 3a (20.2%), 2c (8.5%), 4d (6.2%), 4a (1.2%), and others (1.3%). The 5.8% of individuals was co-infected with HIV and the 41% was cirrhotic. Resistance to any drug class was 34.2% mainly imputable to NS3 Q80K (18.9%, particularly in GT1a), and NS5A F28C (7.9%, all in GT2c), and Y93H (7.9% particularly in GT1b and 3a). Individuals involved in TC/TP were 47 (6.1%), and were composed by 16 TP (76.2%) and 5 TC (23.8%) with 3 sequences. 4/5TC and 5/16 TPs were composed exclusively by DUs, mainly infected by GT1a and 3a (66.7% and 33.3%, respectively). The rest 12 TC/TPs were mainly composed by unknown risk factors (6TPs exclusively and others 5 TPs/1TC mixed mainly with DUs) and infected by GT1a. No individuals infected with GT2c/4a were found in TCs/TPs. Individuals in clusters were prevalently younger [47(38-53) vs 55(48-63), p<0.0001], infected by GT1a (55.3% vs 34.3%, p=0.005), HIV co-infected (14.9% vs 4.7%, p=0.004), DUs (57.4% vs 36.4%, p=0.006), and less resistant (19.1 vs 35.2%, p=0.029) in comparison to individuals out of TC/TP. Multivariable logistic regression confirmed that DUs were positively associated with TC [adjusted odds ratio, AOR (95% CI): 4.7 (0.99-21.9), p=0.05], younger age and GT1a were also positively associated with TC/TP [1.02 (1.01-1.05), p<0.001 and 2.09 (1.07-4.09), p=0.032], while drug resistance was negatively associated with TC/TP [0.45 (0.21-0.96), p=0.040].

Conclusions: Even if our findings show an overall low circulation of HCV clusters between DAA naïve individuals in Rome, we identified factors such as younger age, DU and GT1A as mostly associated with clusters. Further studies are needed to better understand the complex dynamics and phylogenetic relationship between HCV infected individuals.

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Characterization of NS5A and NS5B variability from HCV Genotype 4d patients treated with DAA

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Background: Almost 20% of hepatitis C patients world-wide are infected with genotype 4 (GT4). Among the 18 recognized subtypes, the most common in Europe is 4d. GT4 has long been considered one of the most difficult genotype to treat, but after the introduction of novel direct-acting-antiviral (DAA) agents, higher efficacy rates have been obtained, accompanied by improved safety and tolerability profiles. We evaluated NS5A and NS5B polymorphisms at baseline in patients treated with DAA. Evolution of viral quasispecies was analyzed in failed patients.

Materials & Methods: Conventional genotyping was performed with Abbott RealTime HCV. Baseline NS5A and NS5B analysis was performed by Sanger population sequencing of specimens from 16 (NS5A) and 21 (NS5B) out of 50 GT4 patients treated with different DAA combinations (36 SOF-based). Samples from 2 no-SVR patients, Pt1 (HIV-coinfected, SOF-DCV) and Pt2 (SOF-LDV), underwent quasispecies analysis by ultra-deep pyrosequencing (UDPS) at different time points (T0=baseline; T1 and T2=post failure).

Results: Most patients (19) were subtype 4d, two were 4a. No relevant substitutions were detected at positions known to be associated with drug resistance in either gene.
Conventional genotyping of one failed patient (Pt2) at baseline indicated 1a/4 coinfection. It is interesting that Sanger sequencing at baseline classified Pt2 as GT1a based on NS5A, and GT4d based on NS5B. UDPS analysis of NS5B revealed, indeed, concomitant presence of GT1a and GT4d sequences at baseline (ratio about 1:5), and virtual disappearance of GT1a components after failure.
Regarding NS5A, longitudinal quasispecies data were obtained only for Pt1. The most renowned resistance-associated variant (RAV), Y93H, absent at T0, appeared at T1 and persisted at T2 as predominant variant (77.7% and 62.2%, respectively), coexisting with the minority variant Y93S (T1= 21.4% and T2= 34.9%, respectively). Furthermore, UDPS revealed dynamics of other mutations: T56I, absent at T0, was detected as majority variant at T1 (76.4%) and T2 (62.1%); I101T, present as minority variant at baseline (1.6%), raised at T1 (16.3%) and T2 (29.3%).
Regarding NS5B, sequence data were obtained for both failed patients, except for T1 of Pt1. In both patients, the frequency of the RAV N142S fluctuated around 20-30% at all time points (i.e. at baseline and after failure). To note, in Pt2, the frequency of L204F raised from 1.5% at T0 to 77.0% at T1 and to 91.6% at T2.

Conclusions: The predominant subtype was 4d. Natural variability of NS5A and NS5B was low.
Y93H was observed in NS5A at failure to SOF/DCV, and did not reverse after one month of follow up.
N142S was the only known NS5B RAV detected, whose frequency was stable along the observation period, suggesting no relationship with virologic failure.
Novel mutations (T56I in NS5A and L204V in NS5B) increased their frequency along therapy failure, suggesting possible role in conferring virologic resistance. Further analyses are necessary to support the hypothesis that increased frequency could be related to the evolution pressure conferred by treatment, and to better clarify the role of these mutations in the HCV resistance mechanisms.

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HIV-1 diversity and antiretroviral resistance mutations among treatment naïve patients from 2005-2017: 13-year experience at Umberto I General Hospital in Rome

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Background: Transmitted drug resistance (TDR) in antiretroviral (ART)-naïve patients remains a serious concern since it can reduce the efficacy of treatment and may affect clinical outcomes. The aim of this retrospective study is to describe circulating viral subtypes and determine the trend in prevalence of resistance in drug naïve patients from Sapienza University Hospital.

Materials/methods: Genotypic resistance tests (GRT) was performed on 670 ART-naïve patients attending Sapienza University Hospital, Rome, between 2005 and 2017. GRT were conducted in integrase (n= 52), protease and reverse transcriptase (n=670) sequences (Trugene® HIV-1 Genotyping Kit, Siemens; ViroSeq™ HIV-1 Genotyping System, Abbott). TDR mutations and subtypes (hivdb.stanford.edu) were analysed.

Results: Most of patients were male (76.1%), of Italian origin (70.9%), with a median age of 38 years (IQR 31- 48 years); the median viral load was 4.71 log10 copies/mL (IQR 4.1-5.3) and the mean baseline CD4 cell count was 352 cells/mm3 (IQR 148-570). The most commonly reported transmission risk factors were homosexual (44.2%) and heterosexual contact (43.4%). Phylogenetic analysis revealed the presence of 21 different subtypes and Circulating Recombinant Forms (CRFs). HIV-1 subtype B was most common (67.16%), followed by CRF02_AG (7.7%), subtypes C (6.1%) and F (5.8%). In each of these different clusters are evident no association with risk factors, but clusters are stronger when considering sex or date of isolation. We found a significantly increased overtime in the proportion of non-B strains ($p<0.001$) and in the rates of non- Italians patients ($p<0.001$). Most individuals (n= 621, 92.7%) had no TDR mutations and were susceptible to all drugs studied. The overall prevalence of TDR was 7.3% [nucleoside reverse transcriptase inhibitors (NRTIs)= 2.9%, non-nucleoside reverse transcriptase inhibitors (NNRTIs)= 2.5%, protease inhibitors (PIs)= 1.8%] and was higher in subtype B strains. The overall percentage of TDR increased if we included in the analysis the E138A mutation, a common polymorphic mutation, weakly selected by NNRTIs. The most frequently observed resistant mutations in NRTIs were M41L (22.2%), D67N (22.2%)and L210W (22.2%); in NNRTIs were K103N (56.2%) and in PIs L33F (27.3%). Analysis of the integrase gene showed a low prevalence (3.8%) of major integrase strand transfer inhibitors (INSTI) mutations. In total, 9 (17%) patients had INSTI mutations, 7 with only a minor mutation and 2 with major mutations. Indeed the last two individuals were a couple and share the same INI and RT mutations (G140S, Q148H; M230L). The most common INSTI minor mutations were E157Q (5.7%) and T97A (5.7%).
No significant decrease of TDR was documented overtime.

Conclusions: TDR rate observed in our population is in agreement with the average rate in Europe. The lack of a significant reduction of TDR underlines the importance of a continuous surveillance of resistance mutations. These data on INSTI mutations highlight the importance to perform GRT before commencing treatment, given the increased use of INSTI as a first line treatment. Moreover, the significant increase of non- B viruses suggests the importance to monitor dynamics of HIV-1 transmission, because this may have important clinical and diagnostic implications.

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All-in-one HIV-1 pol PCR for analysis of drug resistant HIV-1 pol quasispecies heterogeneity by Next-Generation Sequencing and Clone-Based Sequencing

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Background: Integrase inhibitors are recommended for first-line therapies and furthermore potent combination partners with a broad activity against multi-resistant HIV strains. Therefore, the interest in analysis of resistance to integrase inhibitors is increasing. The co-amplification of protease and reverse transcriptase and population sequencing are currently state of the art, whereas the 3' integrase region is analyzed separately.
The aim of this work was the development of a subtype independent all-in-one PCR of the complete pol-gene region which allows the analysis of the coevolution of resistance mutations in different gene regions and the comparison of Next-Generation Sequencing (NGS) and Clone-Based Sequencing (CBS) for the analysis of drug resistant HIV-1 pol quasispecies heterogeneity.

Material & methods: The all-in-one pol PCR was established using cell culture supernatants and HIV-1 plasma samples obtained from the RESINA cohort. To amplify the pol-gene, a PCR protocol consisting of a one-step RT-PCR with RNA pre-incubation with T4gp32 (T4 gene 32 protein) and a nested PCR was established. For validation samples with a broad variety of HIV-1 subtypes and viral loads were included.
The comparison of NGS and CBS was performed with pol-fragments of ten multi-resistant HIV strains detected by routine resistance diagnostics. Cloning was carried out by Gibson assembly into a cloning vector with subsequently classical population sequencing of the pol-clones. Next Generation Sequencing (NGS) of the pol-PCR products was performed using the Illumina MiSeq technology. For statistics Pearson correlation coefficient and two-sided Fisher's exact test was used.

Results: A PCR protocol for the amplification of the complete HIV-1 pol-gene (~3kb) was developed and optimized for a subtype independent application. The most critical step for the amplification of large gene product of RNA viruses is the reverse transcription and the stabilization of the cDNA for PCR amplification. This was succeeded by the use of the SuperScriptTM III One-Step RT-PCR with Platinum® Taq DNA Polymerase by Invitrogen and the T4gp32 protein. The protocol was optimized for the detection of a large variety of HIV-1 subtypes including the subtypes A, B, C, D, F, G, H and the circulating recombinant forms 01_AE, 02_AG, 06_CPX. The sensitivity varied depending on the HIV-1 subtype and also reduced with viral loads <1000 copies/ml.
The comparison of NGS and CBS was done by clonal analysis and included 30 clones of each pol-PCR product. The resistance profiles of all POL-clones of the ten patient samples showed a significant correlation between the quantity of detected substitutions by CBS and by NGS, R = 0.9577 (IQR 0.93-0.998), p <0.0001%.

Conclusions: The novel all-in-one pol PCR-protocol facilitates a resistance analysis of the complete pol gene, including the protease, reverse transcriptase and integrase presenting a broad HIV-1 subtype coverage and an adequate sensitivity. NGS delivers not only a sensitive detection rate of drug resistance mutations but also a representation of the heterogeneity of the HIV-1 pol quasispecies.

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New determinants of protease inhibitors-resistance in protease, GAG and GP41 regions

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Background: Viruses of some patients with virological failure to Protease inhibitors (PI) don't harbor any resistance associated-mutations in protease gene. Not only gag mutations (inside or outside cleavage sites, CS) can have implications in PI resistance but also gp41 mutations because of interaction between cytoplasmic tail (CT) of gp41 and non-cleaved protease precursor. We studied selection of protease, gag and gp41 mutations on viruses from patients with unexplained virological failure to PI.

Materials and Methods: We studied patients initiating an antiretroviral treatment and experiencing virological failure (VF, defined as two consecutive viral load >50 copies/mL) to PI-based regimen (NRTI with darunavir/r or atazanavir/r or lopinavir/r) from January 2011 to January 2016, without any transmitted drug resistance (TDR) mutation detected by Sanger sequencing on protease and reverse transcriptase genes according the WHO TDR list. We performed UltraDeep Sequencing (IlluminaTM Nextera®) of protease, gag and gp41 genes in plasma before initiation of treatment and at VF. At failure, only mutations present at more than 20% of total viral population in at least 2 patients and whose prevalence was multiplied by 2.5 between initiation and VF were taken into account.

Results: Among the 32 studied patients, 56.1% were infected with subtype CRF02_AG, 19.4% with subtype B and 29.0% with other non-B subtypes. The median time between baseline and VF was 495 days and the median CD4 count nadir was 220/mm3. Adequate pharmacological measurements were retrieved at VF. Viruses carrying emergent and non-previously described mutations on protease gene was identified in 5 patients at VF: 15V/64M/70R (2 CRF02_AG), 15V/79A (CRF02_AG), 64M (CRF02_AG), 79A (CRF06_cpx). Two patients' viruses showed the emergence of R286K in the capsid region, outside CS (2 CRF02_AG).
In gp41 region, among the emergent mutations identified, 1 mutation was located inside the CT (V321I, 1 subtype A and 1 subtype B) and 4 mutations were outside the CT: K106R (2 CRF02_AG), E109D (1 CRF02_AG and 1 subtype B), T165S (1 CRF02_AG and 1 subtype B) and K172R (1 CRF02_AG and 1 subtype A).

Conclusions: In case of failure to PI/r regimen, despite adequate plasma concentrations, we evidenced the emergence of some protease, gag and gp41 mutations that were not previously described and that could be related to the VF. In particular, the emergence of the same pattern of mutations in protease (15V, 64M, 70R) among CRF02_AG viruses is interesting. These mutations should be studied by site directed mutagenesis and phenotypic studies to better characterize their impact on susceptibility to PI.

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Molecular investigation of the HIV-1 B/G and B/F recombinant forms in Spain: Evidence for local transmissions

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Background: Our previous analysis on 6,632 HIV-1 sequences sampled in Spain revealed that B/G and B/F recombinant forms were among the HIV-1 non-B clades with the higher prevalence in Spain (1.54% and 1.48%, respectively). Our aim was to investigate the patterns of B/G and B/F dispersal across Spain and estimate the spatiotemporal characteristics of their largest regional epidemics, using molecular methods.

Materials & Methods: We studied 102 B/G and 98 B/F sequences, available in the PR/RT regions. Sequences were isolated from HIV-1 diagnosed patients during 2002-2014 from 10 autonomies of Spain. Patients' samples were merged from two datasets: a)CoRIS (2004-2013), and b)Eastern Andalusia Resistance Cohort (2000-2014). We analyzed phylogenetically sequences from our study population along with the most closely related sequences to them (HIV BLAST tool; B/G:N=317; B/F:N=210), using maximum likelihood method with bootstrap evaluation as implemented in RAxML v8.0.20 (GTR+G model). Local transmission networks (LTNs) were phylogenetic clusters including sequences from Spain at proportions >70%. Phylodynamic analysis was performed by using a Bayesian method as implemented in BEAST v1.8.0 (birth-death model).

Results: Navarre (B/G:7.4%; B/F:14.8%) and Basque Country (B/G:4.9%; B/F:4.9%) were the autonomies where B/G and B/F were more frequently found. Phylogenetic analysis revealed that 86.3% (N=88) of the B/G sequences from Spain found within 9 LTNs (CRF14_BG:N=40, 1 LTN; CRF20_BG:N=27, 4 LTNs; URF B/G:N=19, 3 LTNs; CRF24_BG:N=2, 1 LTN). The two largest B/G LTNs included 40 (39.2%; CRF14_BG) and 18 (17.6%; CRF20_BG) sequences. The 94.4% (N=17) of the sequences found within the CRF20_BG LTN were from individuals living in Madrid reported men having sex with men (MSM) as transmission risk group. Analysis revealed Cuba as the most possible source of CRF20_BG subepidemic. Analysis also revealed that 74.5% (N=73) of the B/F sequences from Spain formed 9 LTNs (CRF47_BF:N=32, 1 LTN; CRF12_BF:N=26, 3 LTNs; CRF40_BF:N=6, 1 LTN; CRF44_BF:N=3, 1 LTN; CRF17_BF:N=2; 1 LTN; CRF39_BF:N=2, 1 LTN; CRF42_BF:N=2, 1 LTN). The largest B/F LTN (CRF47_BF) consisted of 32 (32.7%) sequences, the majority of which had been isolated from heterosexuals (N=25, 78.1%) living in Andalusia (N=10, 31.3%), Navarre (N=8, 25%) and Basque Country (N=7, 21.9%). Molecular clock analysis estimated that the time of the most recent common ancestor (tMRCA) of the subepidemics was in 1991 (median estimate; 95%HPD:1985-1996) (CRF14_BG), in 2004 (95%HPD:2002-2005) (CRF20_BG) and in 2004 (95%HPD:2002-2005) (CRF47_BF). The birth-death skylines suggested a large increase in number of infections for the CRF47_BF and CRF20_BG, lasting between 2005 and 2011. For the CRF14_BG the largest increase in number of new infections occurred during 1996-2005.

Conclusions: Our study revealed that the B/G and B/F transmissions are due to regional dispersal at a considerable proportion in Spain. The hot spot for one of the largest B/G regional subepidemics (CRF20_BG) in Spain was in Madrid, associated with MSM risk group and probably originated from Cuba. The tMRCA and transmission dynamics of the three largest outbreaks were diverse. The most recent subepidemics (CRF47_BF, CRF20_BG) showed a rapid increase that lasted for approximately six years, whilst the CRF14_BG epidemic growth occurred over a longer time period.

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Molecular analysis of HIV-1 subtypes A1 and B epidemic dispersal in Greece: A hot-spot for subtype A1 epidemic in Western Europe

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Background: Previous studies have shown that HIV-1 subtypes A1 and B are the predominant clades in Greece. Our aim was to investigate the patters of subtypes A1 and B dispersal in Greece, to identify the proportion of infections occurred locally, and to estimate the factors that are associated with local transmissions, using molecular epidemiology methods.

Materials & Methods: We studied 1,230 A1 and 2,156 B sequences isolated from HIV-1 diagnosed patients during 1996-06/2015 in Greece. Sequences were available in the protease (PR) and partial reverse transcriptase (RT) regions. Maximum-likelihood (ML) phylogeny reconstruction with bootstrap evaluation was conducted in RAxML8, using GTR+G as nucleotide substitution model. Phylogenetic analysis was performed separately on the 1,230 A1 and 2,156 B sequences from Greece along with a random set of globally sampled sequences (A1: N=1,500; B: N=2,000), used as references. Phylogenetic analysis was performed for each subtype in five replicates, using a different reference dataset each time. Local transmission networks (LTNs) were defined as phylogenetic clusters including sequences from Greece at proportions >70% (geographic criterion), found in all five replicates (phylogenetic confidence). Local dispersal was estimated by dividing the number of sequences found within LTNs with the total number of sequences sampled in Greece for each subtype. The statistical analysis was carried out using multivariate logistic regression models. Presence in LTNs was the binary outcome variable, while age, period of sampling, risk group, nationality and gender were chosen as possible explanatory variables.

Results: Phylogenetic analysis revealed that 91.5% (1,125 out of 1,230) of the A1 sequences sampled in Greece formed 23 LTNs. The size of the LTNs ranged between 2 and 1,059 sequences. The 86.1% of the A1 sequences fell within a single LTN consisted of 1,059 sequences. The rest of the A1 sequences (N=105, 8.5%) clustered at different points in the ML tree. The majority of the unclustered sequences had been isolated mainly from heterosexuals with non-Greek nationality. Phylogenetic analysis also revealed that 72.8% (1,570 out of 2,156) of the B sequences sampled in Greece formed 133 LTNs, with a range of 2 to 157 sequences. Multivariate analysis showed that gender (male; OR=1.8; p=0.034), risk group (MSM; OR=7.2; p<0.001) and nationality (Greek; OR=7.2; p<0.001) were associated with regional clustering of subtype A1, while period of sampling (2011-2015; OR=7.21; p<0.001) with local transmissions of subtype B.

Conclusions: Our analysis suggests considerable differences in the levels of regional clustering of HIV-1 subtype A1 and B in Greece. For subtype A1 we found a lower number of introductions than for subtype B. This probably due to that subtype B is more frequently found in developed countries, suggesting that cross-border transmissions are more likely than non-B clades. It was also shown that the majority of A1 unclustered sequences had been isolated mainly from heterosexuals with non-Greek nationality. This finding suggests that this population group was infected with A1 strains with non-Greek origin and it is not related to further dispersal. Moreover we found that local dispersal of subtype B transmissions is mostly associated with recent diagnosis.

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Molecular surveillance for investigating the HIV outbreak among Romanian people who inject drugs

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Introduction: Sequencing (parts of) the genome of human pathogens, in particular RNA viruses, and performing phylogenetic analysis have proved to be very useful, particularly in the field of molecular epidemiology. Romania has faced an HIV outbreak among people who inject drugs (PWID) starting 2011 that was mainly localized in Bucharest and surroundings. During the same period, Greece has reported a similar outbreak among PWID in Athens. Early findings indicated the circulation of two dominant HIV strains among Romanian PWID: CRF14_BG and subtype F1. Phylogenetic analysis suggests that CRF14_BG strains circulating in Athens originated from strains circulating in Bucharest. Our goal was to further investigate the Romanian HIV outbreak by performing molecular surveillance and by using phylogenetic approaches.

Methods: Blood samples were continuously collected from HIV newly diagnosed PWID between 2011 and 2017. HCV co-infection was documented (positive serology, genotypic tests) for all the studied patients included. Different HIV genomic viral regions were used for sequencing that was performed either by Sanger method (used as standard), or NGS. HCV genotyping was performed only through Sanger sequencing. The tools used to phylogenetically analyze and characterize the viral sequences were maximum likelihood (ML) and Bayesian approaches, recombination analysis, intra-host viral diversity.

Results: Analyzing the sequences from PWID diagnosed in Bucharest in this time interval we have observed different transmission dynamics of the HIV and HCV infections in this population. Several transmission networks were identified when analyzing the sequences of the two viruses, namely 3 HIV and 13 HCV clusters. All HIV clusters (subtype F1 and CRF14_BG) were monophyletic. According to molecular clock analysis, the Romanian HIV transmission clusters originated between 2006-2009. The HCV epidemic among Romanian PWID was more diverse (5 different genotypes were co-circulating) than for the HIV, and the estimated time of the most common origin (tMRCA) of the HCV clusters were more dispersed in time, ranging from the 1980s (genotype 1b, 1a) to 2011 (genotypes 3a and 4d). HCV clusters were monophyletic for genotype 4 (a and d) only. Injecting drug was associated with high frequency of dual infections and recombination. The analysis using NGS data revealed 5 cases of dual/multiple infections. We have also indicated the circulation among Romanian PWID of new (unique) HIV recombinant forms between subtype F1 and CRF14_BG.

Conclusion: By using a combination of Sanger and NGS data with molecular epidemiology tools it was possible to estimate the origin of HIV epidemic in Bucharest, to reconstruct different viral population dynamics and to identify transmission networks. The results indicated that Bucharest HIV outbreak preceded the one from Athens, suggesting also that these local outbreaks were diagnosed rather in their early phases. Moreover, molecular surveillance has contributed to outbreak investigation by identifying new HIV circulating viruses and by documenting cases of multiple infections among PWID. Eventually, the results may contribute to improve prevention strategies and future public health programs.

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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 and is a suitable and approved marker for monitoring treatment success. Sustained plasma viral load below the limit of detection is the goal in treatment strategy of HIV patients. In time of successful long-term therapies and upcoming cure strategies there is need for a further marker to analyse the influence of a certain drug combination on the proviral reservoir and also for the long-term monitoring of the proviral reservoir. In order to analyse the dynamics of proviral load (pVL) and evolution of proviruses in patients with undetectable plasma viral load we are collecting and examining longitudinal collected samples from 72 patients. Here we present the preliminary results of this ongoing analysis.

Materials and methods: Three consequent samples from each patient were collected during a period of twelve months and the total proviral DNA from PBMCs was extracted. The proviral load (cop) and PBMC count were measured in a real-time PCR to calculate pVL (log cop/Mio cells). The data was analysed with regard to plasma viral loads, CD4 cell counts, HIV treatment combinations and also the period of undetectable plasma viral load. The treatment combinations were divided into following groups: NRTI-INI (w/o DTG) (24), NRTI-NNRTI (17), NRTI-PI (11), INI-PI (w/o DTG) (2), DTG-containing therapy combinations (8), without treatment (3) and other combinations [NNRTI-PI + NNRTI-INI-PI (w/o DTG)] (2). Sequence distances were analysed using Mega7.

Results: The mean pVL of the measurements from three consequent time points was by trend higher in the NRTI-PI (0.39, σ0.19) and DTG-containing regimens (0.36, σ0.22) compared to the other regimens (0.12-0.29). There was no significant variance between the measurements from three time points. In order to include the CD4 cell count into the analysis of the pVL the quotient of pVL and CD4 cell count was determined. The mean of the quotient was significantly higher in the NRTI-PI group (0.00077, σ0.00070) compared to NRTI-INI (0.00036, σ0.00007; p=0.027) and NRTI-NNRTI group (0.00037, σ0.00028; p=0.045). There were no significant differences in the mean CD4 cell count and mean pVL between these groups.
The analysis of the evolution of proviruses included successful protease (PR) and reverse transcriptase (RT) amplicons (1450 bp) of 56 patients. In 24 cases evolution (as distance from the first sample) could be observed. There was no significant difference in the composition of the treatment between these two groups. 20% of patients showed RT-RAMs and 6% PI-RAMs. In 50% of all patients APOBEC-induced mutations could be found, where M184I and M230I were the most prevalent mutations.

Conclusions: Based on the observation that pVL in patients receiving the standard treatment regimen NRTI-PI is higher compared to other therapy combinations, the role of PI-regimens in sustainment of the proviral reservoir has to be determined. Furthermore, there is also need to examine in detail which factors contribute to the evolution in proviral DNA in spite of suppressed plasma VL. Proviral load could be an easily to perform and useful readout in routine diagnostics.

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Evaluation of virological response and resistance profile in virologically suppressed HIV-1 infected patients switching to integrase inhibitors based treatment in clinical settings

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Background: We evaluated the virological response and the resistance profile in virologically suppressed patients switching to a regimen containing integrase inhibitors (INI) in clinical settings.

Materials & Methods: Survival analysis was used to assess probability and predictors of virological rebound (VR, at least 2 consecutive viremia >50 copies/mL or 1 viremia >1000 copies/mL after switch). Resistance to protease inhibitors (PIs), nucleos(t)ide reverse transcriptase inhibitors (NRTIs), non-NRTI (NNRTIs), INIs was evaluated before (as cumulative plasma resistance) and after switch. Cumulative genotypic susceptibility score for companion drugs (cGSS) was also evaluated (HIVdb algorithm ver.8.4).

Results: Overall, 789 cART-treated patients virologically suppressed from a median (IQR) time of 1.6 (0.5-4.1) years were analyzed. At switch, patients experienced a median (IQR) number of 4 (2-9) previous regimens; 30.8% of patients was previously exposed to INIs (raltegravir [RAL]: 25.2%; dolutegravir [DTG]: 3.8%; elvitegravir [EVG]: 2.5%). Patients switched to the following combinations: DTG+2NRTIs (19.1%); EVG+2NRTIs (19.0%); RAL+2NRTIs (13.9%); DTG or RAL based dual-therapy (31.4%); other (16.6%). Overall, 54.4% had at least one previous resistance mutation (NRTI: 44.7%; NNRTI: 61.6%; PI: 23.1%; INI [N=222] 2.7%). cGSS revealed that 36.8% of patients harbored a virus with intermediate/full resistance to companion drugs received at therapy switch.
By 24 months after switch, the overall probability of VR was 13.8% (median [IQR] viremia at VR: 521 [99-12,836] copies/mL). Longer was the duration of virological suppression before switch, lower was the probability of experiencing VR after switch (<1 year: 24.3%; 1-3 years: 9.8%; 3-5 years: 5.3%; >5 years: 2.9%, P<0.001). Patients showing intermediate or full resistant cGSS had a higher probability of experiencing VR compared to those with a full susceptible cGSS, with a trend toward statistical significance (16.7% vs. 12.2%, p=0.193).
By multivariable Cox-regression (adjusting for demographic, viro-immunological and therapeutic parameters), the unique factor negatively associated to VR was a longer time of previous suppression (adjusted relative hazard [95% C.I.] per 1 year higher: 0.63 [0.52-0.76], P<0.001). No other factors were associated to VR.
Among patients experiencing VR, 31 patients (27, 2 and 2 under RAL, EVG and DTG, respectively) were tested for resistance in a median (IQR) time of 11.6 (5.8-11.6) months after switch. INI major resistance mutations were detected in 15 (48.4%) patients (E92Q: 3.2%; G140A/S: 12.9%; Y143C/H/R/S: 16.1%; Q148H/R: 12.9%; N155H: 12.9%). One patient previously exposed to 4-drug classes (including INI) and with previous INI resistance (N155H, V151I) failed a DTG + ritonavir-boosted darunavir based treatment with the mutations Y143C/H/R and G163R. Concerning companion drugs, 8 (26%) patients accumulated further resistance (PI: 19.4%; NRTI: 9.6%; NNRTI: 6.4%). A significantly higher proportion of intermediate/full resistant cGSS was observed in patients with INI resistance at VR compared to those who failed without resistance (70.6% vs. 21.4%, P=0.011).

Conclusions: INI-based treatment switch in virologically suppressed patients ensures a high rate of virological control regardless the INI used. However, to avoid VR and potential resistance accumulation after therapy switch, patients should be in stable virological suppression since at least 1 year and receive fully active drugs according to previous cumulative resistance.

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Newly diagnosed individuals infected with HIV-1 non-B subtypes are frequently involved in transmission clusters in Italy

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Background: We herein evaluated the characteristics and the dynamics of HIV-1 transmission clusters (TCs) in newly diagnosed individuals, infected by non-B subtypes over the years 2005-2017 in Italy.

Materials & Methods: Phylogenetic analyses were performed on pol sequences from 1,890 HIV-1 newly diagnosed individuals. Subtypes/CRFs were identified by using Neighbour Joining tree. TCs were firstly identified by HIV-1 TRACE (Tamura-Nei model, genetic distance ≤0.015) and then supported by Maximum-Likelihood (ML) tree (RAxML, Generalised Time-Reversible (GTR) model, 1,000 bootstrap support). TCs were divided in small (STCs, 2-3 sequences), medium (MTCs, 4-9 sequences) and large (LTCs, ≥10 sequences). Differences in the prevalence of TCs over time were evaluated by Chi-squared test for trend. Logistic regression was used to define factors associated with TCs.

Results: Overall, the most prevalent variant was CRF02_AG (432, 22.9%), followed by F1 (348, 18.4%), C (332, 17.6%), CRFs_BF (205, 10.8%), A1 (183, 9.7%), G (106, 5.6%); other variants (284, 15%) were present with a prevalence <5%. Individuals were mainly male (69.6%), with a median (IQR) age of 42 (35-50) years; most of them were heterosexual (46.4%) and MSM (24.1%); 45.5% of the individuals were native from Italy. Transmitted drug-resistance (TDR) to any drug class was 6.5%, mainly imputable to NNRTI resistance (4.3%). 172 TCs were identified (including 2-91 individuals), and involved 840 individuals (44.4%). Of note, 436 persons (51.9%) were included in 18 LTCs, 136 (16.2%) in 26 MTCs, and 268 (31.9%) in 128 STCs. Individuals in TCs significantly increased over time (2005-2017: 25.3%-39.3%, p=0.02). Compared to Individuals out of TCs, patients involved in TCs were prevalently Italian (67.2% vs. 28.2%, p<0.001). This correlation between Italian origin and inclusion in TCs was confirmed by considering the following subtypes: CRF02_AG (70.1% vs. 18.2%, p<0.001), and subtypes F1 (64.5% vs. 41.8%, p=0.001), C (66.7% vs. 20.3, p<0.001), A1 (62.3% vs. 24.6%, p<0.001), and G (58.6% vs. 10.4%, p<0.001). TCs were characterized by MSM, overall (37.6% vs. 13.2%, p<0.0001), and by considering CRF02_AG (55.4% vs. 8.1%, p<0.001), C (48.0% vs. 8.8%, p<0.001), and A1 (27.9% vs. 12.3%, p=0.011) subtypes. Individuals in TCs were also characterized by higher CD4+ T-cells at genotyping [cells/mm³, median (IQR)], overall [379 (233-563) vs. 239 (75-412), p<0.001], and by considering CRF02_AG [413 (301-595) vs. [282 (99-305), p<0.001], F1 [329 (208-543) vs. 234 (55-398), p<0.001], C [433 (299-596) vs. 180 (60-336), p<0.001], and CRFs_BF [372 (218-587) vs. 206 (51-512), p=0.002] subtypes. The median (IQR) viremia at genotyping was 4.9 (4.3-5.5) log_{10} copies/mL, without any significant difference between individuals in and those out of TCs.

Multivariable logistic regression confirmed that MSM, Italian origin and higher CD4+ T cells (per 50 cells increase) were all positively associated with the TCs [odds-ratio (95% Confidence-Intervals): 1.50 (1.08-2.07), p=0.015; 2.87 (1.77-4.66), p<0.001; 1.08 (1.05-1.11), p<0.001].

Conclusion: HIV-1 newly diagnosed individuals infected with non-B subtypes are frequently involved in TCs in Italy, mainly in the latest years, highlighting the complex phenomenon characterizing HIV-1 spread. This is important especially in view of monitoring the HIV-epidemic and guiding the public health response.

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Two cases of dolutegravir failure with R263K mutation

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Background: The emergence of resistance to ART is associated with virological failure. The development of resistance to INSTI is a matter of concern. In vitro studies shows that mutation R263K, associated to resistance to DTG, doesn't have impact due to viral fitness cost. However there are rare reports of a different behaviour of R263K mutation selection in vivo.

Case presentation: We present two case reports of similar patients who developed resistance to DTG.

The first is a case of a Portuguese woman aged 29 years, HIV-1 (subtype B), category A3 (CDC Atlanta), diagnosed in 2008 during her first pregnancy. At the diagnosis her viral load (VL) was 48157cp/mL with a negative baseline PR/RT resistance test. In January 2009, she started AZT/3TC+LPVr and in September, after delivering, she was simplified to TDF/FTC/EFV. In September 2010, on a new pregnancy was reintroduced AZT/3TC+LPVr. In November 2010 she had a negative resistance test (VL 153cp/mL). In April 2011, she restarted TDF/FTC/EFV but abandoned ART and follow-up in august 2012. In September 2016, she re-starts ART with ABC/3TC/DTG during six months but abandoned follow-up. In august 2017, during a new pregnancy the same ART regimen was reintroduced and one month later the PR/RT and INSTI resistance test (VL 246793cp/mL) revealed the presence of the mutations M184V, K103N, as well as, the mutations E157Q and R263K. Patient confirmed very poor adherence to the regimen during the first 6 months of use. Therapy was changed to TDF/FTC+DRVr. The subsequent resistance test (VL 447cp/mL) reveals the same mutations with the reversion of M184V and R263K associated with lack of adherence to ART.

The second case is another Portuguese woman, 29 years old, diagnosed in 2008 with HIV-1 (subtype G), category C3 (CDC Atlanta), with a negative baseline PR/RT resistance test. She started ART in 2008 with TDF/FTC+LPVr. In 2011, switched to TDF/FTC+ATVr due to virological failure. In 2014, changed because of virological failure, to TDF/FTC+DRVr. This patient had lack of adherence and maintained detectable VL during all the follow-up. In October 2015, she abandoned ART. A year later, TDF/FTC+DTG was introduced but she only took it for one month. In December she had a negative PR/RT and INSTI resistance test (VL 1315481cp/mL).
In March 2017, with a VL of 8100256 cp/mL, she re-started ART with ABC/3TC/DTG. Because she maintained detectable VL (2030cp/mL), in June 2017, a new resistance test was performed that revealed M184I and R263K mutations. At this time ART was switched to ABC/3TC+TDF+DRVr.

Conclusions: Even if we have only two cases it seems that an intermittent adherence to regimens with DTG are associated with the selection of resistance mutations namely R263K. In opposite to what happens in vitro the R263K mutation may have the ability to lead to virological failure in vivo. On the first patient E157Q mutation may contribute to compensate the low fitness of R263K virus, but the second patient had only R263K.

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Low degree of liver fibrosis in a cohort of young HIV-HBV co-infected patients on long-term ART

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Background: Romania has an important number of young adults, parenterally infected with HIV in early childhood, who associate frequently a perinatally acquired HBV coinfection and who have been exposed to complex antiretroviral treatments. The aim of the current study is to assess the evolution of liver disease and the degree of liver fibrosis in these multi-therapeutically experienced subjects.

Materials & Methods: A cohort of 227 HIV positive patients (median age: 24 years, median duration of HIV infection: 22.5 years, median ARV exposure time: 11,6 years) was tested for the presence of HBV coinfection. Coinfected patients were classified according to the EASL criteria using the presence of serum HBsAg and HBeAg, the level of HBV DNA and the level of ALT. Liver fibrosis was assessed using the noninvasive FIB-4 score. HBV resistance mutations were analyzed using a DNA hybridization line probe assay in all cases with serum HBV DNA >1000 IU/ml.

Results: HBV coinfection was present in 63.8% of the patients, 52.4% of these were chronic HBsAg carriers. In the entire cohort, the rates of liver fibrosis were particularly low, with only 7.8% of the patients having FIB-4 values predictive for fibrosis (>1.4) and only 1.3% values predictive for advanced fibrosis (>3.25). HIV-HBV co-infected patients were significantly more likely to present fibrosis compared with HIV monoinfected ones (p=0.006). The degree of fibrosis was directly correlated with active HIV replication (p=0.001), active HBV replication (p=0.05) and presence of severe immunosupression (p=0.01). The number and type of antiretroviral regimens and the cumulative exposure to potentially hepatotoxic antiretrovirals did not influence the presence of fibrosis. 97.7% of the patients were treated with a regimen including a dual HIV/HBV active ART (96.9% -3TC; 29%- TDF); lamivudine resistance mutations, present in 52.9% of the 17 patients with active viral replication, did not show any influence on the degree of liver fibrosis; no tenofovir resistance- mutations were detected. Only 11.7% of the coinfected patients had moderate to high levels of HBV DNA; severely immune-depressed patients tend to maintain active HBV replication more frequently (p=0.06). Only 3 coinfected patients had HBeAg positive chronic hepatitis, with high levels of HBV DNA, elevated ALT and significant fibrosis, while 11% had HBeAg negative chronic hepatitis, with anti-HBe and moderate levels of serum HBV DNA and a mild degree of fibrosis (mean FIB-4: 1.06). All the other patients had no or minimal fibrosis (mean FIB-4: 0.60), irrespective of the status of their HBV infection according to the EASL criteria: HBeAg positive infection (6.9%) with undetectable or low (<2,000 IU/ml) HBV DNA levels and normal ALT; HBeAg-negative chronic HBV infection (36.3%) with anti-HBe positive and moderate levels of HBV DNA or occult hepatitis B (45.6%) with HBsAg-negative, positive anti-HBc, with or without anti-HBs, very low or undetectable HBV DNA.

Conclusions: Significant liver fibrosis remains a rare occurrence in young-HIV infected patients even in the presence of HBV coinfection and after prolonged antiretroviral treatment; most coinfected patients maintain an inactive hepatic disease under cART.

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Prevalence and Characterization of NS3, NS5A and NS5B resistance associated variants (RAVs) in a cohort of 49 patients who had failed to a DAAs regimen

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Background: Direct-acting antiviral agents (DAAs) have shown to be highly effective and well tolerated for HCV chronic infections' treatment, with a rate of sustained virological response (SVR) >90%. Drug resistance to one or more DAAs classes is associated with the emergence of resistance-associated variants (RAVs) selected during therapy. In this study we describe the prevalence of NS3, NS5A and NS5B RAVs in a cohort of 49 patients naïve to direct acting HCV antivirals who failed a DAAs treatment and were screened in the Laboratory of Hygiene Unit of the Policlinico San Martino Hospital (Genova).

Materials & Methods: Fourty nine serum samples were collected between March 2016 and January 2018 from patients who experienced a virological failure after a DAAs regimen containing an NS5A (Ledipasvir; Daclatasvir; Ombitasvir; Elbasvir) + NS5B (Sofosbuvir or Dasabuvir) ± NS3/4 Inhibitors (Paritaprevir, Grazoprevir) ± Ribavirin. HCV-RNA was extracted from serum samples using NucliSens EasyMAG system (BioMérieux, Boxtel, The Netherlands); genomic regions were amplified with specific HCV genotype/subtype primers, using reverse-transcriptase PCR. Then, cDNA of the NS3, NS5A and NS5B genomic targets were 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) and submitted to Geno2pheno (the latest version available) (http://www.geno2pheno.org/).

Results: Genotypes (Gt) distribution among 49 patients screened for the presence of RAVs was the following: 12/49 (24,5%) 1a, 14/49 (28,6%) 1b, 18/49 (36,7%) 3a, 4/49 (8,2%) 4, 1/49 (2%) 2c. RAVs were detected in 40/49 (81,6%) patients, all of which harboring at least one RAVs in NS5A gene, with the pan-genotypic 93H the most frequently identified variant (27/40 - 67,5%), moreover 11/49 (22,4%) patients showed multi-target clinically relevant RAVs. Main Gt 1a RAVs detected were 80K and 174S in NS3, 93H, 31M and 30R in NS5A and C316Y in NS5B genes. All Gt 1b HCV strains showed a NS5A RAVs with 93H identified in 13/14 (92,8%) samples, NS3 and NS5B RAVs were detected in 64,3% of genotype 1b patients with a high prevalence of 170I and 159F, respectively. Main genotype 3 RAVs was the 93H detected in 12 out 18 (67%) samples. Regarding RAVs distribution among genotypes 4 and 2 an elevated prevalence was found for 93H in NS5A gene, detected in 60% of samples. Pre treatment serum samples were available for 24 out 49 patients (49%), in the half of which the presence of clinically relevant RASs was demonstrated. In particular, RASs classify as significant in vivo for at least one of the licensed NS5A inhibitor were detected in 7 out 12 samples (58,3%) with the highest prevalence in genotypes 1b.

Discussion: Clinically relevant RAVs were demonstrated at failure in more than 80% of patients with the highest prevalence in NS5A region. Notably, the preexistence of NS5A RASs mostly associated with treatment failure, predominantly detected in genotypes 1b. Our results suggest the importance of RAVs screening at failure to determine resistance profile and guide the choice of therapeutic options before starting retreatment.

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Apoptotic effects in Macrophages Infected by CXCR4- but Not by CCR5-Viruses, caused by the involvement of HIV-1 coreceptors.

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Apoptosis induction is associated with (Human immunodeficiency virus type 1) HIV-1 binding with α chemokine receptor 4 (CXCR4 (X4)) or β-chemokine receptor 5 (CCR5 (R5)) (essential step during HIV-1 replicative life cycle) in different target cells. For this purpose, apoptotic induction was evaluated in monocyte-derived macrophages (MDM) infected by X4 and R5 viruses.

To evaluate p38 mitogen-activated protein kinases (p38 MAPK) activation cells were lysed and subjected to immunoblot analysis. To assess apoptosis and survival gene modulation in X4 and the R5 infected MDM, RNA isolation, microarray analysis and flow cytometry measurement (FCM) of apoptotic cells were made.

CCR5-dependent 81A viral load determined a drastic increase from Day 7 up to Day 10. Thus, we investigated if this phenomenon is somewhat linked to the loss of MDM during CXCR4-dependent infection.

Apoptosis induced by X4-viruses starts from day 6, peaks 40–50% at day 10, whereas is 1.65% in R5-infected MDM. In line with this, Western blotting analysis was conducted and the p38 activation at 30' after infection in MDM infected X4-virus, but not with R5 strains was shown. Moreover, the CXCR4 antagonist AMD3100 inhibited apoptosis and proapoptotic MAPK/p38 activation. Microarray analysis showed modulation of proapoptotic and cancer-related genes induced by X4 strains starting at 24 hours after infection, whereas R5 viruses modulated the expression of genes not correlated with apoptotic-pathways.

Overall, these results shed light on the biological mechanism leading to MDM survival during HIV-infection. Abortive infection with X4 strains, and their consequent depletion from reservoirs, may explain a major pathogenic role of R5 viruses in all (but terminal) phases of the HIV-1 disease and provide important implications for the therapy of HIV infection.

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HIV-1 integrase drug-resistance emergence and evolution in patients treated with integrase inhibitors

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Background: We evaluated the emergence of mutations associated to integrase inhibitor (INI) resistance and the integrase evolution in HIV-1 infected patients treated with this drug class.

Materials & Methods: We analysed 107 INI-naïve (19 drug-naïve; 88 drug-experienced INI-naïve) patients in routine care in several Italian clinical centers who started an INI-based regimen and had two plasma genotypic resistance tests (GRTs) available (one before and one under INI-treatment). Amino acid frequencies across all integrase codons were evaluated and compared at the two GRTs using McNemar's test. The emergence of INI-resistance mutations (INI-RMs, panelled in the IAS list 2017) and integrase evolution (estimated as genetic distance) were also evaluated. A logistic regression analysis was performed to evaluate factors independently associated with the integrase evolution under INI-treatment.

Results: Patients were mainly infected by B subtype (72.0%). The most prevalent non-B subtypes were F1 (7.5%) and CRF02_AG (6.5%). 87 patients were treated with raltegravir, 13 with dolutegravir and 7 with elvitegravir. Median (interquartile range, IQR) viremia at GRT under INI-treatment was 3.5 (2.5-4.4) log_{10} copies/mL. No major INI-RMs occurred in the GRTs performed before INI-treatment, while three patients harboured the minor RM T97A and one the R263K. GRTs under INI-treatment were performed after a median (IQR) of 11.4 (6.5-21.5) months from INI start; this time was similar with the different INI types (p=0.462 by Kruskal-Wallis test). The emergence of at least one INI-RM was found in 39 (36.4%) patients (median [IQR] number of INI-RMs: 2 [1-2]). The following major INI-RMs emerged: N155H (17.8%), G140S (8.4%), Y143R (7.5%), Q148H (6.5%), Y143C (4.7%), E92Q (2.8%), Y143H (2.8%), Q148R (1.9%), F121Y (0.9%). Among the minor INI-RMs, the prevalence of T97A significantly increased under INI-treatment (from 2.8% to 12.1%, p=0.006), while the following RMs emerged: E138K (1.9%), L74M (0.9%), E138A (0.9%). In patients with at least two INI-RMs, the most common pathways found were: Q148H/R+G140S (n=5) and Y143R/C+T97A (n=3). No significant differences in all other codons across all integrase region were found by comparing GRTs before and after INI-treatment.
Concerning the integrase evolution, a median (IQR) genetic distance of 0.018 (0.009-0.028) was found between pre and post INI GRTs. This distance was significantly higher in patients with at least 1 INI-RM compared to those without emergence of resistance (0.024 [0.012-0.036] vs. 0.015 [0.009-0.024], p=0.018 by Mann-Whitney test), while it was similar with different INI types (p=0.462 by Kruskal-Wallis test).
A higher integrase evolution was significantly associated with a longer duration of HIV-1 infection (Adjusted Odds Ratio, AOR, [95% Confidence Interval, CI] per 1 year increase: 1.17 [1.07-1.26], p<0.001), a lower number of antiretroviral regimens previously administered (1.12 [1.01-1.24], p=0.034) and non-B vs. B subtypes (3.60 [1.03-12.58], p=0.045).

Conclusions: No major INI-RMs occurred in INI-naïve patients. Under INI-treatment, selection of drug resistance followed the typical drug resistance pathways; a higher integrase evolution was found in GRTs with resistance compared to those without any resistance.

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Molecular epidemiology of HIV-1 shows intermingling among Arab and Jewish men who have sex with men

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Introduction: The increased incidence of HIV among men who have sex with men (MSM) is a major challenge in HIV prevention in developed countries. MSM in Israel comprise ~30% of new HIV infections. Although Arabs comprised a minority (3.7% of 3349) of all new infections reported between 1986 and 2010, 48.1% of them were MSM. In this study we characterized for the first time HIV-1 epidemic in the Arab-MSM population and aimed to identify possible inter-religious contacts.

Methods: Israeli-born HIV-1 positive MSM, reported to the National HIV laboratory between 2005 and 2016 were selected and data were cross-matched with the National civil registry to identify population groups (Jews/Arabs). Partial protease (PR) and reverse transcriptase (RT) sequences from treatment naive patients, taken <6 months post diagnosis, were assessed. Transmitted drug-resistance mutations (TDRM) and HIV subtypes were identified. Phylogenetic analysis was performed via BEAST (GTR+G+I model) to investigate the clustering and ancestral relationships.

Results: Between 2005 and 2016, 1143 individuals that self-identified as MSM, all born in Israel, were diagnosed as HIV positive. 6.4% (73/1143) were Arabs and 93.6% (1070 of all MSM) were Jews MSM. Their median age was similar: 33 years (interquartile range, IQR 26.1-40.4) and 33.3 (IQR 27.1-40.4) for Arab and Jews, respectively, p=0.3. The total number of newly diagnosed individuals reporting MSM exposure increased between 2005 and 2013 with highest numbers of MSM identified between 2011 and 2013. 99 MSM were identified during seroconversion: 96 (9.0%) Jews and 3 (4.1%) Arabs (p=0.2). HIV-1 sequences from MSM were available for 62 Arabs and 440 Jews. Subtype distribution in both MSM population groups was similar. 82.3%, 9.5%, 4.1% and 4.1% of Jews and 80.6%, 8.1%, 4.8% and 6.5% of Arabs carried subtypes B, A, C and none ABC, respectively. Overall, prevalence of TDRM was 13% (66/502) with a non-significant higher rate in Jews compared to Arabs (p=0.1). TDRM rate was not affected by year of HIV-diagnosis or by viral subtype. The most common TDRM were RT-K103N/S, M184V, T215S and PI-L90M mutations. Phylogenetic analysis revealed that HIV-1 sequences from Arabs-MSM intermingled with HIV-1 sequences from the Jews-MSM. This pattern was similar in all subtypes (A, B, C). While subtype A and C sequences formed clusters of >4 individuals, most of subtype B sequences formed smaller clusters (with >0.9 posterior probability) of 2-3 individuals. These clusters as well as clusters of sequences with K103N/S and of T215S TDRM included both Jews and Arabs MSM.

Conclusions: Phylogenetic analysis demonstrated intermingling between the Arab and Jews MSM and revealed clusters of TDRM carriers from both populations. These molecular results corroborated with our previous report that Arab-MSM have sexual contact with Jews. Interventions aimed to prevent further HIV-transmission should address the cultural and behavioral characteristics of these individuals.

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Using phylogeny to identify active HIV-1 transmission clusters for targeted intervention.

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Background: In Denmark HIV infections has a prevalence of 0.12%, and of the 91% estimated patients, who are diagnosed with HIV, 93% are suppressed through antiretroviral therapy (ART). Despite this, the number of newly diagnosed HIV patients have remained stagnant at ?200 per year for more than a decade. It is currently not known how many of these new cases are caused by endogenous transmission and how many are imported and what characterizes these two groups. Phylogenetic analysis can differentiate major clusters of active endogenous HIV transmission from travel or imported cases, with little secondary infections, and can therefore help to identify and profile, which risk groups or locations should be primarily targeted for interventive measures.

Materials and Metods: 1227 HIV-1 POL gene (protease and reverse transcriptase) sequences collected along with epidemiological information between 2009 and 2017 from HIV patients with a recent diagnosis in Denmark were analyzed phylogenetically with Mega 6.0 using the Maximum likelihood GTR setting with 100 bootstrap replicates. Clusters were initially identified using Cluster picker using default settings (cluster support=90%, genetic distance 4.5%) and further refinement and validation of the cluster picking criteria were performed on epidemiologically well-defined clusters. Clusters were classified as active if new infections had occurred within the 2015-2017 period.

Results: In total, 154 clusters with 588 patients were identified. Of these, 75 were active clusters with 351 patients. Patients in active clusters were earlier diagnosed with HIV (63% with CD4+>350) than patients outside of clusters (43% with CD4+>350). Nine active clusters contained 10 or more patients (range 10-46, mean 17.4, total 157), and accounted for ?44% of all patients in active clusters. These clusters were subtype B (8 clusters) and CRF_01 (1 cluster), either MSM (8 clusters with >80% MSM) or IDU (1 cluster with 100% IDU). Most clusters (7 clusters with >70%) were located in the capital region and two were regional clusters.

Conclusion: Around 30% of the recent active endogenous transmission of HIV were identified in clusters, mainly consisting of young MSM in the capital region, which should be targeted for interventive measures.

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Hepatitis E in pigs in Israel: seroprevalence, molecular characterization and potential impact on humans

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Background and Aims: Hepatitis E virus (HEV) is a major causative agent of acute viral hepatitis worldwide. In recent years, reports on HEV-3 infections in Europe have accumulated, and undercooked pig products were found to be a source. In Israel, while clinical cases of HEV-3 infection have not been identified, HEV-3 sequences have been identified in 14 of 169 urban sewage samples collected between 2015 and 2016 from different regions in the country. In Israel, of the 90,000 pigs farmed at any given time, 80,000 are located in 23 farms in the north (West Galilee) and 10,000 in a single farm in the south (Negev). About 200,000 pigs are slaughtered yearly, exclusively for local consumption, yet, the distribution of the virus is unknown. In this pioneer study, we aimed to assess the status of HEV in the swine population and among swine farmers in the country.

Method: Serum samples from 141 pigs, representing different age groups, from 4 breeding farms (3 of which were northern farms) were collected between 2016 and 2017. Pig faeces (n= 39) and 5 raw sewage samples (4 from the sewage pipeline serving all northern farms and 1 from the southern farm) were also collected. Blood samples from 24 swine farmers were assessed. Serum anti-HEV immunoglobulin IgG was detected using the Wantai total IgG (pigs) or IgG (humans) HEV ELISA assays (Wantai Biopharmaceutical, Inc. Beijing, China). Total nucleic acids were extracted from blood (pigs and humans), pig faeces and sewage with NucliSENS EasyMag (bioMérieu, France). HEV RNA was analysed with the RealStar HEV RT-PCR kit, version 1.0 (Altona Diagnostics GmbH, Hamburg, Germany). HEV genotype was assessed in all HEV-RNA-positive samples.

Results: The overall prevalence of antibodies to HEV in pigs was 75.1% (106/141). Seroprevalence was age-dependent, with 85% (17/20) of young pigs (1.5 months old; maternal antibodies) and 97.4% (74/76) of pigs at slaughter (>5 months) but only 34.1% (15/44) of 2-4-month-old pigs, exhibiting seropositivity (Figure 1a). HEV RNA was detected in 2.1% (3/141) of serum samples, 40% (2/5) of sewage samples (from both northern and southern pipelines) and 5.1% (2/39) of faecal samples. All HEV RNA-positive samples were from the 2-4 month-old age group. HEV RNA was not detected in samples from pigs entering the food chain (n=60, age > 5 months). Sequencing was successful in two of the serum samples and revealed clustering with HEV-3 sequences previously identified in sewage samples (Figure 1b). All farmers (median age 42.9) were HEV IgG-positive but RNA- negative.

Conclusions: HEV-3 is endemic in local pigs. The high force of infection yields anti-HEV-positive, HEV RNA-free animals in the age group used for meat consumption. All farmers demonstrate past HEV infections. As no clinical symptoms were ever noted, continuous monitoring of HEV-3 RNA in pig farms should be considered and the potential impact on farmers and on public health should be further explored.

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Characterization of HIV-1 Drug Resistance to Integrase Inhibitors in drug naïve patients in Portugal

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Background: Highly active antiretroviral therapy (HAART) is the only way to prevent AIDS and control the HIV epidemic. First line treatment (FLT) regimens include drugs of two different drug classes, normally 2 nucleoside reverse transcriptase (RT) inhibitors (NRTI) combined with one nonnucleoside RT inhibitor (NNRTI) or protease inhibitor (PI) or integrase inhibitor (INI). INIs, the most recent class of antiretrovirals (ARV), are well tolerated, effective and durable. As such, they have recently been recommended for FLT in Portugal.
Drug resistance (DR) testing before starting therapy is widely recommended for NRTI, NNRTI and PI, including in Portugal, but not yet for the more recently approved INIs. The increasing use of INIs as FLT underscores the importance of evaluating INI resistance in drug naive patients (DNs).

Aim: We aim to characterize HIV-1 drug resistance to INIs for DN patients in Portugal.

Methods: The collection of data was an extension of the BEST HOPE project (2014-2017) and is ongoing in 19 Portuguese hospitals. HIV-1 integrase region (866 base pair, HXB2 genome location: positions 4230-5096) from plasma samples of 90 integrase DN patients was amplified and DR testing was performed using population-based Sanger sequencing at reference lab Laboratório de Biologia Molecular-Hospital de Egas Moniz.
Drug resistance mutations were analysed using Stanford DB database (hivdb.stanford.edu) and grouped into major and minor mutations as assigned by this on-line tool. For subtyping we submitted all sequences to COMET version 2.2 and Rega version 3.

Results: Of 90 genotypes, 40% were subtype B, 24,0% G, 10.0% C, 8,0% F1, 8,0% A1 and 9,0% recombinants. No major INSTI resistance mutations were detected. Accessory mutations occurred at a prevalence of 2.2% and included T97A (1,1%) and E157Q (1,1%). The 2 patients that presented accessory mutations are from Subtype G.
T97A is a polymorphic accessory mutation selected by Raltegravir and evitelgravir. However, it has also been described as an infrequent integrase polymorphism that occurs in 1%-5% of virus from untreated persons, specially among HIV-1 non-B subtypes [1,2]. E157Q mutation is a polymorphic accessory mutation, found in between 1.7% and 5.6% of viral sequences from ART-naïve patients depending on the viral subtype. E157Q mutation is weakly selected in patients receiving raltegravir and selected in vitro by eviltegravir [2,3].

Conclusion: None of the major INI-resistance mutations was found in this study. The mutations we have found correspond to polymorphisms found in non-B subtypes.
Until now INI-based regimens are expected to be effective across the different major HIV-1 subtypes in Portugal, but there is a need for INI drug resistance surveillance in drug naïve patients.

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Baseline resistance testing for integrase inhibitors in HIV-1 treatment naïve patients, 2016-2017

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Background: Transmitted drug resistance (TDR) in naïve HIV-infected patients has been associated with suboptimal response to Highly Active Antiretroviral Therapy (HAART). Integrase Strand – Transfer Inhibitors (INSTI) based regimen represents a first line therapy, even though testing for INSTI mutation has not been routinely performed. We sought to evaluate the prevalence of INSTI resistance in naïve patients at the moment of HIV diagnosis, during free anonymous screening consultation, in "D. Cotugno" Hospital, Naples (Italy).

Methods: All naïve HIV-infected patients that were screened at our Hospital, during free anonymous screening consultation, called "Gruppo C", from January 2016 to December 2017, were involved in this study. Integrase region was amplified and sequenced from plasma samples and FASTA format sequences were obtained. TDRs were defined according to the Stanford University Drug Resistance database algorithm. Categorical variables are expressed as percentages. Fisher's exact test and 2x2 contingency table were applied to compare proportions, as appropriate.

Results: 290 individuals resulted positive for HIV-test, 181 of these were tested for INSTI genotypic resistance during the study period. 121 (67%) were males; median age was 41 yrs. HIV-1 subtype were: B in 82(45,3%) patients, CRF02_AG in 39 (21,5%) patients, G in 17 (9,4%) patients, A in 17 (9,4%), C in 7 (3,9%) patients and other subtypes in 19 (10,5%) patients. 109 subjects were Italian and 72 were non-Italian subjects. 19/181 (10%) had INSTI mutations; only two had major mutations, E92Q and N155K (5% respectively), they both were found in non-Italian subjects. Other mutations detected were all accessory resistance mutations: T97A in 11 (55%) patients, E157Q in 4 (20%) patients, G163K in 1 (5%) patient, H51Y in 1 (5%) patient, Q95K in 1 (5%) patient. Of these mutations, two (T97A and Q95K) were found in the same patient. Although no significant differences in integrase strand-transfer inhibitor resistance were observed, between Italian and non-Italian subjects, the polymorphic mutation T97A resulted more common between Italian patients compared to non-Italian patients (47% and 5%, respectively) (p=0,0055).

Conclusions: The prevalence of major pretreatment INSTI resistance is very low, being only 1% in our study, while the prevalence of accessory pretreatment INSTI resistance is 10 %. This is an ongoing study, therefore a more complete analysis will be performed. However based on our results, it seems that T97A mutation is strongly associated to Italian population.

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Treatment of chronic viral hepatitis C in difficult patients

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Background: One of the goals of antiviral therapy for chronic hepatitis C is to eliminate the virus, achieve a sustained virologic response (SVR), reduce liver fibrosis and improve the quality of life of patients. Treatment of patients with chronic viral hepatitis C 1 genotype has hitherto been difficult. Achievement of a sustained virologic response at 12 and 24 weeks (SVR12, SVR24) on interferon-containing regimens reached 50-70% in patients with 1 HCV genotype. With the advent of direct-acting antivirals (DAA), a greater number of patients increased the chance of cure to 90-99%.

Materials & Methods: clinical cases and variants of antiviral treatment of "difficult patients" with chronic hepatitis C 1b HCV genotype are investigated.

Results: Clinical case 1. Male, 49 years old, white, Ukrainian, infected with HCV since 1999, liver fibrosis - F2, IL28β - CT genotype, RNA HCV - 2.360.000, genotype HCV - 1b, ALT 109 U/l, alpha fetoprotein (αFP) – 1.85 ng/ml, non-responders. Experience of therapy: received simple interferon-α (IFN-α) in 2005 for 24 weeks (24W). The result - is non-responders. Treatment of pegylated IFN-α2a (Peg-IFN-α2a) / ribavirin (RBV) in 2011 48 weeks (48W). The result - is non-responders. The patient was prescribe Peg-IFN-α2b / RBV in 2014 48W. The result is a partial response, relapse. Treatment of ledipasvir / sofosbuvir (LDV / SOF) / RBV in 2016 for a period of 12W is prescribed. The result is a quick response - HCV RNA is not detected at 4W; SVR12, but relapse at 20W. The patient was decided to prescribe in 2017 for 12W a 3D regimen of therapy (ritonavir-boosted paritaprevir / ombitasvir / dasabuvir). The result is a quick response - HCV RNA is not detected on 4W, SVR12, SVR24, liver fibrosis - F1-F2. Patient monitoring continues.
Clinical case 2. Male, 42 years old, white, Ukrainian, infected with HCV since 2011, fibrosis F2, IL28β - CT genotype, RNA HCV - 9.600.00, ALT 86.6 U/l, non-respondent. Experience of therapy: received in 2013 Peg-IFN-α2b / RBV 48W. Before treatment - fibrosis F3, A3, S3, N1, H0, RNA HCV - 9.000.00, αFP - 4.6 ng / ml, ALT 201 U/l. Result - relapse. The patient was prescribe in 2017 for 12W a 3D regimen of therapy (ritonavir-boosted paritaprevir / ombitasvir / dasabuvir). The result is a quick response - HCV RNA is not detected on 4W, SVR12, SVR24, liver fibrosis - F2. Patient monitoring continues.

Conclusions: Despite the difficulties in approaching the antiviral therapy of patients with the 1b HCV genotype, who did not have the elimination of the virus on interferon-containing regimens, we observed the possibility of regression of liver fibrosis by 1 point. The 3D regimen of therapy makes it possible to achieve SVR24 even in "difficult" patients who had a relapse or had no response to previous therapy regimens.

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HCV therapy as part of a low-threshold harm reduction strategy in HIV/HCV active PWID

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Background: The World Health Organization has recently called for hepatitis C virus (HCV) elimination and has identified injecting drug users as a key population. Clinical trials of 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 clinical practice, including patient profiles that may not have been evaluated in clinical trials to date. The aim of this study was to evaluate the efficacy of all-oral HCV therapies among HIV/HCV coinfected active intravenous drug users (IDUs) followed at a harm reduction program in a European shantytown.

Methods: Between May 2016 and June 2017, active IDUs coinfected with HIV and HCV were recruited and integrated in an interdisciplinary care at a low-threshold harm reduction program, addressing medical, psychiatric, social and addiction-related needs. SVR12 rates for all patients who started treatment were reported on an intention to treat (ITT) basis and we included a modified intention to treat (mITT) analysis excluding non virological failures. Reinfections accounted as failures. Data were collected and managed using Research Electronic Data Capture (REDCap).

Results: A total of 28 HCV/HIV coinfected active IDUs with were included in the study. With a mean age of 44 years, 71% were males and 93% were naïve to HCV therapy. Sixty four percent were Spanish and 30% had an active psychiatric illness. All patients were active intravenous users in the last year, with 4 median injections per day. 93% received OST and 92 % received syringes and other paraphernalia.
All the patients were on ART with a DOT approach and had been undetectable for a mean period of 14 months. Median CD4: 415 (171-665). HCV genotype repartition G1a/G1b/G2/G3/G4 was 63%/11%/0%/26% and 0% respectively. Mean fibrosis measured by transient elastography was 6,1 KPa. Five patients achieved EOT viral response but are still waiting for the result of SVR12 and were not included for the analysis. The overall SVR12 rate was 82.6% (19/23) (ITT) and 90.4% (19/21) (mITT). No one stopped therapy due to side effects, 2 patients lost follow-up, 1 patient had a virological failure after EOT and one patient was re-infected (with a different genotype).

Conclusions: The treatment of HIV/HCV-coinfected IDUs as part of a low-threshold harm reduction program is both safe and effective, extending current clinical trial data into a population entirely made up of active drug users.

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Efficacy of switching to Raltegravir from a virologically effective regimen in HIV/HCV coinfected patients, with and without resistance to NRTIs

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Background: In HIV/HCV-co-infected patients, cART often needs to be changed considering drug-drug interactions. In such patients raltegravir (RAL) might constitute an appropriate part of an antiretroviral regimen, as it lacks interactions with any of the DAAs currently in use. Purpose of this study is to evaluate the efficacy of a switch to RAL in HIV/HCV coinfected patients.

Methods: We studied 188 HIV/HCV coinfected subjects receiving effective cART recorded in the Italian ARCA database from October 2007 to October 2016. Demographic and clinical characteristics, comprehensive of previous genotypic resistance test, were analyzed. We included in the analysis patients in cART with an HIV-RNA< 50 copies/mL at the time of switch to RAL from a not including RAL regimen non including RAL. Patients with documented virological failure to any INSTI were excluded. Kaplan-Meier curves and Cox regression analysis were used to estimate the probability to virological failure (defined by two consecutive HIV-RNA>50 copies/mL) and probability to virological failure according to presence of previous drug resistance mutations to NRTIs and PIs. Covariates in the final model included gender, age, HBV/HCV co-infection, CD4 nadir count, viral load at cART initiation, the previous exposure to NRTIs and PIs, the presence of Thymidine Analogue Mutations (TAMs), M184 mutation, K65 mutation, Q151 mutation and major PIs mutations. P-value <0.05 was considered statistically significant.

Results: The median observation time of observation was of 20 months (CI95% 7.0-37.9). One hundred thirteen (60.1%) patients interrupted the RAL containing regimen for any reason during the time of observation [median overall follow-up: 14 months (5.6-34.7)]. The most frequent causes of cART discontinuation were: virological failure [28 patients (14.9%)] and simplification [28 patients (14.9%)] followed by toxicities [12 patients (6.4%)] and patients choice [11 patients (5.9%)]. We observed no evidence of association between the presence of previous TAMs, M184 mutation, K65 mutation, major PIs mutations and the probability of virological failure. No association was also observed considering the combined effect of this mutation. In the multivariate Cox model the presence of previous TAMs, M184 mutation, K65 mutation and major PIs mutations was not didn't resulted associated with a higher probability of virological failure.

Conclusions: Our results suggest that the switch to RAL is a feasible option despite a not negligible rate of discontinuation due to virological failure. Our results support the utilization of RAL in HCV/HIV patients needing treatment with DAAs, whether or not they harbor HIV variants bearing mutations conferring resistance to NRTIs.

57 *(withdrawn)*

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Phylogenetic segregation of distinct HIV quasispecies detected before and after second-line antiretroviral therapy in a Nigerian cohort

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Background: The majority of people who fail second-line antiretroviral therapy (2L ART), which contains a protease inhibitor (PI) do not develop protease resistance mutations. This longitudinal analysis aims to explore HIV evolution under PI selective pressure by comparing the intrahost viral populations detected before and after PI therapy.

Materials & Methods: HIV-positive participants were selected from a cohort at the University of Abuja Teaching Hospital, Nigeria, if they had experienced virological failure (VF) during both first-line (1L) ART, comprising two NRTIs and one NNRTI, and 2L ART, comprising two NRTIs and one PI. VF was defined as HIV-1 RNA >1,000 copies/mL, detected by clinician-driven viral load testing, at least six months after initiation of 1L or 2L ART. If plasma samples were available from both time points then whole genome deep HIV sequencing was performed using an Illumina platform. Consensus sequences were assembled de novo and variants present at >2% frequency were examined to identify all IAS-USA resistance associated mutations. Viral quasispecies were reconstructed from sequence read data using QuRe software, which maps reads against a reference and identifies overlapping variants that are likely to come from the same haplotype. For each participant, haplotypes from 1L and 2L VF samples were aligned with mafft and intrahost phylogenies were created with FastTree.

Results: Ten participants yielded whole genomes from both time points: eight women, median age 32 (IQR 30 – 36). Nine had received lopinavir and one atazanavir during 2L ART for a median of 37.2 months (IQR 29.9 – 54.7) before the 2L VF sample was obtained. Three participants had developed protease mutations (M46I, I54V and L76V; M46L, I54V and L76V; I54V and V82A) and nine had lost reverse transcriptase mutations between 1L and 2L VF. Five participants had successful viral quasispecies reconstruction, with more than two haplotypes each, ranging from 2196 to 8353 base pairs in length. Strikingly, the phylogenies for each participant showed complete separation of haplotypes according to 1L and 2L VF time points. This separation was seen in all genomic regions, including the relatively conserved pol gene, even after stripping drug resistance mutation sites.

Conclusion: The study participants were predominantly young women with infrequent virological monitoring, typical of a resource-limited setting. The phylogenetic segregation observed between pre- and post-2L ART viruses could suggest these quasispecies arose from different cellular reservoirs, rather than representing a continuous evolution from the virus present at 1L VF as would be expected. The loss of reverse transcriptase mutations could represent the emergence of older lineages, or suboptimal adherence. Although definitive conclusions are limited by the small sample size, further longitudinal sampling of additional participants may provide an insight into the source of escape viruses during 2L ART failure.

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Transmitted drug resistance among naïve HIV-1 infected patients in Central Poland in 2012 - 2017

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Introduction/Background: The usage of antiretroviral drugs for HIV-1 infection treatment can lead to selection and appearance of HIV drug resistant genetic variants. Surveillance of transmitted drug resistance (TDR) mutations plays important role in choosing effective initial therapy regimen in naïve patients. The aim of the study was to estimate the prevalence of TDR mutations and subtype pattern among naïve HIV-1 infected patients diagnosed since 2012 to 2017 in Hospital for Infectious Diseases in Warsaw. The Hospital delivers ART to ca. 60% of Polish HIV patients.

Materials/Methods: One thousand four hundred twenty four plasma samples, obtained from treatment naïve patients diagnosed in our center since 2012 to 2017, were analyzed. One thousand three hundreds twenty patients (92,5%) were men. Patients of Polish origin were included, only. The RNA isolation, amplification and sequencing were performed using ViroSeq HIV-1 Genotyping Kit (Celera) and 3130-Avant Genetic Analyzer (Life Technologies); for the data interpretation Stanford HIV DR Database and Calibrated Population Resistance Tool (WHO 2009 mutation list) were used. Subtype determination was done on the base of PR and RT sequences using REGA HIV-1&2 Subtyping Tool. Maximum likelihood method with aLTR for branch support technique were used for phylogeny tree reconstruction.

Results:

The mean prevalence of TDR in 2012-2017 was 6,56%, starting from 10,16% in 2012, decreasing to 3,5% in 2015 and then reaching 7,47% in 2017. In according to drug classes, mean resistance prevalence was: NRTI – 4,2% (2,32% - 5,24%), PI – 1,36% (0% - 3,95%) and NNRTI – 1,24% (0,46% - 2,01%). The most frequent mutations were: NRTI class – T215Y/F/I/S/C/V/E (75%), NNRTI class – K103N (53%) and PI class – L90M (50%). L90M mutation were detected in 5 cases in 2012 and 4 in 2017. Phylogenetic analysis revealed that it was a result of cluster transmission among MSM. Two patients were infected with variants resistant to two (NRTI and NNRTI) and three (NRTI, NNRTI, PI) drug classes. Prevalence of subtype B decreased from 84,7% in 2012 to 72,3% in 2017. The opposite trend was observed in prevalence of subtype A: increase from 6,55% in 2012 to 14,8% in 2017.

Conclusions: Presented data shows dynamic changes in TDR among Polish naïve patients. TDR in Poland is constantly below 10%, except 2012 where reached 10,16%, which is corresponding to result from our Center from 2000-2011 period. Appearance of rare mutations, like L90M in our cohort, which was the effect of confirmed cluster transmission, can affect significantly TDR ratio. Intense economical migration, mainly from Former Soviet Union countries, enriches genetic diversity of viral pathogens. Especially, we observe rapid growth in prevalence of subtype AFSU – phenomenon more precisely described in our previously report.

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The neurotropic potential of HIV-1 subtype F1

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Background. A high prevalence of neurocognitive impairment (NCI) has been previously reported in a Romanian cohort of young adults growing-up with HIV, all infected with clade F1. To assess the possible neurotropic effect of this HIV subtype, we analized the env sequences derived from stored plasma samples collected from ART-experienced HIV-positive Romanian patients, parentally infected with HIV subtype F in early childhood.

Methods: Plasma samples were sequenced in the env V3 region and analyzed using Geneious 7.0.6 and CodoneCode Aligner 5.0.1. Coreceptor tropism was predicted based on V3 sequences using GENO2PHENO version 2.5. The neurocognitive impairment were assessed using a comprehensive, seven-domain neuropsychological battery and a global deficit score (GDS) was calculated for each participant (cut- off 0.5).

Results: 200 HIV infected patients (median age: 24 years, males: 47%, median HIV infection length: 22 years, median duration on cART: 12 years) were analyzed. Median HIV viral load and CD4 T-cell count were 2.78 log10 HIV RNA copies/mL and 440 cells/mm3, respectively. 58% of the participants had a viral load >1000 copies/ml. Neurocognitive impairment was present in 37% of the study participants. Coreceptor analysis identified 31% of the HIV strains as R5, 37.5% as dual tropic and only 12.5% as R4. HIV R4 tropism was associated with immunosuppression, expressed by both CD4 cells number and CD4/CD8 ratio. There was no correlation between the presence of neurocognitive impairment and CD4 count, viral load, AIDS-defining events, duration of HIV infection, cART exposure or coreceptor usage. However, we found an env region of more than 50 nucleotides that is very conserved between the R5/X4 sequences obtained from the HIV- impaired patients.

Conclusions: Conserved genetic elements in the HIV V3 loop may be important in defining a neurotropic signature clade F virus. Further studies are needed in order to establish the predictive value of this potential HIV env signatures in neurocognitive impaired individuals infected with HIV subtype F in early childhood.

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Does baseline (pre-protease inhibitor treatment) susceptibility predict virological failure in patients infected with HIV-1 CRF02_AG and G subtypes?

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Background: Resistance to protease inhibitors (PIs) results from a complex interplay between protease and gag proteins, with gag showing wide variation across HIV-1 subtypes. Other viral determinants of failure to PIs are poorly understood. HIV-1 CRF02_AG/G viruses have been reported to be less susceptible to lopinavir/ritonavir (LPV/r) than subtype B viruses and therefore these patients may be more likely to experience virological failure. Furthermore, an association with baseline PI susceptibility and virological outcome has been demonstrated in participants of first-line LPV/r monotherapy trials. We hypothesised that baseline PI susceptibility, as determined by a full-length gag–protease assay, is also associated with second-line treatment outcome in a clinical setting.

Methods: HIV-1 gag-protease phenotypic susceptibility in CRF02_AG/G viruses circulating in Nigeria, West Africa was characterized. Individuals who experienced second-line failure (HIV-1 RNA >1,000 copies/mL after >6 months on treatment) on a LPV/r- or atazanavir/ritonavir (ATV/r)-containing regimen, without any major PI mutations were selected as cases. They were matched to controls who had achieved virological suppression (HIV-1 RNA <400 copies/mL) with similar age, sex baseline viral load, baseline CD4 count, and duration of treatment. Baseline (pre-PI) plasma samples from these matched pairs was retrospectively retrieved. Full-length gag–protease was amplified from patient samples for in vitro phenotypic susceptibility testing. Susceptibility to three PIs [LPV, ATV and darunavir (DRV)] was measured using cell-based, VSV-g pseudotyped single replication-cycle phenotypic assays. The 50% inhibitory concentration (IC50) was calculated using logistic regression, susceptibility was expressed as a fold-change in IC50 compared with the subtype B reference strain (p8.9NSX+). Two-tailed, paired t-tests were performed, with $p<0.05$ considered statistically significant.

Results: Six matched case-control pairs were studied. There was no significant difference in PI susceptibility between successes vs failure groups among five pairs. Baseline susceptibility to all 3 PIs was lower in viral failures than viral successes, but the differences were not statistically significant. Median (IQR) LPV susceptibility in virological successes versus failures was 4.126 (3.147 - 8.167) versus 4.040 (2.485 – 7.895), p=0.96. The same pattern was replicated for ATV and DRV where median (IQR) susceptibilities in virological successes versus failures were: 4.394 (1.601 - 7.732) versus 2.425 (1.347 – 9.655), p=0.84 and 1.529 (1.136 – 2.319) versus 1.234 (0.8400 - 2.047), p=0.86, respectively. One pair showed some significant differences between the success and failure groups. Fold change in IC50 to ATV, DRV and LPV in this pair was 3.4 vs 26.5, 1.3 vs 4.0 and 3.1 vs 10.7 respectively between success and failure patients.

Conclusion: Baseline susceptibility does not seem to be associated with success or failure of 2L PI in these individuals with CRF02_AG and G subtypes. In addition to reduced baseline susceptibility, evolution of resistance has been shown to be a contributing factor to PI failure, despite the absence of classical PI resistance mutations by standard testing methods. Poor adherence has also been shown to be a major cause of drug resistance. This combination of factors could be responsible for the PI failure observed in this cohort.

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A potential new HIV-1 circulating recombinant form identified in Slovenia

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Background: HIV is well known for genetic diversity due to error-prone reverse transcriptase and recombination events. Nearly 90 circulating recombinant forms (CRFs) together with nine founder subtypes have been characterized within group M, the most widespread of the HIV-1 groups. We performed detailed subtyping analysis based on the divergent subtyping results of the routinely obtained pol sequences to potentially identify a new candidate for CRF. Since some of the newly recognized CRFs had been linked with more rapid disease progression, emerging new circulating HIV variants should be surveyed.

Materials & Methods: Partial pol sequences obtained from HIV-positive persons newly diagnosed in Slovenia in the period 2000–2016 were obtained primarily for the study of transmitted drug resistance. Possible unique recombinant forms were determined by the use of four subtyping tools (Rega 3, COMET, jpHMM, and SCUEAL) and phylogenetic analysis. Among these, a single sample that could most likely represent a new recombinant form was selected for further near-whole genome sequencing. Namely, four over-lapping PCR amplicons were obtained and joined in equimolar amounts, library was prepared using Nextera DNA Flex and sequenced on Illumina MiSeq platform. Initial assemblies of the obtained reads were generated using SSAKE and the longest HIV scaffold was used as a guide in the second stage of assembly with SPAdes. The longest contig was further polished with Pilon to obtain the final sequence. Evidence of recombination was determined by bootscan analysis using SimPlot.

Results: A total of 389 sequences obtained from persons diagnosed with HIV-1 in the period 2000–2016 in Slovenia were subtyped and a total of 35 sequences gave divergent results with at least one of the subtyping tools. Phylogenetic analysis revealed a cluster of three sequences, all subtyped as complex recombinants with REGA 3, COMET and SCUEAL and one of these was selected for further near-whole genome analysis. Four PCR amplicons were successfully obtained and sequenced on Illumina MiSeq. Illumina reads were assembled to form a final contig of 9,070 bp. jpHMM subtyping depicted a complex recombinant with the following composition: A1, G, D, B, A1, G, A2, G, A1, G, A1, G. Next, the sequence was aligned with the pre-generated RIP alignment derived from the Los Alamos HIV sequence database, encompassing all genomes from HIV-1 subtypes and CRFs and further bootscan analysis was performed using SimPlot. Mosaic pattern of the sequence was identified with 9 breakpoints distinguishing most-probable parental strains CRF19_cpx and CRF20_BG. These two CRFs were first recognized in Cuba, making further recombination events plausible. Furthermore, additional Blast search revealed a similar sequence identified in Spain (GU830956), but not yet fully characterized. With two additional pol sequences obtained from another two individuals from Slovenia with a similar complex recombination pattern observed within pol region our findings suggests that this particular strain could be a candidate for a new CRF.

Conclusions: Potential novel HIV-1 circulating recombinant form was identified in three individuals from Slovenia, exhibiting a mosaic pattern of parental strains CRF19_cpx and CRF20_BG, both originating from Cuba.

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Natural polymorphisms of HIV1 sub-subtype A6 variant genes coding accessory proteins NEF, VIF, VPR and VPU cerculating in russia.

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Introduction: There is a unique epidemiological situation has developed on the territory of former USSR. The AFSU variant has recently started to be considered as a separate sub-subtype of the A6 in the world scientific community. This subtype has significant differences in the structure of pol, gag and env genes, but the genes of accessory proteins has not been subjected to detailed analysis, according to the data of literature. The accessory proteins perform many important functions that contribute to the replication of virus and are involved in the interaction of virus with a human immune system. The question of genes polymorphism importance, in particular the presence of differences between HIV subtypes has been little studied, and in the literature only few notes of single polymorphisms and their relationship to the nature of HIV infection can be found.

Materials & Methods: We used blood plasma from HIV-infected patients from different regions of Russia, previously identified as AFSU. We obtained 47 near full-length genome sequences using the MiSeq («Illumina», USA) technology and MiSeq Reagent Kits V2. 54 complete genome sequences were also added to A6 sub-subtype of HIV-1 circulating in the territory of the countries of the former USSR from Genbank. All the sequences were cut into fragments corresponding to vpr, nef, vif and vpu genome regions. A preliminary analysis of the mismatched positions of the HIV-1 genome was carried out using the SimPlot program, v. 3.5.1 (http://sray.med.som.jhmi.edu/SCRoftware/simplot/). The comparison was made with the sequences of the reference strain HXB2 (subtype B), as well as with the consensus sequence of viruses of subtype A1, obtained from GenBank. All sequences were subjected to phylogenetic analysis by MEGA 6.0 program (http://www.megasoftware.net).

Results: In the course of the work, a large number of characteristic mutations of the sub-subtype A6 were detected. One of these mutations in the gene vif is Q136P, which according to the data (De Maio FA, 2011), should be particularly emphasized, causes the accelerated onset of AIDS in newborn infants; this mutation was found in more than 80% of the A6 samples. Phylogenetic analysis showed that the genes nef, vif and vpu of the subtype A6 form a separate clusters on the trees. At the same time, the vpr gene formed a subcluster within the cluster of the A1 subtype. So it can be assumed that the vpr gene is more conservative than the others.

Conclusions: Our study revealed significant differences in the regulatory proteins of the A6 subtype from the A1 subtype and the reference strain HXB2 (subtype B). Further study of the HIV-1 genome, typical for Russia, using modern methods of full genomic analysis will allow obtaining new information and planning studies aimed at elucidating the true role of polymorphisms

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Whole genome deep sequencing of HIV reveals extensive multi-class drug resistance in Nigerian patients failing first-line antiretroviral therapy

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Background: First-line antiretroviral therapy (1L ART) in resource-limited settings is often provided without routine viral load monitoring or drug resistance testing. Whole genome deep sequencing (WGS) could improve understanding of treatment failure and the emergence of resistance by revealing the distribution of mutations throughout the viral population over time. However, this tool is often inaccessible in the non-B subtype epidemics that affect low- and middle-income countries.

Materials & Methods: Adult patients receiving 1L ART (two NRTI and one NNRTI) at the University of Abuja Teaching Hospital, Nigeria, were included if they had experienced virological failure (HIV-1 RNA >1000 copies/mL, at least 6 months after ART initiation, confirmed by clinician-driven testing), and had a stored plasma sample available for WGS. All IAS-USA resistance-associated mutations detected within the intra-host viral population at >2% frequency were considered (to minimise the inclusion of very low-level variants arising from sequencing errors). Mutations were stratified by frequency within the sample and by duration of 1L ART.

Results: Sixty participants were sampled during 1L failure (73% female; median age 30 (interquartile ratio [IQR] 28-35); median CD4+ cell count 110 cells/mm3 (IQR 63-191); median 28 months after ART initiation (IQR 18-41)). The HIV subtypes/recombinants were CRF02_AG (56%), G (37%), CRF06_cpx (5%), and C (2%). The 1L ART agents included AZT (67% exposed), TDF (55%), 3TC (92%), FTC (48%), NVP (85%), and EFV (18%). At 1L failure, 57% of participants had thymidine analogue mutations (TAMs), with 30% harbouring 3 or more TAMs, 95% had other (non-TAM) NRTI mutations and 100% had NNRTI mutations. The most common mutations were M184V, Y181C, G190A, K65R and K103N. Overall, 17% (61/367) of the mutations identified were low-level minority variants (present at 2-20% of the intra-host viral population), which would not have been detected by standard resistance testing methods, 24% (88/367) were present at 20-90% frequency, and 59% (218/367) were dominant majority variants representing >90% of the participant's viral population. The prevalence and number of reverse transcriptase mutations were similar regardless of 1L ART duration, with highly resistant viruses detected less than a year into therapy. None of the participants had major protease or integrase inhibitor mutations.

Conclusions: Diverse Nigerian HIV clades exhibit multi-class drug resistance at 1L ART failure. The predominance of high-frequency mutations suggests that emergent resistance had become fixed in the viral population by the time of sampling. Routine viral load monitoring and adherence support are likely to be crucial from the outset of therapy to prevent the emergence of resistance and to preserve future therapeutic options. The absence of protease and integrase mutations indicates that second- and third-line agents are predicted to be efficacious, however, the clinical effectiveness in this population remains unknown in the context of extensive NRTI resistance.

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Monitoring of HIV drug resistance in Uzbekistan.

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Background: To study the prevalence of HIV drug resistance among patients in Uzbekistan were collected 713 blood samples from adult patients (ART-naive and on ART) for genotyping of HIV and drug resistance assessmants.

Methods: Genotyping of HIV, epidemiological investigation, phylogenetic analysis.

Results: As antiretroviral treatment coverage is expanding among patients HIV the monitoring of drug resistance in Uzbekistan becomes a issue of national importance. In a nationwide study. Totally 713 blood samples were collected from adult patients (344 ART-naive and 369 - those on ART) with 375 of them Pol gene successfully detected. In ART-naive cohort, total number of SDRMs did not exceed 2.7%. Among the patients receiving ART, the following results were obtained. The median duration on therapy was 11-29 months. High resistance against NRTI class drugs was most prevalent against AZT - 88.89%, with less prevalent against 3TC - 49.57%, FTC - 49.57%, ABC - 27.35%, DDI - 26.50%, TDF -20.51%. The prevalence of high resistance against NNRTI class, such as nevirapine and efaverence, 62.39% и 57.26% respectively. PIs are represeted as LPV/r and high resistance to this preparation is a very low prevalence - 0.74%. The HIV subtypes were majorly presented as CRF_02AG (52.27%) and a subtype A1 (38.40%).

Conclusions: The study results reveal high potential for further use of PIs in Uzbekistan. The successful implementation experience should be used for epidemiologic purposes as well as in routine clinical practice to individualize the therapy.

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Evaluation of the efficacy of human antibodies against hepatitis C virus antigens

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Background: Hepatitis C virus (HCV) is a major health problem worldwide particularly in Egypt. The humoral immune response has an important function in the control of HCV infection.

Aim: To study the role of neutralizing antibodies in Hepatitis C Virus (HCV) clearance in infected individuals.

Methods: This study was carried out on apparently healthy blood donors (n=200). Detectable HCV antibodies were assessed by commercial ELISA. Specific human immunoglobulin targeting peptides derived from HCV E1 /E2 regions in blood samples were measured by in house optimized ELISA. Human IgG purification was carried out from both samples positive and negative for HCV RNA in order to evaluate its neutralizing activity invitro using Huh 7 cells.

Results: The studied cohort included 96/200 subjects who were positive for HCV antibodies, among which: 56/96 (58%) samples were positive for HCV RNA (group 1) and 40/96 (42%) samples had undetectable HCV RNA (group 2). ELISA results showed that Human HCV Immunoglobulin (HHI) targeting HCV E1 synthetic peptide (a.a 315-323) was detectable in 63/96 (66%) and HHI targeting HCV E2 (a.a 412-419) were positive in 14/96 (15%) while 19/96 (19%) were positive for HCV E2 (a.a 517-531). (HHI) higher than the cutoff level against peptide HCV E1 (a.a 315-323) were detected in 22/63 (35%) in blood donors group 2 and positive in 41/63 (65%) in group 1. HHI against peptide HCV E2 (a.a 412-419) were positive in 7 (50%) in blood donors group 2 and also positive in 7 (50%) of group 1. In addition HHI targeting HCV E2 (a.a 517-531) were positive in 11 (60%) in group 2 compared with 8 cases (40%) in group 1. Purified human antibodies from cases positive for HCV antibodies and negative for HCV RNA showed invitro neutralization at concentrations 30 and 10 µg/ml while the same concentration of purified human IgG from cases positive for HCV RNA showed no viral neutralization.

Conclusions: The tested epitope (s) derived from HCV envelope E1 and E2, are important for viral clearance and hence can be used for HCV vaccine development.

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The patterns of integrase strand transfer inhibitors drug resistance mutations in HIV-1 CRF06_cpx in Estonia

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Background: The use of integrase strand transfer inhibitors (INSTIs) is in rise across Europe including Estonia. The susceptibility to INSTIs varies among HIV-1 subtypes due to dynamic genetic diversity in different HIV-1 variants. The aim of this study was to describe the distribution of INSTIs drug resistance mutations (DRMs) among ART-experienced but INSTIs-naive patients (INSTI-naive) and those who have failed INSTIs treatment.

Materials and Methods: Genotypic resistance testing was performed using plasma of 50 INSTIs-naive and 34 INSTIs-failed patients from 2013 to 2017. Viral RNA was amplified and sequenced in HIV-1 integrase region. INSTIs DRMs were detected using Stanford University HIV-1 Drug Resistance Database. Subtyping was conducted using REGA HIV-1 & 2 Automated Subtyping Tool (Version 2.0).

Results: INSTIs-naive and INSTIs-failed groups consisted of 68% and 62% males with median age of 35 (IQR 32 – 39) and 37 (IQR 31 – 39), median CD4+ T cell count of 160 (IQR 82 - 359) and 231 (IQR 116 – 342) cells/mm3, and median HIV-1 viral load of 4.4 (IQR 4.1 – 4.9) and 4.4 (IQR 3.6 – 4.9) log10 copies/ml, at the time of DRMs testing, respectively. Of 84 sequenced samples 79% were HIV-1 CRF06_cpx, 15% unique recombinant forms, 5% subtype A1 and 1% subtype B. No INSTIs DRMs were found in INSTIs-naive patients whereas major INSTIs DRMs were detected in 20/34 (59%; 95% CI 42.2 – 73.6) INSTIs-failed patients. The most common INSTIs DRMs were Y143C/R/H (8/20) and N155H (8/20), followed by E138K/A/T (6/20), T66A/I/K, Q148H/K/R/N, E92Q (2/20), S147G (1/20), and G118R (1/20). Most of the detected DRMs were associated with high/intermediate resistance to elvitegravir and/or raltegravir, but low or no effect on the susceptibility to dolutegravir. However, three viruses possess two or more DRMs (Y143CHRY/N155HN; T66A/S147G/ Q148R/E138K and E138K/Q148R) which increased the resistance to dolutegravir to intermediate level.

Conclusions: INSTIs DRM-patterns in CRF06_cpx viruses from INSTIs-failing patients are generally identical to subtype B viruses indicating similar clinical response to the therapy. As no INSTIs DRMs were detected in INSTIs-naive patients there is no need for INSTIs DRMs testing prior to INSTIs initiation. However, the high prevalence of INSTIs DRMs detected among INSTIs-failed patients suggests an essential need for resistance testing in HIV management with INSTI therapies.

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High prevalence of Transmitted HIV Drug Resistance Mutations in a cohort of newly diagnosed HIV-infected patients at entrance to care in the period from 2014 to 2015: the Croatian data

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Background: Croatia has a centralized system of HIV clinical care and all patients are treated at University Hospital for Infectious Diseases "Dr. Fran Mihaljevic", Zagreb (UHID). Previous national study by Grgic et al. showed a high prevalence of transmitted drug resistance (TDR) of 21.6% (in the period 2006 to 2008), mainly attributed to the transmission clusters among MSM characterized by the presence of T215S mutation (1).
In order to continue national TDR surveillance, we analyzed the prevalence of TDR mutations for the time period 2014-2015. Included were newly diagnosed, treatment-naive patients who entered clinical care at UHID during the study period.

Materials and methods: In the period 2014-2015, a total of 221 newly diagnosed HIV-infected persons entered clinical care. The study included 111 patients providing a coverage of 50.2% (111/221) for the observed period.
Based on the stage of HIV infection at the time of diagnosis, patients were divided in two groups: patients diagnosed at the recent stage of HIV infection (47/221, 21.3%) and patients diagnosed at the chronic stage of HIV infection (174/221, 78.7%). In correspondence to this subdivision, TDR was analyzed within two groups (45/47, 96%) and (66/174, 38%), respectively. The entire protease HIV-1 gene (PR, codons 1-99) and a part of the reverse transcriptase HIV-1 gene (RT, codons 1-250) were sequenced by using Viroseq HIV-1 Genotyping System, TruGene HIV-1 Genotyping Kit and a validated in-house method. Mutations were determined by using Surveillance Drug Resistance Mutation list (SDRM) (2). HIV subtype was determined with Rega HIV-1 subtyping tool.

Results: SDRM were found in 26 patients (26/111, 23.4%, 24 men, 92%, 2 women, 8%). All, but one patient, were infected with HIV subtype B. Risk factors for HIV infection for the patients carrying SDRM were MSM (23/26, 88.5%), heterosexual (2/26, 7.7%) and MTCT (1/26, 3.8%). SDRM analyzed for patients with recently acquired HIV infection were detected in 9 participants (9/45, 20.0%), while in a cohort of patients diagnosed at chronic stage of HIV infection were detected in 17 participants (17/66, 25.8%). Mutations conferred resistance to both RT inhibitors (RTI) and PR inhibitors (PI). Eight patients carried SDRM T215S (8/111, 7.2%) and were a part of the local T215S transmission cluster. Other SDRM to RTI found were T215D (7/111, 6.3%), K101E (7/111, 6.3%), K103N (6/111, 5.4%), L100I (6/111, 5.4%), M41L (3/111, 2.7%), G190A (1/111, 0.9%), K219Q (1/111, 0.9%), L210W (1/111, 0.9%), M184V (1/111, 0.9%) and Y181C (1/111, 0.9%). SDRM to PI found were V31I (4/111, 3.6%), I47V (4/111, 3.6%) and I84V (1/111, 0.9%).

Conclusion: We found a high prevalence of TDR in the cohort of newly diagnosed, treatment-naive patients with HIV infection (23.4%). Ongoing national surveillance is necessary to monitor the dynamics of HIV infection and transmission networks in Croatia.

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Frequency, reasons and prediction replacement of antiretroviral drugs

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Background: The introduction of universal access to ART for HIV-infected patients in Belarus, prolongation of their life on ART result in an increase in the frequency of antiretroviral drugs (ARVD) changes in the ART schemes.
The aim of the study was: to present the frequency, causes and prognosis of replacing ARVD in therapy regimens.

Material and methods: A retrospective analysis of the medical records of HIV-infected patients (n = 434) registered in the Grodno Regional Clinical Hospital consulting clinic, which received ART as of December 30, 2017, was performed. Frequency, timing and reasons for the replacement of ARVD in the schemes: virological failure (VF), immunological failure (IF), adverse events in ARVD, correction of the ART regimen were evaluated. The forecast of the timing and frequency of ARVD replacements is made with survival models and Kaplan-Mayer curves, generalized linear models are used.

Results: One change of the ARVD among the total number of patients (n = 434) receiving ART was in 16.4% (n = 71) [95% CI: 12.41-21.27] patients, twice in 8.5 % (n = 37) [95% CI: 5.74-12.49], and 3 or more times in 1.6% (n = 7) [95% CI: 0.65-3.95] patients. In patients receiving ART more than 3 years (n = 196), the frequency of drug substitution in the regimens reached 49.5% [95% CI: 40.72, 58.29]. One-time replacement of ARVD among them was 28.6% [95% CI: 21.27-37.19], double substitutions - 17.3% [95% CI: 11.63-25.08], three and more than once changed ARVD - 3.6% [95% CI: 1.44-8.56].
The causes associated with VF and IF of the current treatment regimen, including cases of drug resistance were 11.7% [95% CI: 8.42 -16.16] (in 51 patients). Adverse events on ARVD - 10.1% [95% CI: 7.07-14.34] (44 patients), correction of the scheme in connection with pregnancy and the presence of concomitant pathology - 2.1% 95% CI: 0.93-4.58] (9 patients), the absence of drugs - 5.2% [95% CI: 3.19-8.67] (23 patients). In 3% [95% CI: 1.53-5.8] (13) cases, the replacement was carried out for simplify the regimen. It was found that for 50% of persons who received a single shift, the replacement of ARVD occurs in the interval from 11.6 to 22.1 months from the start of therapy, while for 50% of persons with a double shift - in the interval from 27 to 48.2 months. The median of the third shift is 56.3 months.
It is projected that the 1st shift of ARVD occurs after 17.1 (11.6, 22.1) months from the onset of ART in 16.4% [95% CI: 12.41 - 21.27], the second shift - after 28.8 (27, 48.2) months in 8.5% [95% CI: 5.74-12.49], the third in 60.6 months in 1.6% [95% CI: 0.65-3.95] patients.

Conclusions: In HIV-infected patients who were on therapy for more than 3 years, the frequency of ARVD replacement in treatment regimens reached 49.5% [95% CI: 40.72, 58.29]. The gradual introduction into clinical practice of new ARVD groups that are highly effective and do not have cross-resistance with long-term ARVD is needed.

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Reality of an immunodeficiency outpatient Portuguese clinic. Who are and how are HIV infected patients being treated?

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Introduction: In Portugal is estimated that 31 thousand people HIV infected are under antiretroviral therapy (ART) and for clinicians who follow these patients, the rate of viral suppression is satisfactory and similar to other European countries. Most of clinicians follow the updated European therapeutic guidelines.

Material & Methods: The authors wanted to analyze what are the main features of their patients regarding treatment and outcome. To be aware of the true viral suppression, the authors made a transversal, randomized analysis of a sample of patients followed in an outpatient clinic of Infectious Diseases, of a central hospital, during a three month period, to find out what are the current therapeutic regimens. With this purpose, only those individuals under ART for more than six months were included. Demographic data, duration of therapy, antiretroviral drugs prescribed and values of current and previous RNA-HIV were collected. As a secondary goal, prevalence of HBV and HCV status were analyzed.

Results: from a total of nearly 2500 HIV infected patients followed in this unit, a total of 444 pt (17,6%) were reviewed. They were predominantly male (72,7%) and the average age was 49,1 yrs (between 21-88 yrs). The average duration of ART was 10,4 yrs, while 43,4% were on ART for more than 10 yrs and 4,5% for more than 20 yrs. ART consisted in NRTI+NNRTI-35,3%, NRTI+II-36,9%, NRTI+PI-18,6% and others 9%. From the total, STR (single regimen tablet) was used in 47,7% of patients (ATR-7,9%, EVP-20,9%, STR-2% and TMQ-17%) and dual therapies in 5,4%. Detectable viraemia (RNA-HIV >20 cp/ml) was detected in 41 patients (9,2%), being the value >200 cp/ml 2,2%. When looking to previous determinations (one or more), detectability was present only in 3,6%. Those with RNA-HIV >200 cp/ml (10 pts) were under treatment for 8,8 yrs and regimens include different ART classes.
The prevalence of AgHBs and HCVab was 2,4% and 29,3%, with an average age of 47,4 and 46,7 yrs respectively. From 115 patients with HCVab+ and RNA-HCV-, the majority were treated for HCV (84,3%) and the remaining 18 (15,6%) had a spontaneous clearance of the virus. Those with detectable RNA-HCV (15 pt) were either on treatment/waiting for treatment (7 pt), had a relapse (4 pt) or had a reinfection (4 pt).

Conclusions: Our patients were mainly treated with STR and Integrase inhibitors were the most frequent drugs prescribed (40,3%), each with NRTI, PI or included in dual therapies. True viral suppression rate was found in 96,4% of the patients, in line with the goals of WHO. In those with detectable viraemia, in addition to blips, non-adherence was the main reason for failure, without responsibility of any therapeutic class. Regarding HCV, 84,3% of the patients had access to treatment with 96% rate of success, which is also in accordance with the proposed objectives.

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Prevalence of baseline resistance associated substitutions in NS3 and NS5A of hepatitis C virus in Slovenia

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Background: Treatment of hepatitis C virus (HCV) had significantly improved due to the development of direct-acting antivirals (DAAs) since their first approval for clinical use in 2011. DAAs are targeting NS3, NS5A and NS5B viral proteins and preexisting resistance-associated substitutions (RASs) play an important role in treatment success. We aimed to investigate the prevalence of baseline RASs in NS3 and NS5A and estimate treatment success with the first and second generation DAAs among the patients in Slovenia.

Materials & Methods: The study included all HCV sequences generated for routine pre-treatment drug resistance assessment in the period 2013–2018 from DAA-treatment naïve Slovenian patients. Obtained sequences were analyzed using HCV interpretation system Geno2pheno [HCV] 0.92 for RASs associated with resistance to eight DAAs targeting NS3 and six DAAs targeting NS5A HCV viral proteins.

Results: A total of 217 baseline HCV sequences were included, namely 186 NS3 sequences and 31 NS5A sequences. The majority of NS3 sequences were subtype 1a (94.6%), since these were obtained primarily for the detection of the Q80K polymorphism, and the rest were subtype 1b (5.4%). RASs were present in 63.1% (111/176) of HCV subtype 1a sequences and in 30.0% (3/10) of HCV 1b sequences, exhibiting 16 different mutations. The most prevalent subtype 1a RASs were: 80K (68/176; 38.6%), 174S (54/176; 30.7%), 54S (11/176; 6.3%) and 174N (10/176; 5.7%). Other RASs were detected in less than 5%, namely 36L, 55A, 55I, 91T, 117C, 117H, 153I, 168E, 170V, 174G and 174H. According to Geno2pheno HCV algorithm 39.2% (69/176), 11.4% (20/176), 1.7% (3/176) of patients with subtype 1a were resistant to simeprevir, boceprevir, and voxilaprevir, respectively. One patient (0.6%) was resistant to asunaprevir, grazoprevir and paritapevir (mutation 168E) and none were resistant to glecaprevir and telaprevir. Reduced susceptibility to telaprevir, voxilaprevir, asunaprevir, grazoprevir, and simeprevir was observed in 39.8%, 38.1%, 3.4%, 3.4% and 2.3% of patients, respectively. The mutation 56F, which exhibits reduced susceptibility to grazoprevir, was the only observed RAS among subtype 1b sequences and was seen in 3 patients (30%).

Among NS5A sequences the following subtypes were detected: 1a (64.5%), 3a (22.6%), 1b (9.7%) and 4d (3.2%). In 3/20 patients (15.0%) with subtype 1a the following RAS were detected: 28T, 30R, 58Y, 93N. Only one patient was observed with RASs in subtype 1b (1/3, 33.3%, RASs: 31I, 58S, 93H) and subtype 3a (1/7, 14.3%, RAS: 93H). All 5 patients with RASs detected in NS5A (16.1%) were resistant to daclatasvir, elbasvir, ledipasvir and ombitasvir. Three patients (9.7%) were resistant to velpatasvir, one per subtype (5.0% 1a, 33.3% 1b and 14.3% 3a). Only one patient (3.2%) was identified with resistance to pibrentasvir (5.0% among subtype 1a).

Conclusions: We identified a low prevalence of baseline NS3 mutations associated with resistance to asunaprevir, glecaprevir, grazoprevir, paritaprevir and voxilaprevir among HCV subtype 1a sequences, in contrast to a high prevalence of resistance to simeprevir and boceprevir. In addition, the determined prevalence of baseline RASs to NS5A inhibitors in Slovenian population was not insignificant, proving the importance of pre-treatment NS5A testing.

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Molecular typing of the HBV epidemic in Poland: Rising genetic diversity.

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Background and Aims: HBV genetic diversity is increasing in Polish hepatitis B patients, which may have public health implications. HBV genetic variants may exhibit differences in several properties including: transmission rates, disease progression, antiviral treatment efficacy, replication capacity, increased potential to create and develop drug resistant variants due to naturally occurring polymorphisms, and accuracy of diagnostic tests. Here we present first in Poland, detailed phylogenetic analysis of HBV pol/S region sequences obtained in years 2011-2017, to investigate the changes in pattern of HBV genetic heterogeneity over time.

Method: One thousand three hundreds thirty eight plasma samples collected during 2011-2017 from newly diagnosed Polish CHB patients were sequenced in pol/S region. Sequences obtained with in-house test and AbiPrism 3130 were verified and corrected with the Seqscape 3.1. Further analysis was performed by geno2pheno algorithm. Fifty HBV reference sequences were downloaded from GenBank to improve the study. Alignment was performed with Kalign, ML method with aLTR for branch support technique was used for phylogenic tree reconstruction; used procedure creates as precise results as bootstrapping but is less time-consuming.

Results: Seven independent phylogenetic trees were constructed with 142, 312, 197, 226, 152, 141 and 168 sequences for the period 2011 - 2017, respectively. Comparison of the data obtained in the year 2011 and 2017 indicated decreasing prevalence of A2 subtype from 72,5% to 64,3%, respectively. The second most prevalent genotype was D2: 16,2% in 2011 and 18,4% in 2017. Prevalence of genotype H increased from 5,6% in 2011 to 9,2% in 2016. Rising prevalence of D1, D3, B3, B4, C1 and C2 subtypes was determined. We identified a clusters of highly associated sequences forming separate groups of subtype A2, D2 and H, which are probably an effect of previous singular introductions. These groups form a compact, homological, separated clades. Second group of sequences exhibits high genetic heterogeneity forming weakly related monophyletic lineage.

Conclusion: Genetic diversity of HBV in Poland becomes more complex. Analysis conducted in our center since the year 2006, when we detected genotypes A (69%) and D (28%), documents rising prevalence of new genetic types, with intensively increasing genetic heterogeneity of isolates. The most common HBV genotype in Poland remains A with predominant subtype A2. Our results document acceleration in HBV genotype diversity in a Polish population over the past years. The most probably the prevalence of new subtypes will continue to increase disproportionately to A2 subtype, as a result of migration intensification caused by geopolitical and economical reasons. Surveillance studies of HBV diversity remain important for diagnostic, clinical and epidemiological reasons.

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First Results: of HIV-1 identification in purified spermatozoa during assisted reproduction programs for serodiscordant couples in Russia

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Background: Development of reproductive technologies significantly reduced the risk of HIV infection of fetus and woman. The new Russian guidelines permitted assisted reproductive technologies (ART) for HIV-infected patients in Russia since 2013. But this permit allotted a complicated task for laboratories to study the sperm for the HIV presence. An urgent need for PCR methods to identify HIV in washed sperm had arisen after the authorization of the ART application for discordant couples when one partner (man) is HIV-positive. HIV infection in a man does not a contraindication today, and after appropriate processing and testing his sperm can be used for artificial insemination, in vitro fertilization (IVF) or ICSI of the partner.
Central Research Institute of Epidemiology (Moscow, Russia) registered for the in vitro diagnostic the AmpliSens HIV-C-FL kit in September 2015. It is the first and exclusive kit which officially adapted for the needs of ART in Russia.
The purpose of our work was to analyze the results of using the AmpliSens HIV-C-FL kit for purified spermatozoa testing from HIV-seropositive men.

Materials & Methods: 174 samples of purified spermatozoa were obtained from 7 IVF clinics. Each clinical sample was tested using the AmpliSens HIV-C-FL kit according to the instruction.

Results: 9 HIV-positive results were obtained: it is 5.3% of all valid tested samples. The number of invalid result was 2.3% (4 samples). It is necessary to note that all invalid results were obtained for clinical samples from one clinic (clinic 1). We suggested that there are special features in the sperm washing protocol that negatively affected on the quality of the PCR study. However, these samples were detected during the first months of the sperm washing procedure implementation. Three months after the beginning of the study, this clinic modified the washing protocol, and we did not receive any invalid result from that moment. Almost half of the HIV-positive samples (4 of 9) were received from the one clinic (clinic 2). All samples were from the patients with high concentration of HIV RNA in the blood plasma (more than 10.000 copies/ml) immediately before sperm delivery.

Conclusions: The first result of AmpliSens HIV-C-FL use in routine clinical practice have shown the necessity of using it during ART for seropositive couples. HIV-positive results indicate the benefit and need for testing of clinical material in order to reduce the risk of infection of the mother and child during ART. We confirmed that the viral load of a HIV-infected man is an important factor (as described in the Procedure for Using ART) because the efficiency of sperm washing is not 100% in this case. It is significant that the lack of a published standardized sperm washing procedure negatively affects on the quality of medical care. A detailed protocol of sperm washing is very important for IVF laboratories which just starting to work with seropositive couples. This protocol will help to reduce a quantity of invalid results in PCR testing and will also simplify to use ART for such category of couples for new clinics.

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Phylogeographic analysis of HIV-1 subtype A infection in Cyprus: The Cyprus HIV-1 Transmission Cohort Study (CHICS) from 1986 to 2012

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Background: HIV-1 subtype A is the predominant non-B clade in Cyprus. Subtype A circulates also at high prevalence in Greece, Albania (sub-subtype A1) and in Eastern Europe (sub-subtype A6). Our aim was to investigate the patterns of dispersal and the origin of subtype-A transmissions in Cyprus by means of phylogeographic analysis.

Materials & Methods: We studied 66 A sequences available in the protease and partial reverse transcriptase regions, isolated from HIV-1 diagnosed patients from Cyprus during 2004-2012. HIV-1 subtyping was carried out using automated subtyping tools (COMET, REGA), and further verified by phylogenetic analysis using 216 globally sampled sequences as references. We analyzed phylogenetically the A sequences from our study population (N=66) along with all the available A sequences from Greece (N=1,230), a random set of globally sampled A sequences available on Los Alamos HIV database (N=3,000) and the most closely related sequences to our study population using the HIV BLAST tool (N=92), used as references. Local transmission networks (LTNs) were phylogenetic clusters including sequences from Cyprus at proportions >70%. Phylogenetic trees were estimated by maximum likelihood (ML) method as implemented in RAxML v8.0.20, using the GTR+G model. In order to verify our results, we performed further analysis based on approximate ML method as implemented in FastTree v2.1 (GTR+cat model). Phylogeographic analysis was performed by reconstructing ancestral states using the criterion of parsimony.

Results: Phylogenetic analysis revealed that 37 (56.1%) sequences sampled in Cyprus clustered within the Greek LTN. Specifically, the 81.1% (30 out of 37) of these sequences formed 7 distinct phylogenetic clusters (LTNs), with a range of 2 to 11 sequences. The majority of sequences found within the 7 LTNs, were from individuals living in Cyprus (N=26, 86.7%) reported men having sex with men (MSM; N=20, 66.7%) as transmission risk group. For 29 (43.9%) sequences from Cyprus we found that they did not cluster within the Greek LTN. Most of these sequences had been isolated from heterosexuals (N=19, 65.5%), originated from Cyprus (N=8, 27.6%), Georgia (N=6, 20.7%) and Ukraine (N=5, 17.2%). Phylogeographic analysis showed that the origin of transmissions for the majority of individuals from whom sequences didn't belong to the Greek LTN, was Russia (N=20, 69.1%; sub-subtype A6), Kenya (N=1, 3.4%) and Cameroon (N=1, 3.4%). For the rest of the sequences (N=7, 24.1%) no specific geographic area was identified as the source of the infection.

Conclusions: Cyprus is one of the few countries in Central and Western Europe where the subtype A circulates at high prevalence. Our analysis showed that the subtype-A epidemic in Cyprus comprises two distinct sub-epidemics: the first originates from Greece and it is mostly associated with Cypriots and MSM transmission risk. The second has been introduced from different locations with Russia to be the dominant source. These patterns can be explained due to the high population mobility between Cypriots and Greeks who share a common ethnicity (first sub-epidemic) and also due to that Cyprus is located the cross-road between three continents, providing an attractive touristic, cultural and business destination for diverse populations.

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Irregular Cases of Hepatitis C Detection in Low-Risk Patients

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Background: In 2017 24 patients infected with virus hepatitis B were under medical supervision in Podil District, together with 170 patients with HCV hepatitis.
Objectives: In the present work, we describe cases of hepatitis C detection in "Diagnostic Center" patients, who all attended infectious disease centers for treatment in 2017.

First Case: Male patient S., 40 years old, referred in June due to a tick bite; a "ring-shaped erythema" was diagnosed, and laboratory tests revealed Lyme disease. The patient underwent antibiotic treatment according to the local protocol. Erythema symptoms disappeared. The results from clinical blood tests were normal. The somatic state improved. However, after seven days, the patient reported pain in the hand joints. The patient was asked to undergo a rapid test for antibodies to hepatitis C that revealed the presence of antibodies, which was confirmed by a PCR in a private diagnostic laboratory. Then, the patient was sent to the Institute of Infectious Diseases for inclusion in the "academic" program for treatment of hepatitis C with direct-acting antiviral drugs.

Second Case: Female Patient P., 57 years old, referred to the surgeon due to the infringement of the umbilical hernias. Upon preoperative examination, antibodies to hepatitis C were detected. After the surgery, the patient was transferred to the infectious disease unit of Prof. Golubovskaya, where a cirrhosis of the liver caused by hepatitis C virus was diagnosed, and direct-acting antiviral drugs were prescribed. Currently, after three months of treatment, the patient's condition is satisfactory. PCR of hepatitis C was not determined.

Third Case: Male patient N., 42 years old, was admitted to the emergency room due to bleeding in the stomach. After being checked out from the surgical unit, the patient was examined by an infectious disease specialist and a surgeon. He was diagnosed with liver cirrhosis, caused by hepatitis C virus. According to the EASL protocol, the patient is not eligible for DAA treatment. Currently, he is under the supervision of a gastroenterologist.

Results: Among 170 patients, 119 were drug addicts and 51 were patients - the origin of infection is unknown. In both groups, we saw a late presentation of liver disease. Three patients were found to have cirrhosis.

Conclusion: In recent years, the number of low-risk-behavior patients infected by hepatitis C virus has increased. The blood of the sexual partners of these patients (wives and husbands) did not reveal markers of hepatitis C. These cases make it necessary to consider expanding the list of studies on parenteral infections, such as the existing HIV testing protocol in the Ukraine, due to the adopted ‘Fast Track Targets and a HIV sustainability strategy’ on ending the AIDS epidemic by 2030. Currently, these tests are performed in all medical governmental institutions in Kiev, upon patient request, or upon the initiative of a doctor.

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Analysis of HIV-1 drug resistance in Republic of Guinea in 2015-2017

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Background: Epidemiological data and statistical approaches applied to HIV-1 sequence data from Central Africa, demonstrate that the homeland of HIV-infection was Democratic Republic of Congo (DRC). It is most likely the first event of infection has been happened around 1920 and then HIV-infection began to spread in Kinshasa. As result by 1980-ss the large number of HIV-1 subtypes was found in this region. Kinshasa is the homeland of subtype B which was imported to US and Western Europe with infected Haitian professionals who worked in the newly independent Congo in the 1960s. At the same time the dominated in Africa subtype C began to spread from Kasai-Oriental Province of DRC to Haut-Katanga Province and then – to boarding Zambia and Angola.

Republic of Guinea (RG) is a country on the western coast of Africa. CRF02_AG is dominating in RG. At present time this recombinant is detected in at least 80% of HIV samples from this country. It was found that CRF02_AG appeared in Kinshasa (DRC) in 1970-ss and was arrived in Cameroon and other countries of Central and Western Africa. The study of this variant is important for Russian epidemiologists because CRF02_AG and other AG-recombinants are spreading in Russia and FSU countries as well. Other subtypes (e.g. D, F and A) were found in RG as well.

Finally Ebola epidemic in RG in 2014-2015 has influenced the effectives of HIV prevention and treatment because of lack of medical personnel and patients' fear of getting Ebola in hospitals. As result the adherence to therapy and its accessibility were decreased. The aim of our study was analysis of drug resistance in HIV-1 samples obtained in 2015-2017 from ART-naïve newly diagnosed citizens of RG. This study was carried out within the framework of the implementation of the Russian Federation Government Resolution #1448-r of 25.07.2015 on the financing of Russian-Guinea scientific and technical cooperation.

Material & Methods: We analyzed pol gene partial sequences (positions 2253-3262) coding HIV-1 protease and reverse transcriptase. The sequences alignment and phylogenetic analysis were performed in MEGA6.0. The drug resistance analysis (SDRM list, 2009) was carried out using HIVdb Program v.7.0.

Results: Totally, 35 samples were studied: 7 was collected in 2015, 18 – in 2016 and 11 – in 2017. All samples formed the brunch on the phylogenetic tree with samples of CRF02_AG and other AG-recombinants. At the same time 6 samples formed separate cluster which may indicate about their complex recombinant nature. 5 samples harbored NNRTIs mutations (2 - K103N, 1 – K101E+K103N and 3 - K101E+K103N+G190A). Only 1 sample harbored M184V mutation to NRTIs. There are no samples with PIs drug resistance.

Conclusions: We found 6/35 (17.14%) samples harbored at least 1 drug resistance mutation. Study must be continued with addition analysis of samples collected in Republic of Guinea to evaluate the rate of drug resistance in this country more accurate. At the same time our data demonstrate that it is important to increase the efforts of anti-HIV measures and drug resistance spreading prevention in this country.

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Analysis of dominant viral populations isolated from two pediatric patients infected by HCV genotype 4

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Background: Hepatitis C virus (HCV) infects approximately 1-8% of pregnant women and 0.05-5% of children worldwide. In 2017, the U.S. Food and Drug Administration approved ledipasvir (LDV)/sofosbuvir (SOF) to treat pediatric patients older than 12 years infected by HCV1, 4, 5 or 6. A 13-year-old Italian female patient and a 16-year-old Syrian female patient living in Italy since 2015 started treatment. Patients were treated with LDV/SOF as scheduled by international guidelines. Since treatment failure may be dependent on resistance-associated substitutions (RASs), target region analysis was carried out.

Materials and methods: Serum samples were collected at baseline. HCV RNA was measured using a routine diagnostic method. Genotyping/subtyping was performed by LiPA assay. Viral RNA was extracted, reverse transcribed and amplified by nested PCR, as previously described. NS5A and NS5B genes were sequenced by Sanger method. HCV genotype/subtype were confirmed using Oxford HCV Automated, COMET subtyping tools and phylogenetic analysis. Newly generated sequences and references sequences, available from Los Alamos National Laboratory HCV Sequence Database, were aligned with MUSCLE algorithm and manually edited using MEGA v7. Maximum-likelihood was inferred in the online version PhyML v3.0, using a general time reversible model of nucleotide evolution and gamma-distributed rate variation among sites. Genetic variability analysis to detect RAS and polymorphisms was performed by Geno2pheno tool.

Results: Viral isolates, classified as HCV genotype 4 by LiPA assay, were correctly subtyped by NS5B phylogenetic analysis. The isolate from the 13-year-old Italian female patient (baseline viremia 7490000 IU/ml) was HCV subtype 4d, endemic in Southern Italy, while the isolate from the 16-year-old Syrian female patient (baseline viremia 242000 UI/ml) was subtype 4a, mirroring infection epidemiology of the Countries of origin. Dominant HCV4d population carried the following substitutions on NS5A region: 31L (polymorphisms at RAS positions related to ombitasvir resistance), 34I, 36L, 41K, 58P, 105N, 126E. 62S, 116V, 127Q, 130N, 189S polymorphisms were found on NS5B region. Dominant HCV4a population carried 28M daclatasvir RAS in NS5A plus 44R, 53M, 56T, 99V, 130I polymorphisms. In contrast, NS5B region harboured quite a lot of polymorphisms not yet related to resistance. Patients started treatment on January, they are still on follow-up without any side effects reported. The lack of RASs related to LDV/SOF therapy suggested good prognosis.

Conclusions: In light of such a delicate situation from a clinical point of view and few data available in real-life in pediatric patients we applied a proactive virological approach. Several substitutions, such as 28M detected in a significant proportion of HCV4 strains (10.7%), were found whose significance merit to be investigated.

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Increment of N-Glycosylation sites in HBV surface antigen favours immunosuppression-driven HBV reactivation in vivo and modify HBsAg antigenicity in vitro

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Background: Mutations introducing NXS/T sequon in HBV surface antigen (HBsAg) determine an enrichment of N-glycosylation sites in the surface glycoprotein. The aim of the study was to investigate additional N-linked glycosylation sites of HBsAg in immunosuppression-driven HBV-reactivation in vivo and to evaluate their impact on HBsAg antigenicity and on HBV replication parameters in vitro.

Material and methods: Mutations associated with the acquisition of N-glycosilation site are investigated in HBsAg genotype-D sequences from 55 patients with immunosuppression-driven HBV-reactivation (defined as Hwang, 2014). The impact of N-glycosilation sites on the levels of pgRNA, core particle-associated HBV-DNA, and extracellular HBsAg is assessed by transfecting Huh7 cells with a plasmid containing wild type (wt) or mutated HBV genotype D full-length genome. The effect of N-glycosilation sites on HBsAg antigenicity is analyzed by transfecting Huh7 cells with a plasmid encoding wt and mutated HBsAg-linked to a streptavidin-tag (strep-tag). The strep-tagged HBsAg amount in supernatants is quantified by a specifically-designed ELISA recognizing the Strep region (thus, not affected by HBsAg mutations) and also by an ELISA directly targeting HBsAg. Tunicamycin, an inhibitor of N-glycosilation, is used on cells transfected with the strep-tagged HBsAg to confirm the role of N-glycosilation in altering HBsAg recognition by antibodies.

Results: At HBV reactivation, median [IQR] serum HBV-DNA and ALT are 6.7[5.3-8.0]log IU/mL and 149[42-630] U/L, respectively. Notably, 12.7% (7/55) of patients remains HBsAg-negative despite HBV reactivation (serum HBV-DNA range:2.9-7.6log IU/mL). >1 additional N-glycosilation site in HBsAg is detected in 5/7 HBsAg-negative patients.
In-vitro, compared to wt, N-glycosilation sites strongly reduce extracellular HBsAg titre without affecting viral replication parameters. In particular, S113N+T131N+M133T determines 80% decrease in HBsAg titre, while ins114+T115N, T115N and T123N cause a 68%, 62% and 32% reduction, respectively. Similarly, N-glycosilation sites decrease strep-tagged HBsAg titre by using the ELISA targeting the HBsAg (% of reduction: 20% to 94%). Conversely, no decrease of strep-tagged HBsAg is revealed by ELISA targeting the Strep-tag, suggesting that N-glycosilation sites hamper HBsAg-recognition by antibodies without affecting HBsAg-release.
Notably, in presence of N-glycosilation sites, tunicamycin restores HBsAg titres observed in wt, confirming the direct role of N-glycosilation in hampering HBsAg recognition.

Conclusions: Additional N-glycosilation sites correlate with HBsAg-negativity despite HBV-reactivation, and profoundly alter HBsAg-antigenicity in-vitro. This supports the role of N-glycosilation in favouring viral evasion from immune response and the importance of HBV-DNA (more than HBsAg) for a proper diagnosis of HBV-reactivation.

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A Surveillance Program of Integrase Inhibitors Transmitted Drug Resistance in Spain during the period 2012-2017.

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Background: Testing for transmitted drug resistance (TDR) in the integrase in newly diagnosed patients with HIV is not recommended by Spanish treatment guidelines. 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 in CoRIS throughout the period 2007-2017. Here we report the results of the prevalence and trends in integrase TDR in Spain during the period 2012-2017.

Patients & Methods: A representative sample of the Spanish epidemic was selected according to transmission risk, age and gender, with a plasma sample available at the Spanish HIV Research Network Biobank. The integrase gene (codons 60 to 270) was Sanger sequenced using in house methods. The prevalence of TDR mutations was evaluated using the 2017 IAS list. Clinically relevant TDR was investigated using Stanford v 8.4 Algorithm. As recommended, potential low-level resistance was pooled into the susceptible category. Clinical, virological and epidemiological characteristics available from the CoRIS database were related to the prevalence and trends on integrase TDR.

Results: Our cohort included 902 patients, 84% male, 71% Spanish, 85% sexually infected, and 80% <50 years of age. Overall, TDR mutations in the Integrase using the IAS list were detected in 24 patients, making a prevalence of 2.7% (T66I, n=1, 0.1%; L74M, n=2, 0.2%; T97A, n=21, 2.3%). Clinically Relevant resistance to integrase inhibitors was lower (0.2%; T66I, n=1; G163K, n=1). Potential low-level resistance to Raltegravir and/or Elvitegravir was found in 35 patients (3.9%), due to the detection of Q95K (n=5), T97A (n=21) and E157Q (n=9). No significant change in TDR or clinically relevant resistance during the study period was observed.

Conclusions: Transmitted Drug Resistance to integrase inhibitors remain at low levels in Spain, even in the most recent years, when regimens based on these drugs are recommended as preferred regimens by Spanish guidelines.

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Mixed Infections in hepatitis C: should we use NGS to confirm Results: provided by commercial tests?

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Background: Accuracy of HCV genotyping with commercial tests has been extensively investigated, but the information on how commercial tests estimate infection by several genotypes at a time (mixed-infections) is scarce. We compared the ability of Abbott Real-time VHC-Genotype-II assay (rt-PCR) to diagnose mixed infections with that of a prototype based on Next Generation Sequencing (NGS) provided by RocheDiagnostics.

Materials/methods: We used Roche prototype to retest samples previously genotyped with rt-PCR. Briefly for the NGS-test, after RNA extraction and purification, cDNA was synthesized (Trancriptor One-Step-RT-PCR kit) and subjected to a 1stround of PCR encompassing a fragment in NS5B(nucleotide positions8254-8641). The 1stPCR product was subjected to a nested-PCR with MID-barcoded-primers, then purified, quantitated, creating a pool(20pM) and resolved in the MiSeq(Illumina) platform using the V3chemistry. Sequences were filtered(Usearch), accepting those with Q>30. Finally, genotypes assignation(Geno2pheno and BLAST). Genotype 1 subtype non-determined samples by Abbot-assay, successfully subtyped by Roche-assay, were scored as discordant.

Results: 139 samples from HCV infected patients were tested. After running the NGS-test, concordance with the Abbot test was 87.05%. For "non-mixed"-genotype cohort (n=114), Roche-test was concordant in all cases except for an rt-PCR Genotype 4 sample genotyped as 1a; in 4 samples the NGS-test could not report the genotype. Genotype1 subtype non-determined samples by rt-PCR were genotyped as Genotype1a in five cases and 1b in one case by NGS. In addition, NGS ascribed the subtype in all genotype 2,3,4 & 5 samples. For the mixed-genotype cohort (n=25) NGS confirmed 22/25 mixed infections and 17/25 samples were concordant with the Abbott test. Discordant cases were: two mixed 4+5 and one 1b+4 by Abbott Real-time that were not confirmed by NGS; and two1b+4 and one1a+3 mixed-infections that were classified as single infections by NGS.

Conclusions: We have observed high concordance between the Roche NGS prototype and the Abbott Real-time PCR (Kappa=0.88). RT-PCR is able to detect mixed-infections. Although no additional mixed-infection was observed by the UDPS-test, further studies may be needed to properly characterize mixed-infection results provided by the Abbott Real time commercial test.

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NS5A inhibitor natural resistance associated substitutions (RASs) detected in a cohort of genotype (GT) 1a and 1b Hepatitis C (HCV) chronically infected patients

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Background: NS5A RASs appear to have an impact on the therapeutic strategies of chronic HCV infection as they can reduce the antiviral activity of the most effective and better tolerated direct-acting antiviral agents (DAAs) currently in use. In this study we describe the prevalence of RASs, detected at baseline, in a cohort of 61 HCV-GT1 DAAs naïve patients followed at the Policlinico San Martino Hospital from June 2015 to June 2016.

Materials & Methods: Serum samples from 61 HCV-GT1 (33 GT1a, 48 GT1b) DAAs naïve patients, were tested for the presence of RASs in NS5A gene. Briefly, HCV-RNA was extracted from serum samples using NucliSens EasyMAG system (BioMérieux, Boxtel, The Netherlands) and genomic regions were amplified with specific HCV genotype primers using reverse transcriptase PCR. Subsequently, cDNA of the NS5A genomic target were purified and sequenced by 3130-Avant Genetic Analyzer (Life Technologies, NY, USA). All 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/). Statistical analyses were performed by SPSS 23.0 (IBM Corp., Armonk, NY,USA).

Results: Clinically relevant RASs for at least one of the licensed NS5A inhibitor were detected in 14/61 (23%) cases, 5/28 (17.9%) in genotype 1a and 9/33 (27.3%) in genotype 1b HCV infection. RASs were not associated with GT1a clade (14.3% in clade I vs. 21.4% in clade II; p=1). We found similar RASs rates in both GT1b and GT1a patients, the most frequently detected RASs were at codon 93 (8/14, 57.1%) with higher rates of Y93H in GT1b patients (21.2% vs. 6.6%, p=0.04). One GT1b-infected patient harbored double mutation 31M/93H. We found no significant association between RASs and HIV status, gender, cirrhosis, HCV genotype and previous exposure to pegIFN. Patients with RASs were significantly older (mean age 62.9 ±9.7 vs. 55.4±11.8 years, p<0.0001) than those without. The effects of aging as modifying factor on the ability of natural NS5A RASs to predict lack of SVR was evaluated. Interaction between NS5A RASs and aging was not statistically significant (p=0.68) indicating that the impact of natural NS5A RASs on SVR do not differ according with age. Overall 9/61 patients (4 GT1a and 5 GT1b) experienced virological failure, following SOF/LDV (N=5) and 3D (N=4) regimens.

Among DAAs treatment failing-patients, 5 harboring the same NS5A variants both at baseline and at failure, 3 patients had RASs only in post therapy samples, and 1 patient did not have RASs at any time.

Conclusion: Our experience confirms previous data on prevalence of natural RASs in GT-1-infected patients, with a significantly higher prevalence of Y93H variant. Although the clinical impact of natural NS5A RASs on sustained virological response following treatment with direct active agents is still under debate, local epidemiology of natural RASs might be helpful for tailoring HCV treatment in difficult-to-treat populations.

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High seroprevalence of HIV diagnosis among patients with mononucleosis-like illness and pneumonia referred to Emergency Department-one center data from Poland

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Objectives: Despite the significant advances in care and treatment of HIV/AIDS, many patients are unaware of their HIV infection in Poland and remain at risk to their own health and of transmitting the virus to others. In such situation, the development and implementation of the innovative, testing strategy such as screening based on the presence of indicator conditions (IC) have a decisive significance and may constitute an additional element of the national HIV testing strategy. The aim of the study is to estimate the prevalence of the HIV infection among patients who present with: mononucleosis-like illness (MONO) and community-acquired pneumonia(CAP) at the Emergency Department, as well as to assess the cascade of care for those tested positive.

Methods: The prospective study concerning patients of 18 to 65 years was conducted at the Emergency Department (ED) of Hospital of Infectious Diseases in Warsaw (from 10 May 2015 until 10 March 2018). Within the programme the HIV DUO test was offered to all patients presenting with MONO and CAP.

Results: A total of 885 individuals referred to the Emergency Department with: MONO–794 (89.7%) and CAP-91 (10.3%), among whom 636 (71.9%) accepted HIV screening and all expressed consent, MONO–573 (72.2%) and CAP-63 (69.2%)
Twenty-five tested positive for HIV, giving an overall prevalence of 3.9%; 3% for MONO and 12.7 % for CAP. All patients were linked to care to the HIV Outpatient Clinic in Warsaw for further diagnostic.
Of those testing HIV positive, Western Blot test was positive in 22/25 (88%) cases. Among those patients 90,9% were male, 60% identified themselves as MSM. The median age of the patients was 32.5 (19-54) years, with the median CD4 count at diagnosis 341cells/ul (4-731). In terms of co-infections two (9.1%) patients were anti-HCV and HCV RNA positive, four (18.2%) of patients had HBV infection in the past, one had HBs antigen and six(27,3%) had positive VDRL test. The comparison statistics for HIV positive persons by Indicator Conditions are shown in Table 1.
Fifty percent of patients were diagnosed as late presenters, after the optimal time for antiretroviral treatment initiation, according to actual European and Polish guidelines. Twenty one patients (95,5%) started cART; the median time from diagnosis to cART was 8 days (2-193). The continuum of care for HIV positive patients is presented at Figure 1.

Conclusion: The rate of confirmed HIV diagnosis among patients who referred to the ED due to MONO and CAP was high (3,5%), confirming clear benefit in routine testing of this group of patients.
The study shows that the implementation of routine IC-guided testing among patients consulted due to MONO and CAP in ED constitutes an essential tool for identifying new HIV infections and seems to be an additional valuable strategy to enhance current testing programmes.

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A new genotype of hepatitis Delta virus identified in Cameroonian patients

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Background: Hepatitis Delta virus (HDV) infection is hyperendemic in Central Africa and the different genotypes of this virus have a characteristic of dissimilar geographic distribution. However, little is known about HDV genotypes distribution in this area. The present study was taken to determine the different HDV genotypes prevailing in Cameroonian patients.

Methods: The study was conducted in the Virology Unit of the Centre Pasteur of Cameroon. Blood samples (n=247) of patient with confirmed HDV infection were analysed. Circulating HDV genotypes were determined using a semi-nested amplification of partial R0 gene and phylogenetic analysis of the different sequences. In order to determine the association between the different genotypes, the viral replication and the severity of liver disease, viral load and ALT level of all patients were obtained from the data base.

Results: Phylogenetic analysis of 84 nucleotide sequences of the Hepatitis Delta Antigen (HDAg) R0 region obtained in this study, showed considerable diversity among the local strains with 84.5% of genotype I, 1.2% of genotype 5, 2.4% of genotype 6, 10.7% of genotype 7 and 1.2% of genotype 8. Interestingly, our study reports for the first time the circulation of HDV-8 in Cameroon, but also the co-circulation of all the others so-called "African" genotypes (HDV-5, 6 and 7). Regarding the association between genotypes and the severety of liver disease, the present study reports that patients infected by genotype HDV-7 had abnormal ALT levels.

Conclusion: This study showed for the first time the circulation of HDV-8 in Cameroon. In addition, HDV genotype 5, 6 and 7 knowned as ‘‘African genotypes’’ and HDV genotype 1 were also identify. Our **Results:** also show that patients infected with HDV genotype 7 are in high risk to develop severe liver disease. Further studies are needed in order to provide more information about the different HDV genotypes and the severety of the infection.

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Drug resistence and genotype in tretmant naive HIV-1 infected patients from montenegro

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Background: Occurrence of transmitted drug resistance (TDR) among treatment naive patients is increasing problem and most common reasons for therapeutic failure. Prevalence of TDR in different geographic settingsis ranging from 0-52%. Prevalence and pattern of primary virus mutations associated with resistance to antiretroviral drugs in Montenegro has not been analyzed so far.

Material/methods: A total of 28 samples from HIV1 infected treatment naive patients aged 18 years and more attended to Clinic for Infectious Disease in Podgorica were collected between 2014-2016. HIV-1 pol gene sequences were obtained using Sanger dideoxi protocol. Analysis was performed at the Institut for Microbiology and Immunology, Belgrade. HIV pol sequences from Montenegro deposited at NCBI database were also used. HIV-1 subtype and genotypic drug resistence were predicted using HIVdb Stanford University algorithm.

Results: Sequences were predominantly of subtype B (85%), followed by C (10.7%), whereas recombinant CRF01_AE was found in one sample. Most patients were male (90%) in CD clinical stage C (53%) TDR mutations were founded in one patient (2.8%). In that patient we founded mutations to different antiretroviral drugs classes. Protease inhibitors (PIs) minor mutation Q58E, witch conduct low level resistence to tiprinavire, and mutation in nucleoside reverse transciptase inhibitors (NRTIs): M41L whitch is associated with potential low level resistence to abacavir, didanosine and tenofovir and law level resistence to zidovudine, stavudin. There was no mutations in NNRTI class in this patient.. In other patients we did not found significant mutations.

Conclusions: Level of resistance founded in treatment naive patients in our country was low (2.8%) and mutation pattern coresponded to the available treatment in Montenegro. Subtype B is the dominant form in our patient, like in other European countries. One patient was CRF01-AE which is mostly founded form in south Asia and has spread throughout the world. This is the first study of prevalence and pattern of primary virus mutations associated with resistance to antiretroviral drugs in Montenegro, research on a larger sample of patients is under way.

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Expression, immunogenicity and diagnostic value of envelope proteins from an Egyptian hepatitis C virus isolate

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Objectives: The present work aimed at 1) characterization of the E1-E2 domain (HCV-E) from an Egyptian HCV-4a isolate on both molecular and immunological levels, 2) in silico identification of the B- and T-cell epitopes responsible for the immunogenicity of HCV-E, and 3) evaluating the diagnostic potentials of both the recombinant HCV-E and antibodies raised against mammalian expression constructs encoding the protein.

Methods: The region encoding the E1-E2 domain was amplified from RNA isolated from blood of human infected with HCV-4 by RT-PCR, cloned into the pSC-TA plasmid, the sequence was verified and used to construct a neighbor-joining phylogenetic tree. Translated nucleotide sequence was subjected to prediction of HCV-E secondary structure using PREDICT-PROTEIN servers and PSI-PRED. The 3D model of the HCV-E sequence was generated using the online tool 3Dpro. B- and T-cell epitopes were predicted using the online tools BCPred and Epijen v1.0, respectively. The HCV-E encoding sequence was later sub-cloned into the mammalian expression plasmid pQE and the generated constructs were used to immunize mice in absence and presence of adjuvants of plant origin.

Results: The maximum degrees of homology obtained by nucleotide and protein blast analysis with previously published HCV-E sequences were 85 and 77%, respectively. The B-cell epitope CFTPSPVVV at position 203 and the T-cell epitope ALSTGLIHL at position 380 were found to be highly conserved among all HCV genotypes. Both ELISA and Western blotting experiments on crude and purified recombinant HCV-envelop proteins as well as mice antisera raised against the HCV-E-mammalian expression construct confirmed the specific antigenicity of the expressed protein. The raised antibodies against the HCV-E encoding construct in mice could efficiently capture circulating antigens in patients' sera with good sensitivities that correlated with liver enzymes levels ($r = 0.4052$, P value < 0.0001 for ALT, and $r = -0.5439$, P value is 0.0019 for AST). Moreover, combining the HCV-E encoding construct with extracts prepared from Echinacea purpurea and Nigella sativa prior to immunizing mice significantly ($P<0.05$) increased both the humoral (14.9 – 20 fold increase in the antibodies) and the cellular (CD4+ and cytotoxic CD8+- T lymphocytes) responses compared to mice which received the DNA construct alone or PBS-treated mice.

Conclusions: Both recombinant HCV-E protein preparations and antibodies raised against HCV-E encoding mammalian expression construct represent useful diagnostic tools that can report on active HCV infection. Also, the immunostimulatory effects induced by the two used plant extracts at both the cellular and humoral levels highlight the potential of natural products in inducing protection against HCV infection. The neutralizing capacity of the induced antibodies is a subject of future investigations. Furthermore, the predicted B- and T-cell epitopes may be useful for tailoring future diagnostics and candidate vaccines against various HCV genotypes.

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Evaluation of the SENTOSA® SQ HIV genotyping assay in the Irish diagnostic setting.

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Background: New HIV diagnoses have been increasing in Ireland since 2013. To inform the appropriate use of effective antiretroviral therapy for HIV treatment and prevention, baseline antiviral resistance testing is routinely conducted. This study evaluates the CE-marked Sentosa® SQ HIV Genotyping Assay system for use in the routine diagnostic environment, to assess whether the extra sensitivity gained by using Next Generation Sequencing translates into better outcomes for patients.

Methods: Forty-four samples, all of which had previously been tested for antiviral drug resistance using a laboratory-developed (LDT) Sanger sequencing assay, were tested using the Sentosa® SQ HIV Genotyping Assay system. A wide range of viral loads (2.6 - 5.9 log10 c/ml) was tested, with 8 samples below the Sentosa® stated limit of detection (3.0 log10 c/ml). Samples representative of the subtypes seen in the Irish HIV-1 population were selected, and included new HIV diagnoses and patients with apparent treatment failure.

Results: The Sentosa® assay successfully generated 44 sequences for Protease and RT gene segments, and 43 Integrase gene segments. The subtype data were 100% concordant. A pairwise comparison of the variants flagged in the Sentosa® report versus the LDT report (with sequence interpretation carried out using the Stanford online tool) identified differences in the Protease region in 2/44 individuals, and in the RT region in 12/44 individuals. Interpretation of the integrase sequences was 100% concordant. For 12/14 patients, increased numbers of resistance mutations were reported by the Sentosa® system, primarily due to the detection of additional DRM, present below a frequency of 6%. One individual's ART regimen was changed based on the new results. In one sample, the Sentosa® assay clearly identified the presence of a mixed-subtype infection which was not apparent in the Sanger data.

Conclusions: The Sentosa® NGS assay performed well in our patient cohort, and represents a significant improvement on our current assay, through quantifying the detection of minor variants, and providing data on dual/mixed infections, thereby informing both treatment of the individual, and enhancing HIV surveillance at the population level. In the absence of clinical guidelines, however, it is not yet clear how best to interpret the relevance of DRM present at low levels.

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Prevalence of HCV/ HBV co-infection and mortality dynamics among people living with HIV in Dnipropetrovsk Oblast (Ukraine)

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Background: Despite some positive trends, there is a high mortality among HIV-infected patients In Ukraine. Among the causes of mortality are those that are due to conditions associated with advanced stages of HIV / AIDS co-infection with tuberculosis, and those that are not directly related to them. Such reasons refers to co-infection of HCV / HBV and HIV. The impact of HCV and HBV co-infection on mortality of HIV-infected patients currently remains not fully established, so this was the goal of our study

Material & Methods: A retrospective study of the mortality dynamics and the frequency of co-infection of HCV/HBV- HIV - in comparison with prevalence of seropositivity in a cohort of newly registered HIV AS and coverage of ART according to the data records in the regional in AIDS center 2014-2016.

Results: During the 2014-2016 years 4213 HIV patients died in Dnipropetrovsk region (1439, 1356, 1418 retrospectively) with a growing mortality rate up to 30.9 per 100 000 of the population in 2016. Of these, about 70% of deaths are caused by progressive HIV infection in 3-4 stage of the disease. With regard to the co-infection of HCV/HBV/HIV, they occupy second place after co-infection TB/HIV. Mortality due to viral hepatitis C, B or cirrhosis remains high, despite the positive trend in last year. From these reasons, 15% of the patients in 2016 had died, compared with 18% in 2015 and 20% in 2014. Reducing mortality by 5% in the year 2016 can be associated with increased access to treatment. Among those who died 50% were IV drug users and less than 50% were receiving ART. Comparison the data mortality caused by HCV/HBV/HIV co-infection with the results of serological survey in patients diagnosed for HIV showed the following: 2695 new cases of HIV were registered in the year 2016 (growth rate compared to 2015- 7%).67% of this group were examined for serological markers of viral hepatitis B and C. Viral hepatitis B markers were identified in 6% of patients, and markers of viral hepatitis in 23%.These data indicate a high prevalence of HCV/HBV/HIV co-infection, especially viral hepatitis C.

Conclusions: The study results showed that co-infection of HCV/HBV- HIV, together with tuberculosis poses a serious threat to patients even in the early stages of HIV. The injecting drug use and the late administration of ART may be also risk factors. Increasing access to screening for viral hepatitis markers can be an important tool for planning further management and designation of adequate prevention and treatment methods.

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The treatment of HCV infection with direct-acting antivirals medicine in 12-year old girl with HIV/HCV co-infection

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Background: The estimating level of co-infections HCV/HIV among people who lived with HIV-infection in Ukraine is near 23 %. Direct-acting antivirals medicine(DAA) treatment, particularly treatment with ledipasvir/sofosbuvir is available now in Ukraine. HCV treatment with DAA in pediatric population is allowed since 12 years old.At the same time there are luck of data of using this regimen in HIV-positive pediatric population.

The first case of treatment child with co-infection HIV/HCV with ledipasvir/sofosbuvir in Infectious Diseases Center «Clinic for treatment children with HIV» at National Specialized Children's Hospital «OKHMATDYT» Kiev, Ukraine are presented.

Clinical case: 12 year girl was admitted in our hospital in January 2018 with complain on the fatigue, nasal bleeding in the morning, pain in legs (since September 2017), which increased during physical activity.
She was vertically HIV infected, on ART since 2006 zidovudine/ lamivudine/lopinavir /ritonavir (AZT/3TC/LPV/r), was switched on tenofovir disoproxil fumarate/emtricitabine/ (TDF/FTC)/LPVr in 2016 because of myelotoxicity.
She was diagnosed with HCV genotype 1b at 4 years old and in 2017 fibrosis 3 stage was found. HCV viral load was 4,38 x10*5 IU/ml. Total bilirubin was 23 mmol/l and alanine aminotransferase(ALT) and aspartate aminotransferase (AST) was slightly increased.
Treatment with ledipasvir/sofosbuvir was prescribed at December 2017. Since that time the pain in legs increased.
CD4 cells- 32%- 760 cell/ml, PCR RNA HIV was less 40 RNA copies/ml
The densitometric diagnostic was made: osteopenia was found.
N-Acetil-b-D-Glucosaminidase (NAG) of urine was made -15,1 (N range 1.64-9.8). TDF was switched on Abacavir(ABC).The patient condition was improved.

Conclusions: Ledipasvir/sofosbuvir increased tenofovir exposure, especially when used together with a pharmacokinetic enhancer (ritonavir).
Patients receiving TDF and a boosted HIV protease inhibitor should be strictly monitored for tenofovir-associated adverse reactions.
The question about changing ART regimen and preferable ART regimen for patient on HCV treatment is still open

90 *(withdrawn)*

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The role of plasma osteopontin as a biomarker of liver fibrosis in children with chronic hepatitis B

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Background and aim: In present world we are in need of more research devoted to the study of potential serum markers of liver fibrosis and the development of biochemical diagnostic tests to determine the degree of its severity. The aim of our study was to determine the plasma osteopontin (OPN) level in chronic viral hepatitis B (CHB) children with acute lymphoblastic leukemia (ALL), depending on sex, age, viral load, degree of fibrosis and inflammatory activity in the liver.

Materials and methods: The study includes 71 children with CHB aged 3 to 17 years, of them 41 CHB patients with ALL (main group) and the control group consisted of 20 healthy children. All patients were under observation at Vinnytsia Regional infectious diseases hospital, Vinnytsia, Ukraine. The verification of the CHB diagnosis was performed on the basis of HBV-DNA detection in the blood of patients using PCR technique and ELISA for HBsAg, HBeAg, anti-HBsAg, anti-HBcAg, anti-HBeAg. The degree of fibrosis was determined on the METAVIR scale using Fibrotest. The level of OPN in serum was determined by ELISA (Human Osteopontin Quantikine ELISA Kit, USA). Statistical analysis of data was done by using STAT6 software.

Results: According to results, the level of this glycopeptide in CHB children with ALL (248,20 [185,18-408,72] ng/ml) is significantly higher than in patients of comparison group with CHB (119,45 [76,50-193,45] ng/ml) and in healthy children (94.50 [74.55-115.0] ng/ml) ($p<0.05$). In the main group, the level of OPN was significantly higher in patients with low viral load (208.06 [185.18-327.53] ng/ml) and high viral load (250.38 [182.47-472.52] ng/ml) compared to healthy children ($p<0,05$). Among CHB children with ALL, only 13 (31.7%) had no liver fibrosis, whereas in more than half of these patients (68.24% (28)) were with various degrees of liver fibrosis, from F1 to F3. It should be noted that minimum level of OPN was observed in children without liver fibrosis, among the main group (185.18 [79.72-215.62] ng/ml) as well as in the comparison group (81.57 [65.62-90.18], ng/ml). In patients from the main group with F3, the level of OPN (472.52 ng/ml) is significantly higher than with F1 (216.23 [196.80-258.25] ng/ml) ($p<0.05$). In the patients with liver fibrosis F2 490.23 [408.72-500] ng/ml, the level of OPN was significantly higher than in children with F1 ($p<0.05$). A similar pattern was also observed in children of the comparison group ($p<0,05$).

Conclusions: The serum OPN levels in CHB children with ALL, irrespective of gender, age, and viral load were significantly higher than in patients without ALL and healthy individuals ($p <0,05$). It's positively correlates with the degree of fibrosis and inflammatory activity in the liver ($p<0,05$) and with the increase in degree of fibrosis the plasma OPN level also increases.Therefore, OPN claims to be a new non-invasive biomarker of liver fibrosis and further study of OPN in detail will give us not only a promising prospective sensitive biomarker of progression of liver fibrosis, but also a criterion for assessment of the effectiveness of treatment and the prognosis of chronic HBV in children

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Morphological Features of Kidneys' Damage in Patients with Chronic Hepatitis C

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Background: Chronic hepatitis C (CHC) is considered to be a systemic disease with not only liver damage but also with a variety of extrahepatic manifestations. The leading place among them belongs to mixed cryoglobulinemia with renal disfunction.

Objectives of our study was to evaluate morphological characteristics of kidneys' damage in patients with CHC.

Materials and Methods. We observed 119 patients with CHC (77 males (64.7%), 42 females (35.3%), mean age – 40,3±12,6y). Presence of clinical and / or laboratory manifestations of chronic kidney disease in patients with chronic hepatitis C for the period of 3 months or longer were the main indications for renal biopsy. Morphological study of renal samples were performed in 22 patients. Statistical analysis of the results was performed by using descriptive statistics.

Results: Renal lesions were diagnosed in 22 (18.5%) patients. Cryoglobulinemic glomerulonephritis (GN) was diagnosed in 13 patients (59.1%) according to the results of morphological studies of renal specimens. Three patients had membranoproliferative glomerulonephritis (MbPGN) without cryoglobulinemia. Membranous nephropathy, focal segmental glomerulosclerosis (FSGS) and mesangioproliferative GN was diagnosed in 2 patients, respectively.

of myointimal hyperplasia of small arteries.

A characteristic feature of cryoglobulinemic GN was the presence of eosinophilic and PAS-positive hyaline thrombi in the lumen of glomerular capillaries. Immunofluorescence investigation in all cases of HCV-associated MbPGN showed small and large granullar deposits of C3, IgM and IgG along the capillary walls and at the mesangium. The highest intensity of luminescence was typical for C3 and IgM. Intraluminal deposits in cases of cryoglobulinemic GN were positive for IgM and IgG, as well as for C3.

Conclusions: Our study showed that the renal manifestations of HCV infection appear in 18.5% of cases. Needle kidney biopsy is important in the differential diagnosis. Levels of serum CGs were elevated in 68.2% patients. The majority of renal lesions in chronic hepatitis C was linked to the CGS, as cryoglobulinemic GN was found in 59.1%. There were also other types of kidney damage – a membranous nephropathy, MbPGN without cryoglobulinemia, focal segmental glomerulosclerosis and mesangioproliferative GN.

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Diagnostic importance of CCC DNA for patients with chronic hepatitis C and D.

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Background and aim: Current methods of antiviral therapy inhibit genomic replication of hepatitis B virus, but are not effective because they do not directly affect the nuclear CCC DNA (covalently closed circular DNA). In our hospital, we wanted to use knowledge of CCC DNA e.g. to predict the outcome of hepatitis B patients. According to some resources occult hepatitis B is diagnosed in 6-7% of severe patients. We have identified occult hepatitis B in one third of examined patients.

Methods: We have examined plasma and biopsy from 39 patients admitted to the intensive care unit of Virology Research Institute in severe conditions with different stage of liver fibrosis and with the following diagnosis - hepatocellular carcinoma, liver failure, autoimmune hepatitis.

Results: The past medical history of part patients had hepatitis B, but the recent HbsAg was found to be negative. Of 39 biopsy samples studied we identified CCC DNA in 13 cases. Among them in 6 cases HCV patients had occult hepatitis B.

Conclusions: Detection of CCC DNA in patients with chronic hepatitis C and D is essential diagnostic and prognostic factor for management and prediction outcome of disease. This high percentage of detection can be explained, apparently, with the suppression of HBV replication in chronic mixed infection with hepatitis C or D.

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An update of hepatitis C prevalence rates in homeless adults after hepatitis C treatment paradigm change: a systematic review and meta-analysis

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Background: An estimated 100 million people are homeless around the world. Concurrent vulnerabilities, such as psychiatric diseases, addictions with unsafe injection practices increase blood-borne infections risks, including HIV, HCV in homeless individuals. A 2012 Lancet Infectious Diseases paper reported HCV prevalence in homeless ranging from 3•9% to 36•2%, and 0•3% to 21•1% for HIV infection, many are co-infected with both, but we know very little about HCV treatment in homeless, aside from the fact that treatment is rarely if at all provided or considered. Old treatment regimens from the "interferon era" had many psychiatric side effects, including increased suicide and major depression rates and were contraindicated in patients, who had pre-existing or secondary psychiatric diseases, addictions, and were unstable. Meanwhile, treatment paradigm has changed in HCV management recently. Current HCV treatment options are not contraindicated in people with psychiatric conditions anymore and can help successfully achieve HCV cure. Additionally, new treatment options are shorter in duration, all-oral instead of injections with easier to adhere regimens, and are recommended by current guidelines in unstable individuals as well. This study objective is to update previous study findings, and examine HCV treatment prevalence in homeless adults.

Materials and Methods: On February 2016 we searched PubMed, EMBASE, and Cumulative Index to Nursing and Allied Health Literature databases for "homeless* and (hepatitis C or HCV)" for studies reporting HCV prevalence in homeless adults published between 31 January 2012 and 15 February 2016. Meta-analysis was conducted following the PRISMA Checklist. Data was tabulated in Comprehensive Meta-Analysis.

Results: Fifteen epidemiological studies yielded. The omnibus prevalence rate for HCV in homeless remains unchanged since 2012, (28%; 95% CI: 23-34; N=15). Only three studies reported HCV treatment investigation, but the data quality could not allow a meta-analysis.

Conclusions: Despite a high HCV prevalence among homeless, HCV treatment prevalence information is limited; some studies mention that treatment is not practically provided. This meta-analysis data can help to estimate the frequency of HCV infection, which can help to plan HCV management services for homeless population infected with HIV in a better way. Together with the recent advancements, paradigm changes in HCV treatment the data from this review can also contribute to the global HCV elimination goal.

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Health related wellbeing in HIV/HCV co-infected drug users in Ukraine

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Background. Global growth of HIV/HCV co-infection remains accompanied with increased attention to quality of life (QoL). Under conditions of the current epidemic among people who inject drugs (PWID) the improvement of QoL is the basic goal of treatment and has considerable practical importance.
The aim of the study was to investigate the indicators of QoL in HIV/HCV co-infected PWID in order to identify the ways of it optimization and improvement.

Materials and Methods. Method of individual interview according to specially developed questionnaire that includes combination of subjective and objective approaches will be applied as a means for collection of data on life quality level of respondents.
The study was conducted anonymously and confidentially among Ukrainian citizens in 2015-2017. The study group consisted of 40 HIV/HCV co-infected PWID registered at Municipal agency of Sumy Regional Council "Regional Narcological Clinic" (Sumy, Ukraine): 30 (75.0 %) men and 10 (25.0 %) women, average age (33.03±0.81) years, 38 (95.0 %) - urban population. 20 (50.0 %) patients received antiretroviral therapy, 35 (87.5 %) - opioid substitution therapy. The comparison group included 44 healthy blood donors: 36 (81.8 %) men and 8 (18.2 %) women, average age (30.68±1.21) years, 38 (86.4 %) urban population.

Results. The total number of HIV/HCV co-infected PWID has a mean score of (61.32±0.53) points, which corresponds to a satisfactory level of QoL; in healthy donors - (71.5±0.19), which indicates its high level ($p<0.001$). Indicators of social and spiritual components of health among drug addicts are lower than among blood donors and account respectively (15.48±0.39) and (14.83±0.57) points vs. (17.88±0.12) and (17.86±0.09) in comparison group ($p<0.001$).
85.0 % of HIV/HCV co-infected PWID and 90.9 % blood donors are satisfied with their health status ($p>0.05$). However, among people living with HIV/HCV, the restriction of physical activity (67.5 %) and the marked impact of the disease course on the viability are followed significantly more often ($p<0.001$).
Signs of isolation and discrimination don't have a marked difference between the groups. Reducing psychological adaptation due to changes in plans for the future is largely attributable to HIV/HCV-infected PWID (27.5 % of respondents), which is observed 3 times more often than among practically healthy persons (9.1 %) ($p<0.05$). A high level of internal stigma and the perception of death among PWID living with HIV/HCV are recorded (77.5 % and 22.5 % respectively) ($p<0.01$).
In assessing the objective criteria of QoL from the doctor's position among HIV/HCV-infected drug addicts we found a significant decline in the capacity to work (45 % of respondents) and physical activity (32.5 %) ($p<0.001$).

Conclusions. The quality of life in HIV/HCV co-infected drug users remains lower than in the general population, but corresponds to a satisfactory level, which is associated with the use of antiretrovirals and opioid substitution therapy. Low level of QoL among PWID living with HIV/HCV is largely due to social and spiritual components, which necessitates strengthening the scope of psychosocial support in the provision of medical care to this contingent of patients.

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Hepatotoxicity and related Risk Factors of Severe Hepatotoxicity Among HIV-1 infected Individuals newly initiated on Highly Active Antiretroviral Therapy in the North West Region of Cameroon

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Background: Since the introduction of highly active antiretroviral therapy (HAART), the life expectancy and quality of life of patients with HIV has improved significantly. However these HAART have also been reported to induce severe or life-threatening cases of adverse effects such as hepatotoxicity. HAART associated hepatotoxicity has gained prominent attention since it can be affected by many factors.
Objectives: The aim of this study was to determine the prevalence of hepatotoxicity and related risk factors of severe hepatotoxicity following HAART initiation.

Methods: A total of 100 newly diagnosed HIV drug naive patients within the age range of 36.53years were recruited and followed up for 24weeks. Socio demographic data was obtained using pretested questionnaires. Venous blood samples were collected to measure hepatotoxicity markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and using colometric enzymatic reaction. Hepatotoxicity was classified based on age and sex. Data was analyzed using SPSS. Chi-square test was used to compare categorical variables while univariate and multivariate analyses used to identify possible risk factors. The level of significance was set at 5%.

Results: Using either ALT, AST, ALP or a combination of any of them 38(38%) and 55(55%) patients presented with hepatotoxicity while 15% and 28% of patients of them had severe hepatotoxicity at 4 and 24 weeks respectively. Serum levels of all enzymes increased significantly ($p< 0.05$) with increased treatment duration. Univariate analysis revealed that the risk of developing severe hepatotoxicity was greater in patients <30 years (P=0.03), males (P=0.04), low BMI (p=0.02), low monthly income earners (p=0.01), and patient on AZT+3TC+ NVP regimen (P=0.01). While multivariate analysis adjusted for univariate variable of $p< 0.09$ showed that age <30 years, Low BMI, low monthly income and the use of AZT+3TC+ NVP regimen was an independent risk factors. Moreover cigarette or tobacco smoking and alcohol consumption showed a supra-additive effect in the development of severe hepatotoxicity.

Conclusions: Low BMI, <30 years, low monthly income and the use of AZT+3TC+ NVP regimen were identifiable risk factors for the development of severe hepatotoxicity. As such these factors should be considered as an important strategy by clinicians in preventing the hepatotoxicity.

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