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Treatment Strategies & Antiviral Drug Resistance

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14th European Meeting on HIV & Hepatitis
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Abstracts
Oral Presentations
Abstract: O_1

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Potent Activity of GS-9883, a Novel Unboosted HIV-1 Integrase Strand Transfer Inhibitor (INSTI), Against Patient Isolates with INSTI-Resistance

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Background: GS-9883 is a potent once-daily unboosted INSTI currently in phase 3 clinical development in combination with TAF and FTC for use in both treatment-naïve and -experienced HIV-infected patients.

Methods: Patient-derived HIV-1 isolates with high-level INSTI resistance from the Monogram Biosciences library were profiled in parallel for susceptibility to GS-9883, dolutegravir (DTG), elvitegravir (EVG), and raltegravir (RAL). A total of 47 isolates were chosen from the Monogram library, including all available isolates with >2.5-fold reduced susceptibility to DTG (n=24) as well as a representative panel of isolates with EVG and/or RAL resistance mutations (n=23).

Results: All of the 47 HIV-1 isolates with INSTI resistance mutations had reduced susceptibility (>2.5 fold change) to at least 1 INSTI and most had high-level reduced susceptibility to EVG and RAL. The number of isolates with full sensitivity to INSTIs defined as fold change <2.5 was 33/47 (70%, GS-9883), 23/47 (50%, DTG), 3/47 (6.3%, EVG), and 1/47 (2.1%, RAL). Only one isolate had >10-fold change for GS-9883 compared to DTG (n=8), EVG (n=43) and RAL (n=45). Susceptibility to GS-9883 was improved compared to all other INSTIs (p=0.042 vs DTG, p<0.001 vs EVG, and p<0.001 vs RAL). The improved resistance profile of GS-9883 compared to DTG was most evident for isolates with the INSTI resistance mutation patterns of E92Q + N155H or G140C/S + Q148R/H/K ± additional INSTI mutations.

Conclusions: GS-9883 is a novel, potent INSTI with an in vitro resistance profile that is markedly improved compared to RAL and EVG. In addition, GS-9883 is more potent than DTG against a number of patient-derived isolates with high-level INSTI resistance and may have broad utility in treatment-naïve patients and those with INSTI resistance.

Conflict of interest financial relationship(s): All authors are employees and stock holders of Gilead.
Abstract: O_2

Treatment Strategies for HIV/ Hepatitis infected Patients

Dolutegravir plus Rilpivirine in cART-Experienced Subjects: a cohort study

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Background: While trials run by the industry are assessing the efficacy and safety of dual combinations of rilpivirine plus dolutegravir, no data is available on this association yet. The purpose of this work is to show the results of this regimen in a switch cohort in clinical practice.

Methods: All HIV-1 infected subjects treated with dolutegravir plus rilpivirine between November 2014 and September 2015 were included in a multicentre observational cohort. Only clinical events, demographic data, CD4+ T-cell counts, HIV-1 RNA, serum creatinine and urinary protein excretion were deemed relevant for this study.

Results: Ninety-eight subjects are in follow-up, one having stopped therapy at week 24 due to headache. The main reason for switch was toxicity (n=31, 10 osteopenia/osteoporosis, 8 hyperlipidemia, 5 gastrointestinal intolerance, 3 cardiovascular issues, 3 glucose intolerance, 1 liver toxicity and 1 mental disturbances), followed by simplification (n=30), drug-drug interactions (n=20, twelve for starting anti-HCV therapy), viral failure (n=7), persistent low-level viremia (n=6) and non-adherence (n=4). Forty-four subjects had no reverse transcriptase mutations, 46 had no protease mutations and one had full INSTI resistance and is taking dolutegravir BID, the choices being limited by drug-drug interactions and cross resistance.

Eleven had baseline viral replication (median 31460 copies/mL), 24 had < 50 copies/mL and in 57 HIV-1 RNA could not be detected. At week 4 in 85 subjects HIV-1 RNA could not be detected and 2 had HIV-1 RNA > 50 copies/mL, respectively 52 and 57 copies/mL. Of the 76 subjects who have a 24-week follow-up 63 (82,9%) had undetectable HIV-1 RNA, while 12 (15,8%) had viremia < 50 copies/mL. One had viral rebound due to missed drug refill, but no new mutations were selected.

CD4+ T-cells decreased in median absolute terms (from 730 to 702/mmc), but not in percentage (from 31% to 31,5%). All the 31 subjects who have a 48-week follow-up had < 50 copies/mL and in 25 HIV-1 RNA was undetectable. CD4+ T-cells increased in these patients from 695,7 to 720,3, as did the proportion (30,8 to 32,0%). One subject had low-level resistance to rilpivirine, one intermediate and four high-level resistance (Stanford median score 50, range 15 - 70), but none failed, all having at least a 24-week follow-up.

The median variation in serum creatinine was +0,1 mg/dL, (range +0,41 to -0,23). One subject had mild proteinuria at baseline, that resolved spontaneously by week 24. During the follow-up only one patient reported headache and insomnia.

Conclusions: A dual regimen of dolutegravir plus rilpivirine proved safe and effective in this cohort of non-naive HIV-1 infected subjects.

No conflict of interest
**Abstract: O_3**

**Treatment Strategies for HIV/ Hepatitis infected Patients**

**Dolutegravir plus Ritonavir-Boosted Darunavir in Highly cART-Experienced Subjects**

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**Background:** Dolutegravir and darunavir oppose a high genetic barrier to HIV-1 resistance. Combining the two drugs makes a solid but simple regimen for patients who are in complex salvage regimens, and helps suppress viral replication in poorly adherent subjects.

**Methods:** All HIV-1 infected subjects treated with dolutegravir plus boosted darunavir between November 2014 and September 2015 were included in an observational cohort. Only clinical events, demographic data, CD4 cell counts, HIV-1 RNA, serum creatinine and urinary proteins were deemed relevant for this study.

**Results:** One hundred and one subjects are at different stages of follow-up, one was lost at six months, two died (one for drug abuse and one for cancer-related sepsis) and one has stopped therapy for grade 2 elevation of liver enzymes. The main reason for switching was simplification (n=44), followed by overt failure (n = 26), toxicity issues (n=20 overall, 12 osteopenia/osteoporosis, 2 cardiovascular issues, 1 gastrointestinal toxicity, 3 metabolic, 1 renal toxicity and 1 CK elevation), persistent low-level viremia (n=6), and lack of adherence (n=5). Seventeen had no RT mutations, 21 had no protease mutations, while 12 had INSTI mutations (6 N155H, 1 N155M, 1 Y143C, 1 T66K and 3 polymorphisms).

Thirty-seven were viremic at baseline (median 948 copies/mL), 23 had < 50 copies/mL and in 31 HIV-1 RNA was undetectable. At w4, 12 subjects still had HIV-1 RNA > 50 copies/mL, with a > 1 log10 decay in all but one, that had stopped the therapy for 3 weeks, while in 61 HIV-1 RNA was undetectable. Of the 85 subjects with a 24-week follow-up, 4 (4.7%) still have measurable viremia (range 53 - 82 copies/mL), whereas 64 (75.3%) have undetectable HIV-1 RNA. The mean CD4+ T-cell count and proportion increased, respectively, from 567 to 619/mmc and from 25.1 to 34.2%. Of the 47 subjects who have a 48-week follow-up, none had measurable viremia and in 42 (89.4%) HIV-1 RNA could not be detected. CD4+ T-cells increased from 600.89 to 643.25/mmc and from 25.48% to 28.57%. Eighteen subjects had some resistance to darunavir (Stanford median score 15, range 15 – 40), but none failed, 6 having a 24-week and 7 a 48-week follow-up. The median variation in serum creatinine was -0.01 (range + 0.2 to – 0.21), depending on the renal impact of the previous regimen, and only one subject had a new onset of mild proteinuria. Three had proteinuria at baseline and none worsened.

**Conclusions:** A dual regimen of dolutegravir plus ritonavir-boosted darunavir proved safe and effective in this cohort of highly drug-experienced patients, partly composed of subjects in overt failure of a salvage regimen (37%) and partly of simplifications of complex salvage regimens (44%). In the latter setting, this regimen provides the simplest salvage regimen ever.

*No conflict of interest*
Abstract: O_4

Novel Diagnostic Technologies & Approaches

Next generation sequencing in routine HIV-1 resistance diagnostic - frequency of additional resistance relevant mutations in 2% and 1% population proportions

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Background: The technologies of next generation sequencing (NGS) have made their way into routine diagnostics in HIV-1 resistance testing. The report of mutations of at least 10% of the viral population is chosen by many laboratories due to its equivalency to Sanger sequencing minority detection. The relevance of mutation detected in lower frequencies is still a subject of debate. We here report the frequency of additional resistance relevant mutations in population proportions of greater than 2% and greater than 1% in routine laboratory testing.

Materials & Methods: All HIV-1 resistance tests of the reverse transcriptase Inhibitors (RTI) and protease Inhibitors (PI) performed with an in house PCR followed by NGS (Illumina MiSeq, sequences reported with >100 reads only) between 10/2014 and 03/2016 were analysed. Sequences were interpreted with the HIV-GRADE online tool (http://www.hiv-grade.de) for resistance relevant mutations using a 10%, 2% and 1% minority cut-off. Besides the subtype and the overall increase in mutations, a specific focus were differences in reported resistance associated mutations. We analysed changes in the percentage fraction and potential increase in resistance level (e.g. additional drug class or further drugs in the same class) for the relevant drug classes.

Results: In the evaluation period, we performed 596 NGS resistance test for HIV-1 reverse transcriptase and protease. 442 (74%) of them were identified as subtype B. No drug resistance associated mutations were reported by the HIV-GRADE tool for 200 (34%) sequences with a cut-off of 10%, 126 (21%) and 83 (14%) with cut-offs of 2% and 1% respectively. With a cut-off of 10% in 195 samples (106 of them with a non-B subtype) only PI relevant mutations could be found. We detected Mutations only relevant for NRTIs in 15 samples and for NNRTI in 75 samples. At a minority cut-off of 2% we detected mutations in 197 more samples as compared to a cut-off of 10%. This increased to 347 samples when utilizing a cut-off of 1%. A relevant increase in resistance levels compared to a 10% cut-off was observed for 102 samples at a cut-off of 2% and for 229 samples in the 1% cut-off group.

Conclusions: A relative high portion (66%) of investigated sequences showed resistance relevant mutations at a minority cut-off of 10%. Even removing the non-B subtype sequences, containing only secondary mutations or subtype specific mutations, still left a proportion of 50% sequences with resistance-associated mutations. This high percentage of resistance increases substantially lowering the cut-off range to 2 or 1% not only by number of mutation but also regarding resistance levels. There is a clear need for clinical evaluation of the relevance of mutations in the low percentage range in NGS for resistance interpretation due to its broader use in clinical routine.

No conflict of interest
Abstract

Transmission of HIV drug resistance mutations varies regionally in Europe


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Introduction: The SPREAD surveillance program, carried out by the European Society for translational Antiviral Research, has been in place for over 10 years, to monitor transmitted HIV drug resistance mutations (TDRM) in Europe.

Methods: Clinical and virological data of >14,000 patients diagnosed in 2002 to 2013 has been collected from 28 European countries. TDRM were defined using the WHO surveillance of drug resistance mutations list. Prevalence of TDRM was assessed by region in Europe (Central: Bulgaria, Croatia, Czech Republic, Latvia, Lithuania, Poland, Romania, Serbia, Slovakia, Slovenia; Mediterranean: Cyprus, Greece, Israel, Italy, Portugal, Spain, Turkey; Northern: Denmark, Finland, Norway, Sweden; Western: Austria, Belgium, France, Germany, Ireland, Luxembourg, Netherlands). Wald tests were used to compare prevalence over the regions.

Results: Overall in Europe the prevalence was stable from 2002 to 2013, with 9.0% in 2011-2013. In these last three years, the lowest prevalence was observed in Northern Europe (5.7%), followed by Central and Mediterranean Europe (6.7% and 7.4% respectively). In Western Europe the prevalence was highest (12.1% in 2011-2013; p<0.0001), driven by a higher prevalence of TDRM to NRTIs (7.4%, p<0.0001) and NNRTIs (4.4%, p<0.025). TDRM to PIs was generally low in all regions. The prevalence of TDRM in MSM was higher in Western Europe (2011-2013: 13.5%, p<0.0001) than the other regions (Central: 7.8%, Mediterranean: 8.5%, Northern: 8.0%), but has remained stable over 2002-2013. The prevalence in heterosexuals was lower than in MSM in all regions, but was again highest in Western Europe, 9.6%, compared to 5.6%, 7.7% and 4.5% in Central, Mediterranean and Northern Europe respectively (p<0.0096).

Conclusion: The prevalence of TDRM varies regionally in Europe and is higher in Western Europe, due to a higher TDRM to NRTI and NNRTIs. To inform clinicians and public health authorities with up-to-date figures from their region, ESAR developed an interactive tool with maps of regional surveillance data from the SPREAD program.

No conflict of interest
Abstract: O_6

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Patients with pre-existent NRTI- and NNRTI-resistance have a higher risk to lose virological suppression under tenofovir/emtricitabine/rilpivirine single tablet regimen

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Background: To evaluate the impact of pre-existent resistance (pRes) on the maintenance of virological suppression (VS) in antiretroviral (ART)-experienced HIV-1 infected patients with viremia <50 copies/mL switching to tenofovir/emtricitabine/rilpivirine (TDF/FTC/RPV) single-tablet regimen (STR) in clinical practice.

Material & Methods: pRes to nucleos(t)ide reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs) and protease inhibitors (PIs) (IAS/Stanford HIVdb 2015) was evaluated in all plasma genotypic resistance tests (pGRTs) available before TDF/FTC/RPV starting. Pre-existent genotypic susceptibility score (pGSS), according to HIVDB version 7.0.1, based on the sum of genotype sensitivities to the three drugs prescribed in the regimen was evaluated by considering all pGRTs available before therapy switching. Kaplan-Meyer were used to evaluate the probability of virological rebound (VR: the first of two consecutive HIV-1 RNA >50 copies/mL) according to pRes and pGSS. Uni-multivariable Cox-regression was performed, separately for pRes and pGSS, including the following variables: age, gender, B subtype, risk factor, VS duration and number of blips before switching, previous treatment, cumulative experience to NNRTIs, baseline and nadir CD4 cell count, number of previous regimes.

Results: 309 patients were analyzed. 126 patients (40.8%) switched to TDF/FTC/RPV from an NNRTI-based regimen (85 already treated with tenofovir/emtricitabine/efavirenz as a STR).

Patients were on VS for a median (interquartile range, IQR) time of 21 (6-50) months before TDF/FTC/RPV switching. Pre-existent major NRTI, NNRTI and PI resistance mutations were observed in 12.3%, 10.4% and 6.5% of patients analyzed, respectively; NRTI+NNRTI pRes was found in 5.8% of patients. Patients harboring a virus fully susceptible to TDF/FTC/RPV were 267 (86.4%), while 31 (10%) showed virus fully or intermediate resistant to RPV or TDF/FTC, and 11 (3.6%) had virus fully or intermediate resistant to both RPV and TDF/FTC. Overall, by 72 weeks after TDF/FTC/RPV starting, the probability of VR was 8.1%. Patients with NRTI+NNRTI pRes had a higher probability of experiencing VR compared to those harboring NRTI or NNRTI pRes and to those without RTI pRes (21% vs. 4.2% vs. 7.8%, p=0.006). Cox uni-multivariable regression confirmed that patients with NRTI+NNRTI pRes had a higher hazard ratio (HR) of experiencing VR compared to those without resistance (adjusted HR [95%Confidence Interval, CI]: 3.27 [1.01-10.6], p=0.048). By considering pGSS, after 72 weeks, patients having a fully or intermediate resistant virus to both RPV and TDF/FTC showed a significant higher probability of experiencing VR compared to those having a virus fully or intermediate resistant to RPV or TDF/FTC, and those having a virus fully susceptible to TDF/FTC/RPV (27.3% vs. 7.8% vs. 7.6%, p=0.002). Cox uni-multivariable analysis confirmed that the HR of experiencing VR was significant higher in patients who showed a virus fully or intermediate resistant to both RPV and TDF/FTC (5.91 [1.48-23.53], p=0.012).

Conclusions: In clinical practice, treatment simplification with TDF/FTC/RPV is associated with a very high rate of virological suppression maintenance (92%), particularly in patients without RTI-resistance. By contrast, patients with pre-existent concomitant resistance to NRTIs and NNRTIs have a higher risk to lose virological suppression, thus highlighting the need for an accurate selection of patients to be switched to TDF/FTC/RPV.

No conflict of interest
Abstract: O_7

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Antiviral Activity of Tenofovir Alafenamide (TAF) against HIV-1 Subtypes and Emergence of K65R

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Background: Tenofovir Alafenamide (TAF), a novel prodrug of the HIV-1 nucleotide RT inhibitor (NtRTI) tenofovir (TFV), has shown potent antiviral activity in clinical studies at lower doses than tenofovir disoproxil fumarate (TDF). Pre-clinical and limited clinical data has shown that TAF is effective against all HIV-1 subtypes. Some reports indicate that in vitro, TFV selects the resistance mutation K65R in HIV-1 RT more rapidly in subtype C than in subtype B, and is associated with specific codon usage at positions 64-65-66 in RT. Here we examine differences between subtype B and C viruses in vitro and the implications they could have on TAF efficacy.

Material & Methods: Panels of HIV-1 subtype B and C clinical isolates without resistance mutations were used to study TAF’s antiviral activity and resistance profile. RT from 5 subtype B (with various 64-65-66 codons) and 4 subtype C (with the most common 64-65-66 codons) HIV-1 clinical isolates were cloned into the pXXLAI viral vector. TAF antiviral activity assays and dose-escalation resistance selections were performed in MT-2 cells. TAF resistance profile was further assessed in viral breakthrough experiments at clinically relevant drug concentrations.

Results: HIV-1 subtype B and C isolates (n=9) were profiled in MT-2 cells. TAF antiviral activity was similar for all viruses regardless of the HIV-1 subtype (ranging from 0.7- to 1.1-fold of the reference value). Over 120 days of resistance selection, K65R emerged in the majority of viral isolates. Duplicate selection experiments showed some variability in the time to gain the K65R mutation (subtype B: 53-118 days for TAF and 42-94 days for TFV; subtype C: 32-46 days for TAF and 32-76 days for TFV). Subtype B viruses with the most common codons at 64-65-66 gained the K65R mutations after 90 days (SD=12.1) for TAF and 69 days (SD=7.2) with TFV. Subtype C viruses developed the K65R mutation after 40 days (SD=1.6) for TAF and 46 days (SD=6.8) with TFV. Of note, subtype B viruses with the 64-65 codons identical to the subtype C viruses gained the K65R mutation in a very similar timeframe as subtype C viruses. In viral breakthrough experiments, all subtype B and C viruses were fully inhibited by TAF and TFV at pharmacologic concentrations. At sub-therapeutic concentrations (2x or 4x reduction), viral breakthrough was seen with TFV with both subtype B and C viruses, but TAF maintained virologic suppression.

Conclusion: In agreement with previous in vitro reports, we observed that subtype C HIV-1 isolates selected K65R more quickly than subtype B viruses and this correlated with codon usage at positions 64-66 in RT. However, when used at pharmacologic drug concentrations, both TAF and TFV were able to prevent the breakthrough of the K65R mutation in both subtypes. At sub-therapeutic concentrations, only TAF maintained antiviral activity against subtype B and C viruses, suggesting that the higher intracellular drug concentrations achieved with TAF may result in reduced resistance development among individuals with lower drug adherence regardless of subtype.

Conflict of interest

financial relationship(s): I am an employee of Gilead Sciences
Abstract: O_8

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Clinical impact of vaccine-escape HBsAg mutants in HBV-infected patients: high rate of atypical serological profiles and increased level of viremia and transaminases

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Introduction: Circulation of vaccine-escape mutations among HBV-infected patients has been extensively described in literature but few data are available regarding the clinical impact of such mutations. This study investigates the prevalence of vaccine-escape mutations and their correlation with virological and biochemical parameters.

Materials & Methods: This study includes 904 viremic patients collected for routine clinical practice. For all patients, HBsAg sequence is obtained by population sequencing. HBV genotypes and subgenotypes are determined by phylogenetic analysis (Mega 6.0). Twenty vaccine-escape mutations(T116N-P120E/S/T-T126A/I/N/S-Q129H/R-T131I/N/M133I/L-K141E-P142S-D144A/E-G145A/R [Lazarevic,2014]) are analyzed. Mutations are defined according to the reference sequence of each specific genotype. The association between vaccine-escape mutations and each mutation in major hydrophilic region (MHR, target of neutralizing antibodies) is assessed by phi-correlation coefficient, using Benjamini-Hochberg for multiple comparison correction. A subset of 21 HBsAg sequences are analyzed using Ultra-Deep Pyrosequencing (UDPS) to assess the intra-patient prevalence of vaccine-escape mutations.

Results: Patients are mainly males (67%), Italians (70%) with a median(IQR) age of 46(35-58) years. Median(IQR) serum HBV-DNA and ALT are 3.8(2.7-5.5) log IU/ml and 43(28-85) IU/L, respectively. A wide spectrum of different HBV genotypes (D:631-A:169-E:39-F:27-C:19-B:14-G:5) is revealed. The percentage of patients with ≥1vaccine-escape mutation varies among HBV genotypes: gen-D has the highest prevalence (17.0% for D vs 5.1% for non-D, P<0.001). Notably, in gen-D infected patients, the presence of ≥1 vaccine-escape mutation correlates with: i) atypical serological profiles (HBsAg negativity despite HBV-DNA positivity; HBsAg/anti-HBs[>10IU/L] co-positivity) (19.0% with vs 5.5% without ≥1vaccine-mutation, P=0.001); ii) higher serum HBV-DNA (median[IQR]: 4.5[2.5-6-6] vs 3.6[2.6-5.0] log IU/ml, P=0.002); iii) higher ALT (median[IQR]: 55[31-131] vs 38[27-67] IU/L, P=0.002). In gen-D, the most prevalent vaccine-escape mutations are: sP120S (3.6%), sT131N (2.7%) and sD144E (2.1%). sG145R is found in 1.3%. UDPS analysis shows that in most patients (76.2%) with ≥1vaccine-escape mutation, these mutations circulate as major viral species with an intrapatient prevalence >80%, confirming their potential contribution to HBV transmission also to vaccinated individuals. By covariation analysis, vaccine-escape mutations lie on divergent genetic pathways involving different MHR mutations: sD144E correlates with sS154P (phi=0.38, P=0.05) sM133I with sT116I (phi=0.38, P=0.05) sT131N with both sM133T and sG130N (phi=0.39 and 0.42, P<0.001). The couples of linked mutations are structurally related: sT116I and sM133I lie in the first loop while sD144E and sS154P in the second loop of MHR. Notably, the third mutational pattern (sG130N+sT131N+sM133T) introduces 2
Abstract

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Immunosuppression-driven HBV Reactivation in Patients with Resolved HBV Infection Correlates with a Relevant Risk of Evolution Towards Active Chronic Infection and Death


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Background: Immune-suppression driven HBV-reactivation can involve patients with chronic infection and with apparently resolved HBV infection. It can lead to severe acute hepatitis, fulminant liver failure and death. This study provides a snapshot of virological and clinical features of patients with resolved HBV-infection, undergoing HBV-reactivation (HBV-R) driven by immunosuppressive-therapy.

Materials & Methods: This study includes 51 patients with HBV-R (defined by Hwang, 2013), all HBsAg and HBV-DNA negative before immunosuppressive-therapy. For 29 patients, virological and biochemical follow-up is available after HBV-R. In a subset of 36 HBV-reactivated genotype D infected patients, HBsAg sequence is obtained by population sequencing. Mean genetic distance (GD) is used to estimate the extent of HBsAg genetic variability. The presence of HBsAg mutations recently associated with HBV-R (Salpini,2015) is investigated.

Results: Before immunosuppressive-therapy, the following serological profiles are observed: 58.8% isolated anti-HBc, 27.5% anti-HBC/anti-HBs pos, 7.8% isolated anti-HBs, and 5.9% negative to all HBV markers. At HBV-R, median (IQR) HBV-DNA and ALT are 6.7(4.5-7.7) log IU/ml and 195(39-762) U/L. Notably, 17.6% (9/51) remains HBsAg-negative despite HBV-R (HBV-DNA: 3.0-7.5log IU/ml). In 9/9 HBsAg negative patients at HBV-R, ≥1 novel N-linked glycosylation, besides the classical at aminoacid 146-148, is detected at HBsAg positions 113, 115, 123, 131. These positions reside in the major hydrophilic region of HBsAg and can affect the recognition by neutralizing/diagnostic antibodies. 52.1% of
HBV-reactivated patients are treated with rituximab (RTX), 12.5% with corticosteroids alone, and 35.4% with other chemotherapeutics. Lam prophylaxis was used in 15.7% of patients with HBV-R (median[IQR]duration: 21[10-33] months). In 59.1% of patients, HBV-R occurs after completing immunosuppressive-therapy (range: 1-48 months). RTX use correlates with HBV-R after completing immunosuppressive-therapy (72% after vs 28% during, P=0.05), while corticosteroids use correlates with HBV-R during immunosuppressive-therapy (0% after vs 100% during, P=0.01). After HBV-R, death for hepatic failure occurs in 9.8% (5/51) of patients. Among 29 patients starting anti-HBV treatment after HBV-R with available follow-up (median[IQR]time: 29[17-47] months), ALT normalization is observed in 79%, virological suppression in 51.7%, HBsAg loss (reflecting return to pre-reactivation status) in only 27.5%. Patients with RTX-related immunosuppression are characterized by a higher HBsAg genetic variability than patients with immunosuppression related to other chemotherapeutics/corticosteroids (median GD±SE: 0.022±0.008 vs 0.008±0.004, P<0.001). 18/20 (90%) patients with RTX-related immunosuppression presents ≥1 HBsAg mutation associated with HBV-R. In particular, the mutation Q129R (known to hamper humoral HBsAg recognition) correlates with RTX use (6/20 on RTX vs 0/16 not on RTX, P=0.01).

Conclusions: In immunosuppressive settings, patients with resolved HBV infection develop HBV-R more frequently than previously thought, which implies a substantial burden of death, and may induce evolution to chronic infection. A relevant proportion of patients remains HBsAg-negative despite HBV-R, highlighting the importance of HBV-DNA (more than HBsAg) in HBV-R diagnosis. A higher degree of genetic variability and specific mutations in HBsAg, such as Q129R, are correlated with RTX use and may favor HBV reactivation in the setting of drug-induced B-cell depletion. Overall, these data support the need of an optimized management of HBV-R in terms of adequate monitoring before and during immunosuppression, and improved prophylaxis.

No conflict of interest
Abstract: O_10

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

HBsAg immune-escape Mutations and Stop-Codons, Circulating among European HBV-chronically infected patients can impact on HBV transmission and disease progression


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Background: In HBsAg, immune-escape mutations hamper HBsAg recognition from antibodies, and stop-codons can increase HBV oncogenic potential. Due to HBV genome organization, some drug-resistance RT mutations correspond to immune-escape mutations or stop-codons in HBsAg. No information is available on the circulation of these mutations in patients with chronic HBV-infection (CHB) exposed to nucleos(t)ide analogues (NA) in Europe.

Methods: This study includes 828 patients (255 genotype A; 573 genotype D) enrolled from 18 European Countries from 1997 to 2012. Inclusion criteria are: CHB with detectable HBV-DNA, exposure to >1 NA, availability of a HBsAg sequence. We analyze the immune-associated escape mutations retrieved from http://hbv.geno2pheno.org, and the NA-induced immune-escape mutations I195M, I196S, and V173L (Torresi,2002). Mutations analyzed are those resulting from an amino acid substitution according to the reference sequence of genotype A or D.

Results: Most patients were exposed to LAM (66.9%) followed by ETV/ADV/TDF (33.1%). We found >1 immune-associated escape mutation in 33% of patients with an increasing trend over time (from 21.9% in 1997-2002 to 36.5% in 2009-2012, P=0.04). Gen-D presented a higher number of patients with >1 immune-associated escape mutation (gen-A: 14.9% vs gen-D: 40.5%, P<0.001). Among
them, T118A is present more frequently in gen-D than gen-A (19.7% vs 0.4%, P=<0.001). Of note, in gen-D, the selection of specific immune-associated escape mutations occurs predominantly during nucleoside- (NRTI) than nucleotide- (ntRTI) analogues treatment (A128V:6.4% vs 1.6%, P=0.02; T126S:1.8% vs 0%, P=0.001; T118A: 18.6% vs 8.6%, P=<0.001).

≥1 NA-induced immune-escape mutation occurs in 29% of patients (gen-A:39.6% vs gen-D:23.7%, P<0.001), with a stable temporal trend. Among them, the vaccine-escape pattern I195M+E164D occurs more frequently in gen-A than D (7.1% vs 3.7%, P=0.03).

Vaccine-escape mutations occur in 14.9% of patients (gen-A:7.1% vs gen-D: 18.3, P=<0.001). Among them, P120S is present more frequently in gen-D than A (5.1% vs 0.8%, P=0.003).

Finally, stop-codons are observed in 8.4% of patients (gen-A: 9.8% vs gen-D: 8%) at 20 HBsAg positions including 172 (corresponding to drug-resistance mutation A181T) and 182, known to increase HBV oncogenic potential.

**Conclusions:** Immune-escape mutations and stop-codons circulate in a relevant proportion of patients exposed to nucleos(t)ide analogues in Europe. Genetic backbone of genotypes and use of specific drugs can influence their emergence. These mutations could favor HBV transmission (potentially including vaccinated persons with inadequate anti-HBs titer) and predispose to a faster progression to liver cancer.

*No conflict of interest*
Abstract: O_11

Spread of Drug Resistance

Substantial presence of natural NS3, NS5A and NS5B HCV-resistance in real practice, and their impact on direct-acting antiviral treatment in genotypes 1-4


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Background: Different HCV genotypes may present different prevalence of natural resistance-associated variants (RAVs), with variable impact on susceptibility to direct-acting antivirals (DAA). This study aimed to analyze the frequency of natural NS3, NS5A and NS5B RAVs in a large real-life Italian multicenter database, within the 4 main HCV genotypes (GTs) and to define the impact of natural NS5A RAVs on NS5A-inhibitors efficacy.

Materials & Methods: Natural resistance in NS3 (N=892), and/or NS5A (N=651) and/or NS5B (N=410) proteins were analysed in a total of 1953 sequences from 1017 chronic HCV-infected patients naïve to NS3- and/or to NS5A-, and/or to NS5B-inhibitors, respectively. Population-sequencing was performed by home-made protocols, specific for each genotype/subtype (591 GT-1a, 866 GT-1b, 99 GT-2c, 258 GT-3a, 20 GT-4a and 119 GT-4d). RAVs with fold-change ≥ 100 were defined as major.

Results: Overall 365/1017 (36%) patients showed natural RAVs with important differences for HCV geno/subtypes. GT-1a, GT-1b and GT-4a showed the highest prevalence of natural NS3 RAVs (46%-23%-37%, respectively), mainly represented by Q80K (16% in GT-1a), and by minor S122A/G/I/N/T RAVs (23% GT-1a; 17% GT-1b; and 38% GT-4a). Notably, the Q80K RAV was never detected in GT-4 patients, eligible for simeprevir treatment. Major D168E/T/V RAVs were rare (prevalence ≤ 1% GT-1a and GT-1b; 4% GT-2c; and 6% GT-4d). GT-3a and 4a did not present major natural NS3 RAVs. Also in NS5A, GT-1a, GT-1b and GT-4a showed the highest prevalence of natural RAVs (16%-26%-29%, respectively). Major NS5A RAVs were detected in 12.2% GT-1a (M28V, Q30H/R, L31M, Y93C/H/N), 8.4% GT-1b (Y93H), and notably also in 7.1% GT-2c (L31M, Y93H), 2.2% GT-3a (Y93H) and 2.4% GT-4d (Y93H), and completely absent in GT-2c and GT-3a (8%-3%-2% prevalence, respectively).

Natural NS5B RAVs were highly prevalent in GT-1b patients, mainly represented by L159F (12%). RAVs were rare in GT-1a and GT-3a (3%), and completely absent in GT-2c and GT-4a/d. The major sofosbuvir S282T RAV was never detected.
Among 295 patients tested in all 3 genes, 48% showed at least one RAV on 1 drug-target, and 8.5% showed RAVs on >1 drug-target, mainly represented by RAVs in NS3+NS5A. Triple-classes RAVs were detected only in 2 GT-1b patients.

Finally, to define a potential role of natural RAVs on NS5A-inhibitors efficacy, 62 patients treated with a NS5A-inhibitor, and with baseline NS5A-sequencing available, were analyzed. All 13 non-cirrhotic patients reached a sustained viral response (SVR), regardless minor RAV presence (N=4, all GT-1b). Among 49 cirrhotic patients, virological-failure was observed in 2/4 (50%) patients with major NS5A-RAVs (GT-1b:Y93H; GT-4d:R30S), 1/5 (20%) patients with minor RAVs (GT-1b:L28M), and in 7/40 (18%) patients without known baseline RAVs (p=.237).

Notably, both failing patients with major natural RAVs were treated with not-conventional/not-recommended regimens, without ribavirin; differently, the 2 SVR patients with major natural NS5A-RAVs (GT-1b:Y93H and GT-1a:Q30R) received a recommended-regimen with ribavirin.

Conclusions: Natural RAVs are common across all HCV-genotypes, and particularly in GT-1 and GT-4. Major natural NS5A-RAVs may affect treatment outcome in cirrhotic patients, when treated with suboptimal regimens (short duration and/or without ribavirin).

No conflict of interest
Abstract: O_12

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Characterization of Resistance-Associated Variants at baseline and failure of All-Oral Antiviral Therapy of Hepatitis C

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Background: As drug resistance can be an inevitable outcome because of the high adaptability of HCV and the failure to maintain a high pressure of inhibition, it is important to characterize in vivo the pre-existence and the evolution of resistance-associated variants (RAVs) in specific genotypes on directly acting antivirals (DAAs).

Methods: We studied 50 HCV-infected patients who started an all-oral DAA regimen and were enrolled in a prospective, observational study performed at the Clinic of Infectious Diseases of L. Sacco Hospital. NS3, NS5A and NS5B genes were amplified with genotype-specific protocols. For each subject plasma samples were tested at baseline and at virological failure (7 subjects). Next generation sequencing (NGS) (Illumina Miseq Tecnology) was used for the detection and analysis of RAVs. Minority species with frequencies above 1% were considered relevant. The χ² test was used to compare differences in RAVs among groups of patients.

Results: Based on NS5B sequencing, 34, 7, 5, 3 and 1 patients were infected with genotype 1a, 1b, 4d, 3a and 4a, respectively. All patients were DAAs naive. Of these, 12 were HCV-infected and 38 HIV/HCV co-infected. At baseline, a higher proportion of patients with genotype 1a (n=16) and 1b (n=6) carried RAVs (p=.003). No differences were observed in the distribution of RAVs in mono-infected versus co-infected subjects. One patient showed a viral breakthrough at week 12 on ombitasvir+paritaprevir/ritonavir+dasabuvir and 6 subjects relapsed after the end of treatment (3 treated with sofosbuvir+simeprevir and 3 with ombitasvir+paritaprevir/ritonavir+dasabuvir).

Among the 7 patients who did not achieve sustained viral response at week 12, three (42.8%) had a baseline sequence showing resistant variants. The two patients with genotype 1a showed variants with Q80K and S122G + M28V, respectively, while one patient with genotype 1b had Y93H + S556G RAVs. All these RAVs were detected in more than 90% of baseline strains. The latter patient, failing ombitasvir+paritaprevir/ritonavir+dasabuvir at week 12, additionally accumulated Y56H and D168V. The patient with Q80K at baseline eventually selected for R155K at failure on sofosbuvir+simeprevir. Of the three subjects with genotype 4d who failed sofosbuvir+simeprevir, none showed variants differing from natural polymorphisms. However, one subject relapsed with D168V, known to confer resistance to paritaprevir. Interestingly, all emerging mutations detected at failure were not present as minority variants at baseline.

Conclusions: The role of HCV baseline resistance mutations in the prediction of virological failure of distinct genotypes is still under investigation. Almost half of patients in our study had RAVs at baseline, but these led to treatment failure only in few cases, as for Q80K in NS3. Other resistance patterns triggered the selection for additional mutations detected at failure. The characterization of natural variation and RAVs in genotype 4 needs further studies. Our data reinforce the need of genotyping at baseline for patients who will undergo to DAAs as RAVs are common in genotype 1a. However, a limited role of NGS at baseline is suggested in this study, since it seems not to predict selection for RAVs emerging at failure.

No conflict of interest
Abstract: O_13

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Virological failures to DAA in real life show frequent resistance associated variants and may require re-treatment with unconventional regimens

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Background: Despite excellent efficacy of direct acting antivirals (DAA) containing regimens, virological failure can occur, often associated with development of resistance-associated-variants (RAVs). Furthermore, RAVs could be present in other drug targets also as natural occurring variants. Therefore, drug resistance could represent an important issue for retreatment options. The aim of this study was to characterize, in a real life setting, the pattern of RAVs in DAAs failures.

Material and Methods: 107 DAA-failing patients were analyzed (GT1a-GT1b-2c-3a-4a/d/n/r=22-39-4-23-19; 41.1% treatment-experienced, of whom 8 with NS3-inhibitors: 74.8% cirrhotic). Sanger-sequencing of NS3/NS5A/NS5B were performed by home-made protocols, at failure (N=107) and where possible also at baseline (N=32).

Results: The majority of patients experienced a virological relapse (78.6%), other 14 patients (13%) had a breakthrough and 9 (8.4%) were non-responder. 64/107 patients failed a suboptimal/not-recommended regimen according to current guidelines, mainly: sofosbuvir+/-ribavirin+/-PEG-interferon (N=46), simprevir or asunaprevir+daclatasvir+/-ribavirin (N=11). Among the 43/107 patients failing a recommended regimen, the most common regimens were: simeprevir+sofosbuvir+/-ribavirin (N=29), daclatasvir or ledipasvir+sofosbuvir+/-ribavirin (N=7), paritaprevir/r+ ombitasvir+dasabuvir+/-ribavirin (N=4). Overall, 59/107 patients (55.1%) showed at least one RAV related to the DAA-regimen at failure. In particular, RAVs prevalence was significantly higher in breakthrough/non responders than in relapsers (87% vs 46.4%, p=0.001). No difference in RAVs prevalence was observed according to ribavirin use in recommended regimen, where its use is optional (83.3% without vs 72.7% with, p=0.48). RAVs related to the DAA-class at failure varied in prevalence according to the inhibitors used: 100% NS5A-RAVs in NSSA-failing patients (N=25), 72% NS3-RAVs in NS3-failures (N=50), 26.7% NS5B-RAVs in sofosbuvir-failures (N=86) and 28.6% NS5B-RAVs in dasabuvir-failures (N=7) (p<0.001). Notably, 25/54 (46.3%) patients treated with ≥2 DAA classes showed RAVs on ≥2 DAA-targets at failure, including...
11/11 (100% NS3+NS5A-RAVs) in NS3-NS5A-failures, and 4/7 (57%) in paritaprevir/r+ombitasvir+dasabuvir+/-ribavirin-failures. Furthermore, 3/4 sofosbuvir breakthrough patients showed the major sofosbuvir S282T RAV (1 GT-3a: sofosbuvir alone, 2 GT-4a: simeprevir or ledipasvir+sofosbuvir). Interestingly, the potentially sofosbuvir RAV L159F was found in 17/80 (21.2%) sofosbuvir-relapsers (14/29 GT-1b, 3/19 GT-3a). Of interest, 4 non-responder patients discovered, by performing a resistance test at failure, a different HCV genotype from the previous obtained by commercial genotyping-assays. Three, previously classified as GT-1a or GT-1b, failed with resistance to paritaprevir/r+ombitasvir+dasabuvir+/-ribavirin, due to non-1 genotype infection (1 GT-2c and 2 GT-3a). The other patient, found infected with GT-4d (previously defined as GT-1b), failed with resistance to daclatasvir+asunaprevir+ribavirin. Among 32 patients with available resistance test at baseline, 6 patients showed baseline major NS3 and/or NS5A RAVs or putative sofosbuvir RAVs (GT1b: NS3-D168V+NS5A-Y93H; GT1a: NS3-R155K, GT1a: NS5A-L31M; GT4d: NS3-D168E+NS5A-R30S; 2 GT1b: NS5B-L159F+C316N). In all patients, baseline RAVs were confirmed at virological failure, and in cases of baseline NS3 and/or NS5A RAVs, new additional RAVs were also observed.

**Conclusions:** Re-treatment options in failing patients to DAA regimen are not yet well defined. HCV drug resistance test at virological failure in all 3 genes (NS3/NS5A/NS5B) should be recommended for selecting a new appropriate DAA re-treatment. The high prevalence at failure of major RAVs involving 2 or more DAAs-targets advocates for unconventional, resistance-based regimens, for appropriate re-treatment.

*No conflict of interest*
Abstract

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Treatment emergent variants to combined direct antiviral agents therapy against hepatitis C virus

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Background: Treatment emergent variants (TEVs) to first line direct antiviral agents against HCV represent a problem of major concern. The information on real life setting is scarce and may vary from that of clinical trials. AASLD-IDSA guidelines now recommend testing for NS3 and/or NS5a RAVs in patients that, having failed first line regimens, have cirrhosis and/or are in need of urgent retreatment. Here we describe the first data of TEVs in a real life setting in Spain.

Patients & Methods: We conducted an observational study, including all patients referred to our Reference Center for antiviral resistance from across Spain, from July 2015 to March 2016. TEVs were investigated using population sequencing on the failing sample, and in parallel stored baseline samples, when available. Genotype specific primers were used for NS5a (codons 1-99; positions 28, 29, 30, 31, 32, 58, 62, 92 & 93) and NS3 sequencing (codons 1-181; positions 36, 43, 55, 56, 80, 122, 155, 156, 168 & 170). Pangenotypic primers were used for NS5b sequencing, which was also used for re-genotyping.

Results: Eighty-seven patients failing an interferon free DAA combination were included. Most of them were male (83.1%) with a median age of 52 (IQR 48-58), and a median HCV viral load of 6.0 logs (IQR 5.6-6.5). Twenty-four patients were HCV-1a, 31 HCV-1b, 17 HCV 3a, 3 HCV 4a and 12 HCV-4d. Twenty-eight patients had failed to Sofosbuvir-Simeprevir, 19 to Sofosbuvir-Daclatasvir, and the rest to other combinations. We only observed the S282T Sofosbuvir mutation in one patient. TEVs were observed in 17 of the 28 patients (60.7%) failing Simeprevir based regimens, in 17 out of 19 patients (89.5%) failing Sofosbuvir-Daclatasvir, and in 18 of the 24 (75%) failing Sofosbuvir-Ledipasvir. NS5b, NS3 & NS5A sequencing, gave discordant results from the reported genotype in 7 patients. Reinfection was confirmed using massive parallel sequencing and phylogenetic analysis on 3/27 patients with baseline and relapse samples available.

Conclusions: We present the first results on the genetic barrier and TEV characterization of patients that relapse to Sofosbuvir based regimens in Spain. The high genetic barrier to Sofosbuvir was confirmed, as well as a high prevalence of NS5a associated TEVs. We have also shown the ability of resistance assays to detect errors in genotyping by commercial genotyping assays, as well as to document reinfection rather than relapse.

No conflict of interest
Abstract: O_15

Treatment Strategies for HIV/ Hepatitis infected Patients

Total HIV DNA increases in lymphomonocytes of HCV/HIV co-infected patients during and after DAA treatment

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Background: Latent HIV reservoir is stable in long treated chronic patients. DAA treatment is complex in HCV/HIV co-infected subjects when considering drug-drug interactions, recommendation of previous cART switch, and viro-immunological interactions. Aim of this study was to evaluate HIV reservoir in HCV/HIV co-infected patients undergoing DAA treatment.

Materials and methods: Forty-three HIV/HCV-infected subjects receiving effective cART were evaluated. Patients were treated with DAA for 12 or 24 weeks according to guidelines and drug availability. In all patients, plasma HIV RNA, total HIV-DNA, and CD4 were performed at baseline, at end-of-treatment (EOT) and 3 months after DAA treatment. HIV RNA was measured by the Abbott Real Time assay and classified as follows: a) detected <40 copies/mL (RNA(+)), b) RNA not detected (RNA(-)). Two patient groups were identified according to HIV RNA results: a) complete suppression (CS) = baseline and all following tests HIV RNA(-); b) incomplete suppression (IS) = all other HIV RNA test combinations. Total HIV DNA was measured in PBMC with a Real-time PCR targeting LTR gene and referred to a million cells by using a Real-time PCR targeting a cellular gene (hTERT). Paired Wilcoxon test and logistic regression were used for statistical evaluation.

Results: Baseline median CD4 cell count was 603/mmc (IQR, 338-820). Median months of HIV RNA suppression previous DAA treatment were 85 (IQR, 55-144) and a cART change was necessary in 24 (55.8%) patients before anti-HCV treatment. DAA were prescribed for 12 or 24 weeks in 29 (67.4%) and 14 (32.6%) patients, respectively. HCV RNA <12 UI/ml was found at EOT in 42/43 (97.6%) and 3 months after treatment in 35/37 (94.6%). A significant transient decrease of CD4 count was observed only at EOT (median count 435/mmc (IQR, 254-811), p=0.013). CS was registered in 21 subjects, while the remaining 14 had IS with HIV RNA ranging from detectable <40 cp/ml to 79 cp/ml. Median log₁₀ HIV DNA cp/million PBMC was 3.54 (IQR, 2.48-3.95) at baseline, 3.76 (IQR, 3.51-4.03) during DAA therapy, 3.77 (IQR, 3.50-4.04) at EOT, and 4.01 (3.72-4.18) 3 months after DAA. An increase of HIV DNA during follow-up was observed in 27 cases. Compared to baseline values, median HIV DNA during DAA treatment (p=0.006), at EOT (p=0.021), and after 3 months of DAA (p<0.001) were all significantly higher. At univariate analysis, increase of HIV DNA was associated with baseline HIV DNA (OR 0.22; 95%CI 0.07-0.72; p=0.012) and use of sofosbuvir (OR 3.74; 95%CI 1.00-13.92; p=0.049). No association was found with age, gender, time of HIV RNA suppression, CD4 cell count at baseline, cART switch, type of cART, specific antiretrovirals, level of fibrosis, type of DAA. At multivariate analysis, only level of HIV DNA at baseline maintained a significant association (OR 0.26; 95%CI 0.07-0.92; p=0.07).

Conclusions: Our findings show an increase in HIV DNA in some patients receiving anti-HCV treatment and suggest an impact of DAA on HIV reservoir. Further investigation is needed to assess whether replicative-competent virus is involved and if DAA may act as latency-reversing agents.

No conflict of interest
Abstract: O_16

Treatment Strategies for HIV/ Hepatitis infected Patients

Ex vivo determination of stem cell transplantation graft-versus-HIV reservoir effects

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Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is one of the few strategies that substantially reduces HIV-1 reservoir size. Graft-versus-host (GVH) responses likely result in clearance of residual recipient cells harboring HIV. Beneficial GVH responses, which permit donor cells to clear tumor or residual host hematopoietic cells, may be mediated largely by the innate immune system. To investigate the role of NK cells and other lymphocytes in reactivating and eliminating latent HIV following HSCT, we designed a novel ex vivo assay to determine the activity of HLA-matched, post-HSCT donor effector cells on latently infected, pre-HSCT host CD4 T cells.

Methods: We adapted a latency model to enable infection of high numbers of CD4 T cells from individuals with hematopoietic malignancies prior to HSCT with an iGFP-gag HIV viral strain. The infected pre-HSCT CD4 T cells were then co-incubated with PBMC obtained from the same individuals 9-12 months after HSCT, and following full donor cell chimerism. We then determined lymphocyte activation, proliferation, viral reactivation and death over a two week period using flow cytometric analyses.

Results: We included samples from a total of 30 HIV-negative individuals who received either full myeloablative or reduced intensity HSCT. Up to 95% pre-HSCT CD4 T cells were infected with iGFP-HIV-1, with subsequent resting resulting in large numbers of latently infected cells. Flow cytometry was performed 0-13 days following lymphocyte mixing and co-culture. Of note, higher levels of non-proliferating HIV reactivated cells were found in the autogeneic setting compared to that of the allogeneic samples. Conversely, higher levels of proliferating HIV-infected cells were seen in the allogeneic samples, peaking at day 7. While expression of activation markers increased on NK, NKT and CD8 T cells, there were no differences found between the autogeneic and allogeneic groups. However, CD8 T cell activation was strongly correlated with HIV production (R²=0.975).

Conclusions: Our findings suggest that lymphocytes, including NK and NKT cells, may play an important role in surveillance and clearance of residual HIV-infected cells following HSCT. Presented at Miami HIV Persistence Meeting 2015

No conflict of interest
Abstract: O_17

Viral Evolution & Genetic Diversity

Clonal Integration Site Frequency and Replication Competent Virus in Patients on ART

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Background: The latent HIV-1 reservoir remains one of the major obstacles to cure HIV. To monitor HIV cure strategies, a validated biomarker is needed that can evaluate the reservoir over time in vivo. Consequently, numerous assays are now being investigated to estimate the size of the replication competent provirus.

Materials & Methods: A comprehensive study was designed to evaluate and compare potential HIV-1 reservoir biomarkers. A cohort of 25 ART treated patients was sampled in which plasma viral load (<50 cp/ml) was suppressed for median of 7 years (IQR: 5-11). Total, integrated HIV-1 DNA and unspliced (us-) HIV-1 RNA were quantified in peripheral blood. A quantitative viral outgrowth assay (qVOA) was performed. Subsequently, a nested substudy of 10 patients was set-up to analyse HIV-1 integration sites and tat/rev induced limiting dilutions assay (TILDA). The selected patients had low level of total HIV-1 DNA (<250 cp/10^6 PBMCs) or high level of qVOA (> 2 IUPM). The percentage of integration site clonality was estimated based on results from the integration site analysis, and the frequency of cells with inducible HIV RNA transcription were estimated using maximum likelihood method.

Results: Integrated and total HIV-1 DNA were detected in all patients, and both measures correlated well (p=0.002, R²=0.85). Replication-competent virus was detected in 80% of patients by the qVOA and this correlated with integrated and total HIV-1 DNA (p=0.05, R²=0.44; p=0.019, R²=0.54; respectively). In total 317 integration sites were analysed. A wide range of percentage of clonality (25.0-91.4%) was observed between patients. The majority of patients had an average of 30% clonality, which on average comprised of 3 to 4 predominant clonal integration sites per patient. Patients with higher clonal diversity (≥ 6 clones) had higher estimate for TILDA (frequency of cells with inducible msRNA > 200 cells /10^6 PBMCs).

Interestingly, one patient had an extremely high clonality (>90%), which was represented by a single clone. This patient also had no inducible virus in both VOA and TILDA, suggesting that the clonal provirus in this patient is replication incompetent. The single predominant clone is thought to be associated with the extremely CD4 nadir count in this patient (3 cells/ul.)

Conclusions: We observed a good correlation between VOA and integrated and total HIV-1 DNA. Integration site sequencing revealed that most of the ART treated patients in this study had an HIV DNA reservoir consisting of 30% clonally expanded cells. These levels of clonal provirus did not affect the amount of inducible virus, except for the one patient with >90% clonality.

No conflict of interest
Abstract: O_18

Treatment Strategies for HIV/ Hepatitis infected Patients

Viral dynamics and post-mortem analysis of HIV-1 Reservoir after Allogeneic Transplantation using Stem Cells with a nonfunctional CCR5 (CCR5?32) co-receptor

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

Methods: We investigated the impact of SCT on the size of the HIV-1 reservoir in EpiStem patient #11. SCT for acute myeloid leukemia was performed using an HLA-matched unrelated CCR5Δ32 donor. The patient was re-transplanted with cells from a CCR5Δ32 heterozygous donor after graft failure at ten weeks. Before SCT we performed: 1) Phenotypic and ultradeep genotypic (gp120-V3) co-receptor analysis; 2) Quantitative analysis of the HIV reservoir in different CD4+ T-cell subsets (TN, TCM, TTM, TEM and TSCM) and bone marrow using ddPCR 3) Quantitative viral outgrowth assay (qVOA) 4) Single copy assay (SCA) on plasma. Post-SCT viral dynamics and post-mortem viral reservoir analysis on tissue were performed using ddPCR.

Results: Patient #11 was on effective cART for 18 years and harboured a subtype B CCR5-tropic virus population (false positive rate, 33-49%). Before SCT, no viral RNA was detected in routine diagnostics while 2 c/ml plasma were observed in SCA. qVOA showed presence of replication competent virus (1.6 IUPM). Proviral DNA was detected in PBMCs (295 c/10⁶ cells), bone marrow (80 c/10⁶ cells), CD4+ T cells with stem cell-like properties (490 c/10⁶ cells), naive T cells (579 c/10⁶ cells) and memory T-cell subsets (TCM, TTM and TEM, 2237, 2854, and 4687 c/10⁶ cells, respectively). Four and eight weeks after the first transplantation an increase in the proviral DNA was detected in PBMCs (378 and 533 c/10⁶ cells, respectively), whereas the number of CD4 cells in the PBMCs declined as compared to baseline. At the same timepoints viral RNA could be detected in plasma using the ultra-sensitive SCA (3 and 2 c/ml, respectively). Five weeks after the second SCT, at time of full chimerism, proviral DNA declined to undetectable levels (<1 c/10⁶ PBMCs). Unfortunately, the patient thereafter died with a pneumonitis. Post-mortem analysis revealed that proviral DNA could be detected in lymph node tissue (40 c/10⁶ CD4 cells) but not in ileum.

Conclusion: Within EpiStem, we show that after 18 years of effective cART HIV DNA can readily be detected in various T-cell subsets. In the neutropenic phase post-second SCT, HIV DNA could no longer be detected in PBMCs nor in ileum. In contrast, viral DNA was still found in lymph node tissue indicating that this tissue may serve as a long-standing viral reservoir after SCT.

No conflict of interest
Abstract: O_19

Clinical case

Treatment of HIV and acute myeloid leukemia by allogeneic CCR5-d32 blood stem cell transplantation

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The Berlin patient is presumed to be the only person cured from HIV-infection by hematopoietic stem cell transplantation (HSCT) from a homozygous CCR5-d32 unrelated donor. Attempts to reproduce cure by HSCT have failed because of either viral rebound or death due to the underlying malignancy. We here report a patient alive, well and negative for proviral DNA 900 days after allogeneic CCR5-d32 HSCT.

A 41y old HIV-infected male patient was diagnosed acute myeloid leukemia (AML, inv16, CBF-MYH11) in 01/2011. Since the diagnosis of HIV-infection in 10/2010 he had been treated with TDF/FTC+ DRV (01/2011 VL 44 cop/mL; CD4+ 474 cells/µl). To avoid interactions with chemotherapy DRV was switched to RAL in 03/2011. He achieved CR of the AML after 1 induction course (ICE) and received a 2nd induction and 3 consolidation courses according to AML-SG 07/04. In 09/2012 AML relapsed and he was treated with A-HAM and a 2nd cycle high-dose cytarabine. While in 2nd CR he received 8.74x10E6/kg unmodified peripheral blood stem cells from a female 10/10 CCR5-d32 DKMS-donor after conditioning with fludarabine and treosulfan in 02/2013. Before transplant HIV resistance analysis was performed for PR, RT, IN and viral tropism was determined. There were no significant resistance mutations and the coreceptor-usage was predicted as R5-tropic (Sanger sequencing: FPR 44.5%; NGS: 0.14% X4 at 3.5% FPR; geno2pheno). The proviral DNA load was 29400 cop/mL and in the western blot all anticipated bands could be detected.

During transplant and until today the patient remained on ART (since 06/2014 ABC/3TC/DTG) and the viral load remained undetectable in plasma and liquor. He had a 2nd relapse of AML in 06/2013 but re-entered molecular remission after a total of 8 courses of 5-azacytidine and 4 donor lymphocyte infusions. Concerning HIV, all collected samples were negative for proviral DNA by conventional and digital droplet PCR* in two different labs, namely PBMCs (12/2015, 06/2014, 01/2015* and 02/2015), rectal biopsy (04/2015) and bone marrow (08/2015*). Western blots from 06/2014, 02/2015, 07/2015, 12/2015 and 01/2016 showed incomplete patterns with fading bands. Recent biopsies from ileum and rectum are under investigation.

Like in the Berlin patient, all tests from the Duesseldorf patient so far suggest that HIV may have been eradicated and that he may be the second individual cured from HIV by allogeneic CCR5-d32 HSCT. Further investigations will be performed before considering the discontinuation of ART.

No conflict of interest
Abstract: O_20

Treatment Strategies for HIV/ Hepatitis infected Patients

Breakthrough of preexisting X4-tropic HIV after allogeneic stem-cell transplantation.

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Background: HIV infection can effectively be treated in most patients nowadays, but cure has only been achieved once. Even though this case is a great inspiration, the underlying mechanism resulting in this sterilizing cure has not been completely resolved so far. Recently we reported the case of an HIV-infected patient diagnosed with T-cell lymphoma who subsequently underwent allogeneic stem-cell transplantation (alloSCT) from a CCR5 delta32 homozygous donor. In this case, HIV replication could not be controlled by the immune system and the patient died after a relapse of the T-cell lymphoma.

Material and Methods: The co-receptor tropism of HIV was determined from viral RNA and proviral DNA using the Illumina MiSeq platform for deep sequencing of gp120 V3. Samples were taken before (-287d: RNA, -103d: RNA/DNA, -20d: DNA) and after alloSCT (+20d: RNA, +280d: DNA, +373d: RNA). Viral tropism was predicted by using geno2pheno[coreceptor] indicating the probability of classifying a R5-tropic virus falsely as a CXCR4-capable virus (X4-capable FPR<5, R5-tropic FPR>10). To address the phenotypic properties of selected V3 sequences, these sequences were cloned in the pHXB2-Δgp120-V3 vector and chimeric viruses were grown in PBMCs from healthy donors in the presence of either maraviroc or AMD-3100. In addition these viruses were analyzed in cell culture using the U373-MAGI-CCR5E and U373-MAGI-CXCR4CEL cell lines.

Results: Several distinct virus populations could be observed before and after alloSCT, which harbored specific mutational patterns (I14L,A19V,G24-,H34Y). One HIV variant detected in proviral DNA(4.4%) before alloSCT(-103d) had a unique mutational pattern (N7K,K10K,S11A,H13T,F20Y, T22T,T23K,Q32K) predicted as clearly X4-capable (FPR 0.4). This sequence was identical to the sequence of the dominant HIV variant replicating after alloSCT. Interestingly another isolated detected at day -287 in RNA (2.8%) carried characteristic patterns of mutations of this afore mentioned X4-capable HIV variant but was still predicted to be R5-tropic (FPR 6.9). Consequently this sequence was placed in the phylogenetic tree in between the two clusters of R5-tropic viruses dominating before alloSCT and of X4-capable HIV variants replicating after alloSCT. However in phenotypic analysis this virus remained R5-tropic, only HIV sequences with FPR<1 were tested X4-tropic. In cell culture experiments using the U373-MAGI cell lines, the X4 tropic viruses showed to some extent the property of still using the CCR5 co-receptor, which was not observed in PBMC. All chimeric HIV variants proofed do be replicative competent and especially the X4-tropic HIV variant (FPR:0.4), which was detected before and after alloSCT, was well replicating in PBMC and cell lines.

Conclusion: The selective pressure exerted by the transplantation of allogeneic stem-cells homozygous for the CCR5 delta32 mutation resulted in the selection of an already preexisting X4- capable HIV variant. In contrast to the Berlin these X4-tropic viruses were highly replicative competent and might still have been capable of using the CCR5 receptor. Therefore even the presence of minor X4-capable and replicative competent HIV variants can be responsible for the lack of control of HIV replication from therapy regimens aiming at the functional knock-out of the cellular CCR5 co-receptor of immune cells.

No conflict of interest
Abstract: P_21

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Features of HIV Persistent Viremia After the Start and Restart of Antiretroviral Treatment Regimens

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Background: After the start of antiretroviral therapy (ART), HIV RNA levels normally fall below the limit of detection (LOD) within 24 weeks. Under certain circumstances, however, the drop in viral RNA may take longer, which we define as persistent viremia (PV). At the moment it has remained unclear whether this prolonged decline is related to preexisting drug resistant variants, the emergence of new drug resistance mutations (DRM), compliance issues, or the size of the cellular reservoir. Thus, in this retrospective observational study, we analyzed clinical and diagnostic features of HIV PV after the start and restart of ART.

Material & Methods: Next generation sequencing (NGS) of viral RNA and DNA was performed to study viral evolution and the emergence of DRM in 20 HIV-PV infected patients, who had viral loads above 50 HIV-1 RNA copies/ml for at least 24 weeks after the start (n=15) or restart of ART (n=5). Moreover, immunological parameters (CD4+, HLADR+, CD4+/CD8+ ratio) and clinical follow-up of the patients were compared. Furthermore, it was investigated whether the quantity of total viral DNA, integrated proviral DNA or 2-circle LTR DNA correlates with the risk of developing LLV.

Results: We identified viruses carrying therapy associated DRM in six patients, although only one of them had insufficient drug adherence. In nine patients (45%) we found DRM, which were not related to the current therapy regimen (ancient therapy associated or primary DRM). Here, drug levels were insufficient in only 25% of the patients. Independent of whether the therapy regimen was switched or not, we observed an improvement of the immunological status in almost all PV patients under ART. Strikingly, after reaching HIV levels below the LOD more than one-third of the patients showed episodes of LLV (n=6/17, 35.3%) and one-sixth (n=3, 17.6%) blips. We quantified non-integrated 2-LTR-circles, integrated proviral DNA, as well as total HIV-1 DNA. However, based on the measurement of proviral DNA no connection between the subsequent emergence of blips, LLV or aviremic phases could be determined.

Conclusions: According to our data in 30% of the PV patients (n=6/20) DRM were detected. Adherence issues were not the causative reason for the prolonged RNA decline. ART switching did not affect immune recovery and HIV RNA decline. More than one-third of the patients with PV showed up with low level viremia (LLV). Furthermore, the amount of HIV DNA seems not to be a suitable marker to predict episodes of LLV or blips emerging after PV.

No conflict of interest
Abstract: P_22

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Primary Resistance to Recommended Initial Antiretroviral Regimens in Spain in the period 2007 to 2015

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Background: Recommendations for what treatment to start have evolved in the last years. In Spain, current first line regimens are based on integrase inhibitors. Here we describe primary resistance to the antiretrovirals recommended for treatment initiation in the Spanish HIV antiretroviral treatment guidelines (GESIDA-SEIMC) during the period 2007-2015.

Methods: CoRIS is a multicenter cohort of adult HIV naïve patients. By 2015, 27 sites from Spanish centers participating in the cohort have contributed with 4734 patients to the study. Resistance to first line drugs was investigated using Stanford HIV Db algorithm (v.7.0). HIV Subtype was obtained using REGA HIV-1 subtyping tool V3. Transmitted drug resistance was evaluated using the WHO 2009 list of mutations.

Results: Overall TDR was 7.6% (6.8-8.4), being 3.5% (2.9-4.0) for NNRTIs, 3.5% (3.0-4.1) for NNRTIs and 1.8% (1.4-2.2) for PIs. TDR remained stable throughout the study period, moving from 6.2% (4.2-8.3) to 8.5% (6.0-10.8) in 2014, and from 3.0 to 4.3 for NRTIs, 2.3 to 4.6 for NNRTIs and 0.7 to 2.5 for PIs. TDR mutations to more than one ARV family were uncommon (0.9%; 0.6-1.1). Overall primary resistance to first line drugs was 10.9% (10-11.8), again remaining stable throughout the study period, except for NRTIs, where we found a significant trend to decrease from 2.3% in 2007 to 1.7% in 2015 (p=0.033). When primary resistance to the GESIDA guidelines calendar specific recommendations was investigated, we found a decrease from 9.1% in 2007, when preferred first line was based on 2NNRTIs+ NNRTI or PI, to 1.7% en 2015, when a 2 NNRTIs + INSTI based regimens are recommended. We found a significant (p<0.001) increase in non-B subtypes in Spain, rising from 10.2% in 2007 to 17.4% in 2015.

Conclusions: Transmitted Drug Resistance (TDR) in Spain remains low and with no trend over time during the period 2007 to 2015. Although Primary Resistance to all first line drugs also remains stable, resistance to first line NRTIs has decreased in Spain, as well as primary resistance to the antiretrovirals that are currently recommended for first line treatment. Non-B subtype infections are on the rise with a majority of CRFs.

No conflict of interest

Abstract: P_23

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Patterns of low-level-viremia and blips in HIV-positive patients with continuous antiretroviral therapy (cART)

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Background: Modern antiretroviral therapy regimens lead to sustained suppression of viral replication in the majority of patients. Accordingly, treatment failure is associated with disease progression, whereas the clinical significance of low-level-viremia (LLV) is less clear. In clinical practice it would be crucial to
distinguish between ongoing viral replication and viral release during LLV, since the assumption of ongoing viral replication would result in interventions, but the mere release of viruses would not.

**Materials and Methods:** This retrospective analysis of HIV-positive patients treated at the University Hospital, University Duisburg-Essen from 2006 to 2015 comprises of a clinical data set using the HIV-RNA levels, treatment history and immunograms from routine diagnostics. We defined low-level-viremia (<50/50-1000/50-1000), blips (<50/50-1000/50) and viremia (<50/>1000) using two or three consecutively measured viral loads (copies/ml).

**Results:** More than half of all patients (n=740) showed at least one episode of blip (n=521), of LLV (n=231) or of viremia (n=258). After an initial blip (n=436, 31.4%) neither the risk of an additional blip (n=146, 33.5%), nor LLV (n=63, 14.4%), nor viremia (n=69, 12.3%) was significantly increased. But after an initial LLV (n=133, 9.6%) the risk of an additional LLV (n=36, 27.1%) was three fold higher. Interestingly, after an initial viremia (n=171, 12.3%) the risks of additional LLV (n=35, 20.5%) or viremia (n=47, 27.5%) were also increased. The changes of immune cells measured before and at the time of the initial detection of HIV RNA revealed characteristic patterns. In patients with viremia the CD3+ cells (-103(+/−571), the B-cells (-89(+/-143), and the CD3/CD4+ cells (-134(+/-166) dropped significantly, whereas the CD3/HLA-DR+ cells (+188(+/-406) significantly increased. Statistically significant differences between the two groups of blip and LLV showed up in HIV RNA levels (116(+/-134) vs. 176(+/-197) and in an increase of CD3/CD8+ cells (+14(+/-306) vs. +73(+/-315)).

After correcting for the length of treatment (days of treatment per drug in the cohort), the likelihood of detecting blips during cART was almost identical for all antiretroviral drugs (one blip per 10 years of treatment). In contrast both nevirapin and efavirenz containing treatment regimens were less likely associated with LLV and viremia.

**Conclusions:** Blips were found to be an independent event neither influenced by previously observed blips nor by the kind of treatment regimen. In contrast LLV significantly increased the risk of further LLV episodes and was also associated with viremia. By assuming that LLV is at least in part related to incomplete adherence, the selection of more robust treatment regimens for patients with suspected adherence problems might be an important confounding factor for the negative correlation of specific drugs with LLV in retrospective studies.

**Conflict of interest**

financial relationship(s): The study was supported by Janssen.

**Abstract:** P_24

**Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)**

**Virological suppression and cART discontinuation and the role of baseline genotypic resistance and tropism on in HIV-positive subjects with primary HIV infection (PHI)**

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**Background:** Diagnosis and treatment during the early stages of HIV infection (stage I to V according to Fiebig classification), is crucial for the reconstitution of the immune system and the control of viral replication.

**Methods:** Patients with primary HIV infection (PHI) were enrolled between 2008-2014 in an Italian Network AcuTe HIV InfectiON (INACTION). In this study we present an analysis including 74 patients from 4/24 centers with available genotypic sequence data and immunological and virological data. Patients that started cART within 3 months since PHI diagnosis were classified as early cART group, while other patients were included in late/no cART group. Characteristics of groups (X4 vs R5) were compared using Student T-test and continuous variables are described as median.
Abstract

The majority of patients were diagnosed in Fiebig V stage and symptomatic patients were 87.8%, with fever as the most common symptom. Median plasma HIV-RNA was 5.81 log10 copies/ml while median BL CD4 T-cell was 443 cells/µl. During follow-up, all patients started cART, which was initiated during the first 3 months in 82.4% of patients. The majority of patients showed a R5 (72.3%) vs X4 (27.7%) tropic virus. 25.5% (12/47) of patients with a R5-tropic virus had a FPR>60. Univariate and multivariate analysis showed that early cART start was associated with the year at diagnosis 2012-2015 vs 2008-2011 with a higher frequency in the most recent years. Multivariate analysis demonstrated an association between factors associated to early cART and HIV-RNA (value at baseline) As far as prediction of time to virological suppression, univariate and multivariate analysis showed association with symptomatic acute HIV infection. Moreover, this analysis showed an association between prediction of time to virological suppression and with HIV-RNA. Furthermore, predictors of time to first-line regimen discontinuation were associated with first cART regimen including NNRTI (lower probability) and entry inhibitor (higher probability). Besides, univariate and multivariate analysis demonstrated an association between an optimal immunological recovery and GSS of the first regimen.

Conclusions: Our preliminary data show a predominant presence of R5-tropic viruses in the acute phase of HIV infection and suggest that years of diagnosis (2012-2015) was associated with earlier cART initiation. The presence of R5 vs X4 tropic viruses did not influence the virologic success. Of note, first regimen GSS was associated to an optimal immunological recovery.

No conflict of interest

Abstract: P_25

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Integrase Strand Transfer Inhibitors (INSTIs) Resistance Mutations in HIV-1 Infected Turkish Patients


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Introduction: Integrase strand transfer inhibitor (INSTI) is a new class of antiretroviral (ARV) drugs designed to block the action of the integrase viral enzyme, which is responsible for insertion of the HIV-1 genome into the host DNA. The aim of this study was to evaluate for the first time INSTI resistance mutations in INSTI naïve Turkish patients.

Material and Methods: This study was conducted in Turkey, between April 2013 and April 2015 using 169 HIV-1 infected patients (78 ARV naïve patients and 91 ARV experienced patients). Laboratory and clinical characteristics of ARV naïve and ARV experienced patients

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were as follows: gender (M/F): 71/7 and 80/11, median age: 38 and 38.4; median CD4+ T-cell: 236 and 216 cells/mm³, median HIV-1 RNA: 1.1+E6 and 2.4E+6 IU/ml. Population based sequences of the reverse transcriptase, protease and integrase domains of the HIV-1 pol gene were used to detect HIV-1 drug resistance mutations.

**Results:** INSTI resistance mutations were not found in recently diagnosed HIV-1 infected patients. However, ARV experienced patients had major resistance mutations associated with raltegravir and elvitegravir, the following results were generated; F121Y, Y143R, Q148R and E157Q (6/91 – 6.5%).

**Conclusions:** Our results demonstrate that in HIV-1 infected treatment in naive Turkish patients transmitted or naturally occurred therefore, INSTI resistance related mutations did not exist. The prevalence of INSTI resistant mutations in ART-experienced patients suggested that resistance testing must be incorporated as an integral part of HIV management with INSTI therapies. However, INSTI could be a strong option for initial or switch ARV therapies in HIV-1 infected individuals.

*No conflict of interest*

**Abstract: P_26**

**Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)**

**The prognostic significance of low level viremia among HAART treated patients in Belgrade**

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**Background:** Even though most of the HIV-infected patients treated with highly active antiretroviral therapy (HAART) achieve and maintain undetectable HIV RNA values, resistance to antiretroviral therapy, with virologic failure of HAART, is a major challenge in the management of HIV. The virologic failure is defined by a HIV viral load of more than 50 cp/ml of plasma six months after starting therapy, and/or any detectable viremia of above 50 cp/ml of HIV RNA in those who already achieved undetectable viremia. It has been found that only viremia above 200 cp/mL of plasma could be associated with emergency of resistance. Many HAART treated patients who are on a stable HAART regimen may have a so-called 'low level viremia' (LLV), defined as the presence of 50-199 cp/mL of plasma. Additionally, 'persistent low level viremia'(PLLV) was defined as at least two consecutive detectable viremias measured 4-6 months apart. We also wanted to evaluate the significance of detectable viremia between undetectable and 50 cp/ml of plasma, ei 'very low level viremia' (VLLV).

**Patients and methods:** A retrospective study was conducted to analyse the significance of these three categories of low detectable viremia patterns, for the virologic outcome of HAART, among 1002 patients on stable HAART. Patients were observed over 3 years period, during which plasma viral load measuring was performed every 4-6 months. The possible influence of low detectable viremia on the virologic failure to the end of the study period was analysed. Plasma HIV-1 RNA loads were measured by a quantitative real time PCR HIV RNK (Cobas TaqMan HIV Test, version 2.0, Roche Molecular Systems, Branchburg, NJ, USA), with a lower limit of detection of 20 cp/mL (1.3 log10).

**Results:** The prevalence of virological failure to the end of observed period was 6.6%, while low level viremia and persistent low level viremia occurred in 48.7% and 6.7% of patients, respectively. Very low level viremia was demonstrated in 16.0%. In addition, 28.5% of patients maintained undetectable HIV RNK, all over the time. Regarding the adherence to HAART, good compliance was registered in the majority (70.0%), while remaining patients were not always well adherent. Univariate logistic regression analyses showed that good compliance prevented LLV, but not pLLV and VLLV (OR 0.2 95% CI 0.06-0.9, P=0.005). Further analyses revealed that, god compliance, LLV and undetectable viremia were associated with virological failure (OR 0.2 95% CI 0.05-1.2, P=0.1, OR 0.3 95% CI 0.1-0.9, P=0.033, OR 3.9 95% CI 2.2-6.9, P=0.001, and OR 2.3 95% CI 1.2-4.6, P=0.01, respectively), while
multivariate logistic regression showed that LLV was the independent predictor of virologic failure (OR 3.0 95% CI 1.3-6.6, P=0.005). On the other hand, the composition of HAART regimens, neither PI basse, nor NNRTI based did influence the type of virologic response.

Conclusion: Our data suggest that episodes of LLV could predict virologic failure, which was not the consequence of poor compliance, and probably some other mechanisms may contribute to the low level of viral replication.

No conflict of interest

Abstract: P_27

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Low Frequency of Resistance Associated Mutations by Ultra-deep Sequencing in HIV-1 Primary Infected Patients

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Background: Minor drug resistant variants may increase the risk of treatment failure. The aim of our study was to evaluate the frequency of resistant associated mutations (RAMs) using Ultra-Deep Sequencing (UDS) in patients with HIV-1 primary infection.

Methods: Protease (PR), reverse transcriptase (RT), integrase (IT) and V3 loop of envelope genes were Sanger sequenced using ANRS protocol. Same amplicons were used for UDS by Roche/454 GS FLX+. Sequences were analyzed using: i) AVA software (Roche) and in house software PyroPack® for RT, PR and IT, ii) geno2pheno[454] (Max Plant Institute) and in house software PyroTrop® for tropism. Variability of mutations were analyzed using two cut off of presence of mutation (>1% and >20%): i) RAMs were characterized using both the 2009 WHO list of mutations and the 2014 French ANRS algorithm (including rilpivirine-, etravirine- and integrase inhibitors (INI) related RAMs reported in the 2014 IAS-USA resistance mutations list), ii) all mutations reflecting the diversity.

Results: Samples from the 42 patients consecutively enrolled in the French ANRS PRIMO Cohort between 07/2014 and 10/2014 (95% men, 71% homo/bisexual) were studied. At inclusion, median viral load and median CD4 cell count were 5.5 log10 copies/ml [range: 3.2-7.0] and 543 cells/ml [179-1074] respectively. More than 7000 sequences/target/samples (mean length 488bp for RT, PR, IT and 380bp for V3) were obtained for 40 samples and analyzed with cut off validated at 1% with controls. RAMs were identical between Sanger sequencing and UDS with the cut off at 20% (NRTI RAMS: M41L (n=1, 93%), NNRTI RAMs: K103N (n=2, >98%), E138A (n=3, >99%)). When using a cut-off at 1%, we found 1 patient who harbored a resistant virus with a mutation M46I (1.06%) in the PR gene. Tropism was X4/DM in 7.7% of patients including 2 patients with X4/DM population below 20% (1 of them was X4/DM in Sanger). No virus was resistant to INI. Independently of resistant mutations, the UDS revealed a diversity higher for the RT and IT genes (9.0% and 10.6% respectively) compared to the PR gene (6.6%). This could suggest a greater adaptability and ability of the RT and IT genes to allow for the emergence of resistant variants under antiretroviral pressure.

Conclusion: In this study, few differences were evidenced in the rate of transmitted RAMs using UDS compared to classic Sanger sequencing. These results strongly support the establishment of a clonal viral population at the time of primary HIV-1 infection.

No conflict of interest

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Abstract: P_28

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Evolution of Resistance Associated Mutations to Integrase Inhibitors in HIV–1 infected patients failing an Integrase Inhibitor-based regimen in Portugal

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Background: Integrase Inhibitors (INstIs) have been available for HIV treatment since 2007. The first drug to be introduced was raltegravir (RAL), followed by elvitegravir (ELV). Recently, a 2nd generation drug was released – dolutegravir (DTG). Our aim was to access the evolution of RAMs to INstIs, in HIV–1 infected individuals who experienced virological failure in the presence of RAL or ELV, over a period of seven years (2009-2015);

Materials & Methods: Viral Isolates (integrase region), sampled from 119 subjects, 70 (59%) male and 49 (41%) female, aged between 10 and 73, were retrospectively analyzed. Viral subtype was determined using the Rega HIV-1 subtyping Tool. The mutations considered here were the ones described by the International AIDS Society (IAS) as associated with integrase resistance; c² statistics and Spearman correlation analysis were used to identify patterns of mutations associated with subtype.

Results: In the studied population, 40% (n=47) of the subjects were infected with subtype B virus and 38 % (n=45) with subtype G. The remaining 22% (n=27) were infected by other subtypes. The prevalence of RAMs was relatively low (46%). The mutation frequency was variable, being N155H the mutation with the highest frequency, followed by T97A. A statistically significant correlation was found for the mutations G140SA and Q148HRK in subtype B versus subtype G. The combination of these two mutations is much more frequent in subtype B than subtype G (p= 0.037; p= 0.04). There was a time trend in the incidence of some mutations, with Q148HRK more common in the first years of this evaluation, with a subsequent sustained decline. This finding is important for the sequential use of Dolutegravir, since this mutation was shown to have a significant impact on the efficiency of this drug.

Conclusions: Integrase resistance prevalence is low on our population. The most frequently found mutation was N155H. The combination of mutations G140SA and Q148HRK is significantly more frequent in subtype B than in other subtypes. Q148HRK mutation has been less found in the last few years, making the use of Dolutegravir in patients previously treated with other INstIs more efficient. With the current increased usage of INstIs, it will be important continuing this surveillance to evaluate future trends on INstI resistance.

No conflict of interest

Abstract: P_29

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

HIV-1 drug resistance testing in low level viremia samples in Warsaw center

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Introduction/Background: In routine laboratory practice HIV drug resistance (HIV DR) testing with ViroSeq system is performed on samples with viral load >1000 copies/mL. Sequencing samples below 1000 copies/mL is tied with low success rate. However, there is a challenging diagnostic problem with patients who do not achieve complete virologic suppression despite treatment and present viral load between 50 to 1000 copies/mL. Therefore,
we decided to improve DR test performance by use larger amounts of plasma for HIV RNA isolation.

**Materials/Methods:** Since 2015 till now, we have been tested 37 plasma samples obtained from ARV-treated patients with low level viremia (mean VL – 398 copies/mL, ranging from 102 to 899 copies/mL); 32 (86.5%) were men. The RNA isolation, amplification and sequencing were performed using ViroSeq HIV-1 Genotyping System (Celera) and 3130-Avant Genetic Analyzer (Life Technologies); for interpretation of the data ViroSeq HIV-1 Genotyping Software 3.0 and Stanford HIV DR Database were used. To improve sensitivity of testing increased volumes of plasma (1-1.5 mL) were analysed.

**Results:** Overall, sequencing was successful in 28/37 (75.6%) low VL samples. In below indicated subgroups obtained results were slightly different. Group a) - VL 100-300 copies/ml – 11/15 (73%); b) - 301-500 – 7/11 (63%); c) - 501-700 – 3/4 (75%); d) -701-1000 – 6/7 (85%). Wild HIV-1 variants were detected in 21 (75%) samples. HIV DR- associated mutations were detected in 7/28 (25%) samples; 5 isolates contained single mutations - E138A (in two cases), Q58E, V106A, T215D; 2 isolates harboured multiple mutations (M184I, G190E and D30N, M46I, D67G, K70T). No multiclass DR variants were recognized. Following HIV subtypes were detected: B - 22/28 (75%), A - 3/28 (10.7%), C - 1/28 (3.5%), CRF01_AE – 1/28 (3.5%) and CRF02_AG – 1/28 (3.5%). In samples group where the sequence analysis was not successful (9/37 – 24.4 %), previously obtained DR results were reviewed for subtype verification. Five samples were subtype B (55%), 1 CRF02_AG (11%), in case of the 3 samples subtypes were not determined (33%).

**Conclusions:** Persistent low level viremia is a very challenging issue in clinical and laboratory practice. Our results show that there are also other factors, than emergence of DR mutations, which influence on this occurrence, i.e. not sufficient adherence, pharmacokinetics or pharmacogenomics. Such ongoing low level replication caused by wild type strains can result in DR developing. Therefore, it is very important to confirm the presence of DR mutations, which can help clinicians to change therapy scheme or drug dosage. Even in cases with very low level of HIV RNA (<200 copies/mL) it is still possible to receive good quality sequences just only by increasing volume of the samples used for RNA isolation. In our research we excluded samples between 50 – 100 copies/mL. However, we plan to analyse an effect of increased plasma volume for RNA isolation on success ratio in this difficult-for-testing group of patients. Recognised percentages of non-B subtypes support previously reported changes in subtype patterns in Poland since the year 2000.

No conflict of interest

**Abstract:**

**Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)**

**New Resistance Mutation to Nucleoside Reverse Transcriptase Inhibitors at Codon 184 of HIV-1 Reverse Transcriptase (M184T)**

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**Background:** The HIV reverse transcriptase (RT) mutation M184V confers resistance to two nucleoside reverse transcriptase inhibitors (NRTIs), lamivudine (3TC) and emtricitabine (FTC). M184I, a less common mutation, has also been reported to show a similar resistance profile as M184V. Here, analyzing a large database, we report the in-vivo selection of another variant M184T in patients failing to NRTIs containing regimens.
Method: The prevalence of mutations at HIV RT codon 184 was evaluated using three large independent RT sequence databases from treatment-experienced (TE) and treatment-naive (TN) individuals. The data were collected retrospectively from 3 centers in Italy and Paris between 1997 and 2016. In order to investigate the role of this mutation, a structural-analysis was conducted using the X-Ray crystallographic coordinates of HIV-1 RT complexed to DNA deposited in the PDB with the code 4R5P. M184V and M184T mutants were generated by single-residue replacement in both RT chains. In order to evaluate the NRTIs thermodynamic advantage in presence of the mutants, docking-simulations were performed and the ΔEmodel variation, expressed in kcal/mol, has been calculated between the WT and the mutated complexes.

Results: Among 32440 RT sequences isolated from TE and 12365 isolated from TN patients, the prevalence of HIV RT codon 184 substitutions in each group was 31.21% (n=10125) and 0.72% (n=89) respectively. As expected, the most common occurring mutation at position 184 was M184V irrespective of exposure to therapy (TE patients: 90.90%, n=9204; TN patients: 95.51%, n=85). The less common substitution M184I was also found in both groups (10.22% in TE and 5.62% in TN patients). Interestingly, the new mutation M184T (n=12) have been observed only in TE patients (0.12%). We also found in TE patients other rare substitutions, that have never been reported to our knowledge: 22 M184L, 2 M184C, 1 M184Y and 1 M184R/M mutations. Almost 67% of M184T were subtype B (n=8), 17% subtype CRF02_AG (n=2), the other subtypes being equally represented (n=1+1; 8% for CRF06_cpx and A1).

Regarding the association with antiretroviral therapy, in all cases but two (missing information for one patient and no treatment the year before M184T selection for the other one), the M184T mutation was present under NRTI treatment. The most frequent antiretroviral drugs present at failure were 3TC (n=5) or FTC (n=4), or tenofovir (TDF, n=4). Finally, the computational analysis seems to corroborate the link between this mutation and 3TC and FTC failures. Indeed, docking-simulations with both M184T and M184V mutations showed both FTC (ΔEmodelM184V= -6.17; ΔEmodelM184T= -11.78) and 3TC (ΔEmodelM184V= -5.72; ΔEmodelM184T= -9.23) thermodynamic profiles remarkably unfavorable in comparison to the WT sequence.

By contrast, M184T mutated complex was associated with an increased binding-affinity, as M184V, after TDF (ΔEmodelM184V= +3.71; ΔEmodelM184T= +2.88) and ABC (ΔEmodelM184V= +15.91; ΔEmodelM184T= +9.45) recognition, if compared to the WT model.

Conclusion: In this study, we identified a new resistance mutation (M184T) for NRTIs resistance. The low frequency of this pathway can be related to high impairment of replicative capacity induced by this mutation. Phenotype experiments in B and non-B subtypes should be conducted to better characterize the relevance of this mutation.

No conflict of interest

Abstract: P_31

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Rare detection of R263K integrase mutation in vivo

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Background: The substitution R263K in HIV-1 integrase (IN) has been identified as dolutegravir (DTG)-associated mutation selected in cell culture. Meanwhile, the R263K has been detected in treatment-experienced patients after therapy failure on the first INI-containing salvage therapy with DTG and in combination with the M50I polymorphism in patients who failed raltegravir (RAL) treatment. Since the emergence of R263K mutations in vivo is only rare reported, we screened the IN sequences of the AREVIR cohort for the existence of the R263K mutation and the possible factors for its appearance.

Materials & methods: The study included 2010 IN sequences of plasma viral RNA, cellular proviral DNA or total nucleic acid from whole blood of treatment-naive and –experienced
patients of the AREVIR cohort in Germany. The sequences were obtained by classical Sanger sequencing or since 09/2014 by NGS analyses routinely performed by the Illumina MiSeq technology. Samples presenting the R263K mutation in the IN gene were sequenced by both techniques for control.

Results: The screening of the IN sequences in the AREVIR database presented the R263K substitution in 3/2010 samples (0.15%) detected by both, Sanger and NGS analysis. All three samples were obtained of three different treatment-experienced patients, two infected with subtype B and one with subtype F1. Sample preparation of those R263K positive samples was performed by total nucleic acid extraction comprising viral RNA and proviral DNA.

2/3 samples presented the R263K with detectable plasma RNA of 122 or 7203 cop/ml, respectively. Noticeable, both patients were treated with an INI-containing therapy, named RAL or elvitegravir (EVG). The resistance profile of the EVG-failure strain presented additionally to the R263K the EVG-associated mutation H51Y, and the RAL-failure strain the described DTG-associated mutation L234V. The third sample with suppressed viral load presented the R263K combined with the described DTG-associated mutation L74I in the integrase, probably in proviral DNA. The corresponding patient was on a PI-based therapy at the time of sample collection and not pretreated with INIs. As no selection of the R263K by INIs occurred, an induction of the R263K by APOBEC-mediated hypermutation cannot be excluded. Regarding the NGS analyses of the three patients’ samples, the R263K was detected in 5.1-56.2 %. The variants found with low or suppressed viral loads reflect almost certainly variants archived in cellular proviral DNA as their amplification from plasma RNA is not expected. The described DTG-associated mutations L74I and L234V seem to be polymorphisms with a detection rate of >99%.

Conclusions: The frequency of the R263K mutation in the AREVIR cohort with 0.15% is quite low. It was detected in patients with detectable plasma RNA on RAL- or EVG-inhibitor containing regimens and in proviral DNA without INI treatment history. Thus, the appearance of R263K is not restricted to a DTG-containing therapy and could also be attributed to cellular APOBEC-mediated hypermutation, indicated by their detection with suppressed plasma RNA and without INI pretreatment. In summary, although the R263K mutation is rare detected in vivo, resistance analysis of the integrase gene region should also be considered prior a DTG-based therapy.

No conflict of interest

Abstract: P_32

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

CFS and plasma: comparison of resistance associated mutations

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Background: Close relationships between HIV-1 virus and central nervous system (CNS) disorders have been established since the very beginning of HIV epidemic history. Despite the introduction of antiretroviral therapy (ART), HIV associated neurocognitive disorders (HANDs) are still observed. As penetration of antiretroviral drugs is restricted by the blood-brain barrier and the blood cerebrospinal fluid (CSF) barrier, their concentration may not be optimal in CNS, therefore HIV-1 RNA levels and also resistance patterns may differ in the two compartments. HIV-1 RNA levels and HIV-1 resistance associated mutations (RAMs) in plasma and in CNS were evaluated in HIV-1 infected patients with neurological disorders enrolled in a multicenter study in Liguria, Italy.

Materials & Methods: Sixty-seven HIV-1 infected patients who performed a lumbar puncture (LP) for acute or sub-acute
neurological symptoms from 2007 to 2014 were enrolled. HIV-1 RNA was quantified in each samples and HIV-1 RAMs in CSF and in plasma were compared in every single subject. Sequencing was performed by using the Trugene HIV-1 Genotyping kit (Siemens HealthCare Diagnostics). RAMs for NRTI, NNRTI and PI, according to the 2015 IAS list, were evaluated.

**Results:** HIV-1 RNA was detected both in CSF and in plasma in 35/67 (52.2%) patients. In fact, HIV-1 RNA were detected in plasma but not in CSF in 12/67 (17.9%) and undetected in both compartments in 22/67 (32.8%). Twenty/35 (57.1%) patients were ART naïve, 9/35 (25.7%) on ART and 6/35 (17.1%) in spontaneous interruption treatment. Subtype B was the prevalent (31/35 - 88.6%). Twenty-one/35 (60%) obtained sequences resulted without RAMs both in CSF and in plasma, 12/35 (34.2%) with RAMs both in CSF and in plasma, 1/35 (2.9%) with mutations in CSF and no in plasma and 1/35 (2.9%) the opposite. Among ART naïve patients, 2/20 (10%) had, in RT gene, the same RAMs in CSF and in plasma, 1/20 (5%) had RAMs only in the plasma. No RAMs in PI gene were detected in ART naïve patients. Among ART patients, 5/9 (55.5%) had mutations in RT gene, one of these patients had RAMs only in the CSF, 2/9 (22.2%) had mutations in PI gene in both compartments. Four out of 6 (66.6%) patients who had interrupted treatment had mutations in RT gene and 2 out of 6 (33.3%) in PI gene. The 184V (6/35 - 17.1%) was the most prevalent RAM detected (5/6 in both compartments and 1/6 only in CSF). Only in 2 patients was a different number of PI mutations found. In both cases they were higher in plasma than in CSF (1 on ART and 1 in spontaneous interruption treatment).

**Conclusions:** Despite successful suppression of HIV-RNA by ART in plasma, HIV-1 virus may replicate in CSF, with development of resistance. In the majority of cases RAMs were present and comparable in both compartments, only in two cases substantial differences were detected. Nevertheless, the results support the use of HIV-1 genotypic resistance test when a LP was performed.

No conflict of interest
genes were amplified on HIV DNA from whole blood using ANRS protocol. RT (804 bp) and PR (548 bp) amplicons were sequenced using UDS by Roche/454 GS FLX+. Sequences were analyzed using AVA software (Roche) and in house software PyroPack® for RT and PR. Detection of mutations were analyzed using two cut off (20% and 1%). Drug resistance interpretation was according to the 2015 French ANRS algorithm. We present here the results provided from the 84 first patients. The decay time of resistance mutations was estimate by calculating the time between the last detection in RNA and the time of non-detection by UDS.

Results: We obtained 82 PR sequences (mean reads 5300, mean length 474 bp) and 78 RT sequences (mean reads 9000, mean length 496 bp). We found 19.5%, 11.5% and 10.2% of additional patients respectively resistant to PI, NRTI and NNRTI using UDS at 1% comparing to DNA Sanger. However, respectively 12.4%, 15.0 and 15.5% of patients for PI, NRTI and NNRTI remained non-resistant using UDS comparing to cumulative RNA.

A large diversity of decay was observed between classes and mutations: (i) for PI, 17% (M36I) to 40% (L10F); (ii) for NRTI, 10% (M41L) to 30% (M184V); (iii) for NNRTI, 26 % (Y181C) to 54% (K103N) of patients harbored no resistance mutations in DNA by UDS 5 years after last detection in RNA.

Conclusion: In this study, archived DRM detection was observed more frequently for each resistance codon in RT and PR gene using UDS compared to classic Sanger sequencing in DNA. Resistance to RT and PI inhibitors increased for 10 % to 20% and could jeopardize antiretroviral regimen in case of treatment switch, particularly for patients harboring mutations with rapid decay. UDS sequencing may be used to detect archive DRM in HIV DNA in clinical practice.

No conflict of interest

Abstract: P_34

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Development of an in-house phenotypic HIV-1 coreceptor tropism assay

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Background: HIV-1 envelope (Env) uses CD4 and a chemokine coreceptor (CCR5 and/or CXCR4) for viral entry. The efficiency of receptor/coreceptor mediated entry has important implications for HIV pathogenesis and transmission. The advent of CCR5 inhibitors in clinical use also strengthens the need for quantitative and predictive tools that can guide therapeutic management. The only currently licensed HIV entry inhibitor, maraviroc, targets CCR5, and the presence of CXCR4-using strains must be excluded prior to treatment. Co-receptor usage can be assessed by phenotypic assays or through genotypic prediction. Currently, the proprietary Trofile assay by Monogram Biosciences is the only commercially available and validated phenotypic assay for determining coreceptor usage in the clinical setting. Here we developed a sensitive and quantitative phenotypic assay based on generation of pseudotyped particles (PPs) for characterizing HIV-1 tropism.

Material and Methods: The single-cycle assay (SCA) is based on the generation of PPs using an overlap PCR to attach the CMV immediate early enhancer/promoter to the 5’ end of a population of patient derived env amplicons. To assess co-receptor tropism, U87.CD4.CXCR4/CCR5 cells were infected and luciferase activity (RLU) was measured. Different viral load input was evaluated to determine the limit of detection of the assay when the starting material is viral RNA extracted from a clinical sample. The ability of the system to detect minority populations of X4 viruses in the background of predominantly R5 virus was assessed by using mixtures of plasmids carrying the X4 and R5 env at different ratios. Finally, 8 plasma samples, previously analyzed

No conflict of interest
Abstract

with Trofile as 4 D/M, 2 R5 and 2 X4 virus, were tested in order to verify its accuracy and a quantitative results was generated interpolating the RLU obtained from X4 positive samples on the curve generated by X4 serial dilutions.

Results: The lower detection limit of the SCA was 1% for X4 minority variant. The sensitivity to detect separately X4 or R5 strains was high: in both cases the limit of detection was 10 copies of HIV DNA. The final protocol was able to generate the expected amplicon from as few as 400 HIV RNA copies/ml. Seven of the 8 (87.5%) samples previously analyzed with Trofile gave concordant results. X4 variants were quantified as 14.2% and 10.7% in the two Trofile X4 samples and 0.6%, 4.5% and 5.3% in the three Trofile D/M samples. A discordant strain scored D/M by Trofile but R5 by our system. The FPR for the corresponding sequence was 40.0.

Conclusions: The in-house system showed good sensitivity (down to <1%) in the detection of X4 minority species, similar to the Trofile (0.3% with the enhanced sensitivity version). The only discordant case could be attributable to a presence of X4 at very low levels. Thus, this assay provides a sensitive, efficient and relatively low-cost approach suitable for use by research laboratories for assessing co-receptor usage in vivo and could be conveniently applied to small to medium scale patient cohorts.

No conflict of interest

Abstract: P_35

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Preliminary evaluation of antiviral activity of candidate HIV inhibitors targeting human DDX3 protein

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Introduction/Background: The human ATPase/RNA helicase X-linked DEAD-box polypeptide 3 (DDX3) participates to several cellular functions such as RNA metabolism, cell cycle regulation, cancer progression, germ line development and antiviral innate immunity. In addition, recent studies demonstrates that DDX3 proteins is an essential host factor for the replication of viruses belonging to different families, such as Cytomegalovirus, Human Immunodeficiency virus type 1 (HIV-1), Hepatitis B and C viruses, Dengue virus, West Nile virus, Japanese Encephalitis virus, Vaccinia virus, and Norovirus. In particular, DDX3 was found to interact with HIV-1 Rev protein promoting the nuclear export of unspliced and partially spliced HIV-1 mRNA. By contrast, knock down of DDX3 expression was associated to a significant reduction of Rev function as well as HIV-1 replication. In this work we describe a preliminary phenotypic evaluation of the anti-HIV activity of candidate DDX3 inhibitors.

Material & Methods: A library of molecules targeting the RNA binding site of DDX3 protein were synthetized after docking studies starting from a previously developed DDX3 inhibitor showing a modest antiviral activity against HIV-1 (EC50 =10 μM). Molecules were firstly screened through a helicase assay based on fluorescence resonance energy transfer (FRET) and then positive compounds were further evaluated through a phenotypic cell based assay. IC50 values were calculated using the HIV-1 wild type reference strain NL4-3 through a phenotypic assay (named BiCycle Assay)
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Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

HBV resistance mutation and genotype prevalence in Liguria from 2005 to 2015

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Background: Hepatitis B virus is a global health problem that affects approximately 2 billion people worldwide and more than 240 million people are chronic carriers (CHB) at risk of developing cirrhosis and liver cancer. For this reason the aim of the treatment is to eliminate the virus, thus reducing the possibility of progressive liver damage, even if a complete virus eradication is not achievable due to the persistence of cccDNA form. The approved drugs for chronic patients are 2 immuno-modulators and five antiviral agents: Lamivudine, Telbivudine, Entecavir, Adefovir Dipivoxil and Tenofovir Disoproxil Fumarate. Ten HBV genotypes (A-J) have been identified and are related to clinical course, geographical distribution and way of transmission. We investigate the evolution of mutation and genotype prevalence over 11 years of clinic surveillance in the Liguria Region, Italy.

Material & Methods: A retrospective survey was performed on genotypic resistance tests obtained in 537 consecutive samples collected from January 2005 to December 2015. Sequences were obtained by Trugene® HBV Genotyping kit (Siemens Healthcare Diagnostics) and Abbott HBV Sequencing (Abbott Molecular) according to manufacturer’s instruction. In order to define genotype and resistance mutations, sequences were submitted in the Geno2pheno [hbv] 2.0 algorithm available at the following website: hbv.geno2pheno.org/index.php.

Results: Thirty one candidate DDX3 inhibitors were tested through the BiCycle Assay and 10/31 molecules showed a weak activity against HIV-1 NL4-3 strain (IC50 > 50 μM), while 9/31 compounds had IC50 values in the low micromolar range (0.2 – 3 μM). One of these most active inhibitors retained full activity against six HIV-1 viral clones resistant to PIs, NRTIs, NNRTIs and INIs (fold change values range 0.2 – 0.9).

Conclusion: Molecules belonging to a newly discovered series of human helicase DDX3 inhibitors were found to be active against both wild type and PI/NRTI/NNRTI/INI resistant HIV-1 viral strains, suggesting a new antiviral strategy that could overcome resistance to the currently approved antiretrovirals. Since DDX3 is involved in the replication cycle of several different viruses, these inhibitors represent valuable candidates for the development of molecules with a broad antiviral activity against well-known and emerging viral diseases.

No conflict of interest

Abstract

consisting in a first round of infection of MT-2 cells in presence of serial dilutions of the investigational compounds followed by a second round of infection of the reporter cell line TZM-bl with MT-2 supernatants. By using the same approach, antiviral activity was further evaluated against a panel of HIV-1 recombinant viruses carrying mutations conferring resistance to drugs approved for HIV-1 treatment.

Results: Thirty one candidate DDX3 inhibitors were tested through the BiCycle Assay and 10/31 molecules showed a weak activity against HIV-1 NL4-3 strain (IC50 > 50 μM), while 9/31 compounds had IC50 values in the low micromolar range (0.2 – 3 μM). One of these most active inhibitors retained full activity against six HIV-1 viral clones resistant to PIs, NRTIs, NNRTIs and INIs (fold change values range 0.2 – 0.9).

Conclusion: Molecules belonging to a newly discovered series of human helicase DDX3 inhibitors were found to be active against both wild type and PI/NRTI/NNRTI/INI resistant HIV-1 viral strains, suggesting a new antiviral strategy that could overcome resistance to the currently approved antiretrovirals. Since DDX3 is involved in the replication cycle of several different viruses, these inhibitors represent valuable candidates for the development of molecules with a broad antiviral activity against well-known and emerging viral diseases.

No conflict of interest
Results: From 2005 to 2015, respectively, 25, 18, 39, 51, 58, 49, 70, 64, 62, 59 and 42 sequences were analyzed. With regard to genotype prevalence, through the years the rate of D genotype declined from 92% to 71.4%, whilst, among the others genotypes, a spread of A, B, C and especially E genotypes were observed. The last one showed an increase from 4% to 9.5%.

With regard to mutation prevalence, 147/454 (32.4%) sequences revealed the presence of at least one mutation conferring drug resistance. In the last 2 years we observed that mutations conferring resistance were found only in 8/101 (7.9%) patients and during the period of observation the prevalence of wild type sequences increased from 56% to 88.1%.

Conclusions: This study highlighted that over the last years we are observing a progressive decrease of mutations conferring resistance, likely due to the latest international practice guidelines that recommend the use of drugs with high antiviral potency and high genetic barrier such as ETV or TDF. It also showed that, although, A and D genotypes are currently the most prevalent in Liguria, as well as in the rest of Italy and Europe, C genotype is progressively increasing in Ligurian patients over the last 11 years. Since a recent study in East Asia (where B and C genotypes are predominant) demonstrated that C genotype was associated with a higher risk of developing HBV-related HCC, the spread and increase of C genotype in our region should be carefully monitored to perform adequate prevention and/or treatment aimed to reduce the future cases of liver damage. In the last year the increase of the E genotype has been probably due to migration from Africa. Being, as recently reported, the most difficult genotype to treat, it also needs monitoring.

No conflict of interest

Abstract: P_37

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Subtype analysis and the impact of resistance associated variants on 2nd generation DAA treatment outcome in a cohort of HCV GT1 carriers in Israel

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Introduction: HCV GT1 treatment regimen remains dependent in part on the viral subtype. Resistance associated variants (RAVs) emerging prior to treatment are also subtype dependent and might compromise HCV treatment response. Subtyping performed by either 5’ UTR sequencing or commercial assays inefficiently differentiates between the various GT1 subtypes. Using population sequencing of NS5B or NS3 genes we have assessed GT1 subtypes and have investigated the prevalence of baseline NS3 and NS5A RAVs and their impact on 2nd generation DAAs treatment outcome in a cohort of HCV GT1 patients in Israel.

Methods: GT1 subtype was analyzed in samples from 267 patients using either the non-structural 5’ region of NS5B (n=88) or the NS3 gene (n=254). Baseline RAVs (NS3 and NS5A) were assessed in 118 Israeli GT1 carriers which were subsequently treated with either the combination of ombitasvir, paritaprevir, and dasabuvir (n=95), grazoprevir and elbasvir (n=17) or sofosbuvir with either daclatasvir, simepravir or ledipasvir (n=6). Geno2Pheno and bio Africa tools were used for subtype determination and RAVs were identified based on the IAS-USA 2012 list. Phylogenetic analysis was performed using Mega 6.0.
Results: All samples from 270 GT1 patients were successfully subtyped. Comparison between the NS3 and NS5B sequences in 60 of the cases revealed identical subtypes. 17% (9/53) of the 5' UTR defined GT1a were found to be GT1b and 1 of 138 5' UTR GT1b defined subtypes was a GT1a virus. NS3 or NS5B sequencing subtyped 79 GT1 samples which were undetermined by Abbott assay as follows: 62 GT1b, 12 GT1a, one GT1m and three GT1d (all immigrants from Morocco). Baseline RAVs identified in NS3 (V36M, T54S, V55A, Q80K, R155K, D168E) and in NS5A (M28V, L31M and Y93H) had no impact on treatment outcome of any of the 2nd generation DAAs even when several RAVs were identified in both NS3 and NS5A proteins of the same HCV carrier.

Conclusions: None of the baseline RAVs interfered with successful treatment outcome (12 or 24 SVR), suggesting that mutation analysis prior to the 2nd generation DAAs is unnecessary. GT1 subtype should not be determined by sequencing the 5' UTR HCV region and in cases where subtype cannot be defined using current commercial tools, sequencing of either NS5B or NS3 should be performed.

No conflict of interest

Abstract: P_38

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Prevalence of NS3, NS5A and NS5B resistance associated variants in DAA treatment-naïve patients

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Background: Treatment of chronic hepatitis C (HCV) has rapidly evolved from (pegylated) interferon and ribavirin to more potent highly effective direct-acting antiviral agents (DAA) combination therapies. While DAAs are widely used for treatment of HCV, the role of resistance associated variants (RAVs) is becoming more clear. In this study we evaluated the prevalence of baseline NS3, NS5A and NS5B RAVs in samples received for routine testing.

Materials & Methods: Genomic regions were amplified from HCV-RNA using reverse transcriptase PCR followed by a nested PCR. Amino acids 1-181 of HCV NS3 protease, amino acids 1-213 of NS5A and amino acids 219-347 of NS5B were included. Purified PCR products were sequenced using 3130-Avant Genetic Analyzer (Life Technologies, NY, USA); sequences were aligned by SeqScape Ver. 3.3 Software (Life Technologies, NY, USA). Mutations and predictions of phenotypic resistance were obtained using Geno2pheno tool (latest version available at the time of our analysis).

The mutations associated with Protease Inhibitors drug resistance considered were 36AGLM, 43ISV, 54AS, 55AI, 56H, 80KLR, 122AR, 155IKMCGQT, 156GSTV, 168EGNTVY and 170AT. Were also considered RAVs at positions 28, 30, 31, 58, 92 and 93 for NS5A inhibitors and 159, 282, 289, 316, 368, 395, 554 and 556 for NS5B inhibitors.
The substitutions observed include variants isolated and sequenced from baseline consecutive serum samples of patients with chronic HCV infection who were DAA-naïve.

**Results:** NS3, NS5A and NS5B sequencing analyses were performed from 71, 144 and 111 patients, respectively. NS3 RAVs were identified in 23/43 (53.5%) GT1a and 4/23 (17.3%) GT1b viruses. Of the 23 GT1a samples with RAVs, 15 (65.2%) harbored a single RAV and 8 (34.8%) had more than one RAV. Among the GT1a viruses, the most frequent substitution was Q80K (27.9%). The polymorphism Q80K was exclusively detected in clade I of subtype 1a (54.5%). 168E, 168V, 122T and 80L were observed in 4.3% of GT1b patients.

Regarding NS5A, RAVs were detected in 43/116 (37.0%) HCV-1, 1/7 (14.3%) HCV-3 and 3/16 (18.7%) HCV-4-infected patients. Of the 43 GT1 samples with RAVs, 31 (72.1%) harbored a single RAV and 12 (27.9%) had more than one RAV. Different patterns of resistance mutations have been observed for two HCV-1 subtypes: RAVs at positions 30 (41.7%), 31 (10.0%) and 93 (16.7%) predominated among HCV 1b viruses, while substitution at position 28 (12.5%) was the most common in HCV 1a samples. Within NS5B, RAVs were observed in 23.4%, 8.3% and 12.5% of GT1, GT2 and GT3 patients, respectively. The 316N variant was mostly found in GT1b (46.1%), followed by 159F and 556G (7.7%). Substitutions in position 395 were found in 7.7% of G1a samples.

**Conclusions:** Natural polymorphisms associated with resistance to NS3, NS5A and NS5B inhibitors have a considerable prevalence, that is variable and depends on HCV genotype and subtype. Resistance testing at baseline is not yet indicated, as more data are needed to demonstrate its role in clinical practice, but may be useful to individualize the best treatment option for each patient.

*Some of the data were presented at HELISA, Genoa 16-Sep-2015*

*No conflict of interest*
sequencing (Sanger or ultra-deep) and subsequent interpretation of the corresponding viral gene with geno2pheno[HCV].

Results: 1295 HCV-infected patients have been enrolled until Nov 17th 2015. We could obtain 992 NS5B sequences, which were used for genotyping: GT1a= 40.8%; GT1b=36.6%; GT1d=0.2%; GT2=2.8%; GT3=14.2%; GT4=5.3%. Baseline NS3/protease-inhibitor susceptibility was determined from 446 baseline sequences and used for baseline resistance prediction (Fig. 1). Baseline resistance was found for ASV=3.0%; BOC=8.8%; GZV=0.6%; PTV=2.8%; SMV=23.2%; TVR=3.0%. For TVR, 20.9% of the samples were predicted as possibly resistant. Baseline NS5A-inhibitor susceptibility was analysed for 251 sequences. The percentage of resistant samples was similar for all four NS5A-inhibitors, 4.4%-6.0%. Baseline NS5B-inhibitors susceptibility: The sequence sets used for the analysis of each of the NS5B inhibitors varied, since the described RAM-patterns for each drug comprise different amino acid residues. While 10.8% of the 278 sequences used for DSV screening were reported as resistant, none of the 742 samples screened for SOF were predicted as resistant. Baseline RAM analysis: The mutation NS3 80K was found in 20.0% of the samples. It led to the high proportion of samples resistant to SMV. Substitutions on additional four amino acid positions were found in ≥0.9% of the samples. In the NS5A, the most frequent amino acids exchanges were at positions 30 and 93. In the NS5B, the mutations 556GNR were found in 12.0% of the cases.

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

No conflict of interest

Abstract: P_40

Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)

Frequency of the NS3 drug resistance variants among HIV-HCV genotype 1 infected patients from Poland

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Background: In the era of direct antiviral drug (DAA) use for the treatment of the hepatitis C, preexisting drug resistance variants may impair its clinical efficacy. HIV-HCV coinfected patients remain the challenging group from the perspective of the DAA treatment optimization and possible sexual or injection drug use (IDU) related transmission of drug resistance. In this study we aimed to analyse the frequency of the NS3 drug resistance variants among HIV-HCV infected patients, among IDU and men-who-have-sex-with-men (MSM) infected with HCV genotype 1.

Material and methods: One-hundred NS3A genotype 1 sequences were obtained from HIV/HCV coinfected patients by bulk sequencing and analyzed using geno2pheno HCV tool with identification of the preexisting HCV protease inhibitor resistance associated variants. Key resistance mutations were defined as any of the following: V36M/A, T54A/S/V, V55A, Q80K, S122 A/G/N/R, R155K, A156T/S/V, D168Q/A/H/T/V/N/Y, V170A/T/L. For statistics Chi2 test, two-sided Fisher's exact test or U-Mann-Whitney test was used, as appropriate.
Results: Sixty-three (63%) patients were infected with subgenotype 1b and thirty-seven (37%) with subgenotype 1a. Twenty-eight patients had history of acute MSM-transmission associated hepatitis C, with subgenotype 1a being more prevalent (n=14, 58.33%) compared to subgenotype 1b (n=14, 22.2%), p=0.001 in this group. No significant differences in subgenotype distribution was observed for gender (subgenotype 1a noted in 11.42% and 1b in 21.31% of females), CDC category at HIV diagnosis (category A found in 35 (62.5%), B - 14 (25.0%), C-7 (12.5%) among subgenotype 1b versus 17 (54.84%), 10 (32.26%), 4 (12.9%) for subgenotype 1a infected cases, respectively). Main resistance mutations to NS3 inhibitors were found in 15.0% of sequences, and were notably more common in subgenotype 1a [n=11 (29.7%)] compared to subgenotype 1b [n=4, (6.25%)], p= 0.0028. For subgenotype 1a ten (27.2) Q80K and one (2.7%) V55A variants were identified, among subgenotype 1b two S122N (3.2%), one S122G (1.6%) and one T54S (1.6%) polymorphisms were found. All Q80K variant containing sequences were detected in clade I of subgenotype 1a. Resistance variants were also equally distributed across genders (17.65% for female and 15.19% for male cases), CDC category (15.38% for among asymptomatic patients, 16.67% among symptomatic, and 9.09% for AIDS), or transmission route (n=8, 18.6% among MSM compared to n=6 (11.76%) among IDU) with no differences in the frequencies noted for the patients with the history of acute hepatitis C compared to the chronic infections (n=4, 14.29% vs n=8, 13.56%, respectively). In subgenotype 1a drug resistance variants were observed in 7/21 (33%) cases with HCV acquired by MSM-route and 3/16 (18.75%) of IDU-associated transmissions.

Conclusions: 1. Among HIV-HCV genotype 1 coinfected patients subgenotype 1a is associated with MSM transmissions and acute hepatitis. 2. Frequency of drug resistance variants among HIV-HCV subgenotype 1a infected patients is common, higher then observed among monoinfected cases, remaining infrequent in subgenotype 1b with the Q80K variant being the most prevalent.

No conflict of interest
protocol and the methodological recommendations of Cochrane and the Cochrane Hepato-Biliary Group. We assessed risk of bias and performed Trial Sequential Analysis to reduce the risk of random errors. Intervention effects were evaluated with GRADE.

Results: We identified 9358 references, of which 527 remained after exclusion based on titles and abstracts. At least 110 trials will be included in our analyses. The analyses will be completed before the 21st of April. We will assess the following primary outcomes at maximum follow-up: 1. Hepatitis C-related morbidity. This composite outcome will be defined as the proportion of participants with either: with newly acquired cirrhosis, ascites, variceal bleeding, hepato-renal syndrome, hepatocellular carcinoma, hepatic encephalopathy, or death from all causes. 2. Serious adverse events. 3. Quality of life. We will assess the following secondary outcomes as proportion of participants with: All-cause mortality, ascites, hepato-renal syndrome, hepatocellular carcinoma, hepatic encephalopathy, non-serious adverse events, sustained virological response. Additionally, we will perform a predefined list of subgroup analyses, between trials with: high risk of bias compared with low risk of bias, different kinds of direct-acting antivirals, with and without HIV, and with and without ribavirin.

Conclusions: We will base our primary conclusions on our primary outcomes in trials with low risk of bias.

Abstract: P_42

Treatment Strategies for HIV/ Hepatitis infected Patients

A systematic review & meta-analysis in the effectiveness of mobile phone interventions used to improve adherence to ART in HIV

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Introduction: There are over 36 million people in the world with HIV. Antiretroviral therapy is effective in preventing the progression of HIV to AIDS but more than 95% adherence is required to suppress viral load. Adherence to HIV medication is lower than ideal. Mobile technologies could deliver low cost interventions to increase adherence to medication. A previous Cochrane review included two trials and concluded that SMS interventions increased adherence to HIV medication, but further trials with mixed results have since been published.

Our review aims to provide an up to date synthesis of the evidence and report the effects of interventions delivered using different mobile phones functions (phone calls / SMS) and frequencies of contact. We explore the effects of interventions which include interactivity, links to services and interventions which include at least 3 behaviour change techniques.

Methods: We searched Cochrane, Medline, CINAHL, EMBASE and Global for randomised control trials of interventions delivered by mobile phone designed to increase adherence to antiretroviral medication. All reference lists of relevant articles were manually screened and risk of bias assessed. Primary outcome adherence measures were objective (CD4, viral load) and the secondary outcome measure was self-reporting. We calculated relative risk ratios (RR) or standardised mean difference (SMD) with 95% confidence interval (CI). Where appropriate meta-analysis using STATA version 11 has been used to pool data. Two independent reviewers extracted data.
Results: Thirteen articles were selected. Interventions included up to 6 behaviour change techniques. No primary outcomes could be pooled due to lack of objective measures. Secondary outcome measures were pooled but at risk of bias as participants could not be blinded and measures are subjective. The pooled effects of interventions delivered by text messages on self-reported adherence was RR 1.11 (1.00 to 1.24) and weekly messaging was RR 1.16 (0.98 to 1.37). Voice call interventions increased self-reported adherence RR 1.20 (1.06 to 1.37) and SMD 0.32 (0.06 to 0.58). Subgroup analyses showed that neither interactivity RR 1.07 (0.81 to1.40) or links to service support RR 1.11 (0.91 to 1.36) improved self-reported adherence. Interventions with at least 3 behavioral change techniques increased self-reported adherence, SMD 0.77 (0.08 to 1.46).

Conclusion: Existing interventions include few behaviour change techniques. Individual trials report beneficial effects of adherence interventions, but pooled analyses of SMS interventions is heterogeneous. The overall pooled effect almost achieved statistical significance at p=0.05. Pooled analysis showed statistically significant increases in adherence to medication for interventions employing mobile phone calls. WHO should reconsider current advice that text messaging in general is effective and should focus on specific interventions, which have been shown to be effective. High quality trials of optimal interventions on objective measure are needed in high middle and low income countries.

No conflict of interest

Abstract: P_43

Treatment Strategies for HIV/ Hepatitis infected Patients

Establishment of a latent HIV-1 infected human LTiT4 (long term inducible T cell positive for CD4) cell line and latency in CD4+ memory T cells by interferon-a.

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Introduction/Background: Human immunodeficiency virus type-1 (HIV-1) persistently infects CD4+ T-lymphocytes and reduces the number of CD4+ T-lymphocytes in vivo. Although long-established T-cell leukemia-derived T-cell lines have been used for the targets of in vitro HIV-1 infection, the responses of these cells to various stimulations differ from primary T-cells in the physical conditions. Here we established a non-leukemia LTiT4 cell line, susceptible to HIV-1 from a healthy individual. Latent HIV-1-infected cells under antiretroviral therapy are reported to be resting memory CD4+ T cells; however the mechanisms of HIV-1 latency is unclear. We demonstrate that long-term culture of interleukin (IL)-2-dependent CD4+ T cells with a memory phenotype mimicked latently HIV-1-infected cells in the presence of interferon-a. These cells are mostly resting and contained HIV-1 proviruses that could be re-activated by stimulation.

Materials and Methods: Peripheral CD4+ T-lymphocytes isolated from a healthy individuals were stimulated with retinoic acid followed by anti-CD3 and anti-CD28 antibodies, then cultured in the presence of rhIL-2 and rhTGF-beta with periodical anti-CD3 and anti-CD28 antibodies stimulations for longer than 6 months. Cell surface phenotype was assessed by flow cytometry. The LTiT4 cells were infected with pseudotype reporter HIV-1, VSVG/NL4-3-luc, and the effects of various stimulation on HIV-1 expression were evaluated by luciferase activities. The effect of INF-a on cell cycle and
latency were evaluated by flow cytometry and RT-PCR respectively.

**Results:** The LTiT4 cells were positive for CD4, and CD25, and initially expressed Foxp3 but lost it during culture. This cell line exhibited high luciferase activity following pseudotype virus infection, which was further enhanced by CD3-stimulation, and suppressed by type-I interferons or antibodies to co-stimulatory molecules such as CD28 or ICOS. We found in the presence of IFN-a, the HIV-1-infected cells entered a resting stage to a greater extent, and the HIV-1 existed as integrated proviral DNA but was hardly replicate.

**Conclusion:** This cell line can be a useful tool for screening the effects of physical and chemical stimulations on HIV-1 expression in normal CD4+ T-lymphocytes. Our findings indicated that LTiT4 cells can mimic resting memory T cells in the presence of IFN-a, exhibiting post-integration latency following HIV-1-infection.

**No conflict of interest**

**Abstract: P_44**

**Treatment Strategies for HIV/ Hepatitis infected Patients**

**When we have Started Antiretroviral Therapy in the last 6 years? Impact of HIV Treatment Guidelines.**

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**Background:** Current HIV treatment guidelines recommend antiretroviral treatment (ART) initiation for all HIV-infected individuals regardless of CD4 count. This study evaluates the immunological and virological status and the clinical characteristics of patients who have started ART in the last 6 years in the Northwest of Spain.

**Materials & Methods:** All HIV-infected patients who started ART between 2009 and 2015 at a reference hospital in the Northwest of Spain were included. Epidemiological, clinical, laboratory and ART regimen were recorded. A statistical analysis was performed using the SPSS v.19. software.

**Results:** A total of 411 HIV-infected patients who started ART in the study period were recorded. 84.2% were men with a mean age of 39 years. Main risk factors were: 48.2% men who have sex with men, 33.8% heterosexual, 13.6% injection drug users, and 4.4% unknown. Mean time since HIV+ diagnosis to first contact with HIV specialist medical team (FirstSMT) was 276.7 days [range 0-5945] and until ART initiation (FirstART) 606.1 days [range 0-5390]. These figures were maintained during the study period. Of note, in 42.5% of patients the time between FirstSMT and FirstART was >180 days. From them, those who required start treatment (50.9%) delayed it due to rejection of ART initiation (26.9%) and lack of compliance with the scheduled visits (24%). During the time between FirstSMT and FirstART, mean ARN-HIV viral load increased (4.9±0.9 vs 5.1±0.8 log copies/mL, respectively; p<0.001), and mean CD4 cell count decreased (386.2±297.9 vs 258.7±195.9 cel/mL, respectively; p<0.001). Mean CD4 cell count at FirstART was maintained during the study: 168.7 (2009), 203.6 (2010), 197.6 (2011), 196.1 (2012), 210.6 (2013), 201.7 (2014), 255.3 (2015) cel/mL (p=0.007). The overall mean nadir CD4 counts was 243.4±179.1 cel/mL. Overall, 71.8% patients started ART with CD4 count <350. From these, most of them (64.4%) had <350 at FirstSMT, 19.7% were lost of follow-up because lack of compliance with the scheduled visit, 15.9% had no indications for ART initiation in the last visit. The rest of patients (28.2%) started ART with CD4 count between 350-500 (20.2%) and >500 cel/mL (8%). While in 2009 only 1.8% of patients started ART with CD4>500 cel/mL, during 2015 they did 26.2% (p<0.001). Regarding the drugs used as FirstART, the main NRTI backbone were TDF/FTC in 85.2% and ABC/3TC in 11.9%. FirstART combinations...
were based on PI in 45.5% of patients, NNRTI in 39.2%, and INSTI in 15.1%. During 2015, 58.5% of patients initiated ART with an INSTI-based therapy compared to < 10% in the previous years (p<0.001). The rate of mortality at the time of completion of the study was 4.4%.

**Conclusions:** High proportion of HIV-infected patients (71.8%) had initiated ART with CD4 counts <350 cel/mL in the last 6 years. However, a trend towards an earlier start of ART was observed during 2015, likely influenced by the last treatment guidelines update. These findings highlight the need to promote and facilitate HIV testing to reduce the late diagnosis (<350 CD4) as well as counseling on HIV prevention, treatment and care.

*No conflict of interest*

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**Abstract: P_45**

**Treatment Strategies for HIV/ Hepatitis infected Patients**

**Undetectable - An achievable goal or is persistent viremia still a problem? Results from a large multicenter observational HIV cohort study in Germany (1999-2014)**

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**Background:** Despite of effective antiretroviral therapy (ART), persistent HIV viremia, PHV, (viral load > 50 copies/mL) remains an ongoing issue in HIV treatment. The aim of the study is to evaluate the proportion of observation time with continuous ART documented with PHV in a large observational HIV cohort study in Germany.

**Materials & Methods:** Data from the German ClinSurv HIV cohort from 01.01.1999 until 31.12.2014 were included. The ClinSurv HIV cohort study is a multi centre, open, long-term observational cohort study with 15 participating HIV treatment sites in Germany. Information on clinical status, laboratory results and ART is collected and sent to the RKI biannually. In order to achieve monthly viral load values for each patient we used a 30 day grid model based on consecutive viral load measurements within ≤ 180 days. The first 180 day after treatment initiation and monitoring gaps > 180 days were not considered for analysis. Persistent viremia was defined as two consecutive viral load measurements above>50 copies/ml within 180 days or one viral load measurement above >1000 copies/ml.

**Results:** In total, 24,231 HIV patients (19,231 men and 4,958 women) were included into the analysis. 20,484 (84.5%) of the patients received ART, 3,747 (15.5%) were missing or treatment naïve patients. 1999, 63% (2983 person years, PY) of the observation time in the cohort was covered with information about viral load measurements, VL. 57% (1693 PY) of this time was documented with PHV. 44% (757 PY) of the initial therapy time was recorded with PHV. In comparison in 2014, 80% (8728 PY) of the observed time was documented with VL in the cohort. 15% (1305 PY) of this time showed PHV. 6% (335 PY) of the initial therapy time was recorded with PHV.

**Conclusions:** From 1999 until 2014 the proportion of observation time receiving ART in the ClinSurv HIV cohort documented with PHV decreased. However, in 2014 still 6 % of observation time with initial therapy and 15% of the total observation time receiving ART were documented with remaining PHV and therefore infectious. The reasons for this might be drug resistance, non-adherence or false documentation regarding treatment interruption. These results show that a monitoring of the proportion of patients infected with HIV with persistent viremia is crucial to evaluate effectiveness of ART and to determine the potential infectiousness.

*No conflict of interest*
Abstract: P_46

Viral Evolution & Genetic Diversity

Different HIV-1 Subtypes are Predominant among MSM and PWIDs in Bulgaria

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Introduction: In Bulgaria 1606 cases with HIV/AIDS were diagnosed from 1986 until 2012. Epidemiological data indicated greater heterogeneity of HIV-1 positive population, including recent increase of men who have sex with men (MSM) 269 (16.7%) and people who inject drugs (PWIDs) 385 (24.0%). The aim of the present study was to investigate molecular epidemiological picture of HIV-1 diversity among the most vulnerable transmission groups - MSM and PWID in Bulgaria.

Materials & Methods: HIV-1 diversity was analyzed in 295 individuals with HIV/AIDS of the studied populations, including: 139 (53.1%) of diagnosed MSM with HIV-1 and respectively 156 (40.5%) PWIDs. HIV-1 pol sequences were generated with TruGene and/or ViroSeq Genotyping Systems. The sequence alignment contained Bulgarian sequences and reference sequences from Los Alamos database. HIV-1 subtypes were first classified using REGA v3 and COMET v1.0. Phylogenetic relationships were inferred by ML analysis with FastTree program. Recombinations were analyzed with bootscan analysis using SimPlot software.

Results: Three major different clades: subtype B, CRF02_AG and CRF01_AE were the most prevalent HIV-1 subtypes in the studied populations. The most widely disseminated was subtype B - 42.8%, followed by CRF02_AG - 22.4% and CRF01_AE - 20.3%, all together representing 85.1% of all sequences in the study. In addition, 17 other subtypes, CRFs and unique recombinant forms (URF) were found, including subtype A1, CRF14_BG, CRF03_AB and CRF12_BF. Interestingly, the subtypes were not equally distributed among these patients and at different transmission groups, different HIV-1 subtypes were disseminated. Thus, in MSM subtype B was dominating with 84.3% while CRF01_AE and CRF02_AG were only 2.2% and 1.4% respectively. In contrast, in PWIDs, CRF01_AE and CRF02_AG dominated with 36.5% and 41% respectively, while subtype B was identified in only 5.1% of these individuals. In addition, in MCM significantly less HIV-1 diversity were found while in PWIDs, a number of 17 different HIV-1 subtypes, recombinant and URFs were defined. Moreover, CRF01_AE and CRF02_AG that were found to be the major HIV-1 clades among PWIDs were distributed unevenly in two separate geographic areas among these groups of individuals. Thus, CRF01_AE were dominating in the capital Sofia (74.6%), and CRF02_AG in Plovdiv (82.7%). Phylogenetic analysis revealed multiple clusters demonstrating rapid development of sub-epidemics in different transmission groups and in geographic areas. Moreover, we found that various viral clades in MSM as well as in PWIDs were introduced in Bulgaria from abroad by migrants.

Conclusions: Our analysis revealed that HIV-1 epidemic among MSM and PWIDs in Bulgaria is dominated by three different HIV-1 subtypes unequally introduced and disseminated in these populations. Three local epidemics of MSM in Sofia, and two distinct epidemics of PWIDs in Sofia and Plovdiv were identified. Phylogenetic analysis revealed multiple clusters demonstrating development of local-epidemics in different regions and transmission groups. Significant number of HIV-1 clades were introduced from abroad by migrants. Our findings indicated that providing of detailed molecular epidemiological surveillance of HIV-1 in Bulgaria is of great importance to better understand the epidemic in the country.

No conflict of interest
**Abstract: P_47**

**Viral Evolution & Genetic Diversity**

**Spatiotemporal characteristics of the largest HIV-1 CRF02_AG outbreak in Spain**

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**Introduction/Background:** Our previous analysis on 6,632 HIV-1 sequences sampled during 2000-2014 from Spain (CoRIS and Eastern Andalucia Resistance Cohort), revealed that the CRF02_AG was the predominant clade among the non-Bs with a prevalence of 5.97%. Phylogenetic analysis on 391 CRF02_AG sequences along with all globally sampled CRF02_AG sequences (N=3,302) revealed that the 61.7% of the subtype CRF02_AG sequences sampled from Spain formed 81 monophyletic clusters, with a range of 2 to 79 sequences. Our aim was to estimate the spatiotemporal characteristics of the largest CRF02_AG subepidemic in Spain.

**Materials & Methods:** The largest CRF02_AG monophyletic cluster included 79 sequences sampled during 2003-2013 in Spain. Phylodynamic analysis was performed by a Bayesian method using HKY+G, an uncorrelated lognormal relaxed clock model and a Bayesian skyline non-parametric plot demographic model with 10 groups as implemented in BEAST v1.8.0. The MCMC convergence was checked by estimating the effective samples sizes (ESS>100). Phylogeographic analysis was performed by reconstructing ancestral states using the criterion of parsimony.

**Results:** For 58 out of 79 of clustered sequences (73.4%) the sampling area was from Spain. Phylogenetic analysis revealed the existence of two nested subclades from Japan (N=7, 8.9%) and Sweden (N=3, 3.8%). Molecular clock analysis suggested that the origin (tMRCA) of the CRF02_AG subepidemic was in 1998 (median estimate; 95% HPD: 1991-2002). The Bayesian skyline plot showed that the CRF02_AG epidemic growth occurred during 2006-2013. We found significant clustering within the CRF02_AG subepidemic according to the ethnic origin. Specifically we found four distinct subclades: i) the first sampled from Spain (N=51) including as nested the cluster from Japan (subclade I). The majority of Spanish lineages were from individuals living in Madrid (N=29, 56.9%) reported men having sex with men (MSM; N=37, 72.5%) as transmission risk group. Expect from Madrid, Spanish sequences were isolated across five different provinces from Spain, ii) the second was sampled from Hispanics from Spain (N=2, 33%) and L. America (N=3, 50%) (subclade II), iii) the third consisted of sequences (N=6) originated from Western Europe (Sweden, Switzerland and Germany) (subclade III) and iv) the last (N=4) was from Colombia, Spain and Switzerland (subclade IV). Subclades III and IV were more genetically divergent than the two others.

**Conclusions:** The hot spot for the largest CRF02_AG regional epidemic in Spain was in Madrid associated with MSM risk group. The existence of subepidemics from five different provinces within subclade I, suggest that several spillovers occurred from Madrid to other areas. Notably CRF02_AG sequences from Hispanics clustered in a separate subclade suggesting no linkage between the local and Hispanic subepidemics. Finally the highest divergence of subclades III and IV including sequences outside Spain are highly suggestive that this epidemic founded first outside Spain.

No conflict of interest
Abstract: P_48

Viral Evolution & Genetic Diversity

Molecular analysis of HIV-1 subtype B regional subepidemics in Bulgaria

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Introduction/Background: Previous studies suggested that the HIV-1 epidemic in Bulgaria is complex consisting of several different subtypes and recombinants. Subtype B was the predominant form among men who have sex with men (MSM), while CRF01_AE was the most common non-B clade found at high proportions in women and intravenous drug users (IDUs). The aim of the current study was to estimate the levels of regional clustering for HIV-1 subtype B and to infer the transmission dynamics of regional subepidemics in Bulgaria.

Material & Methods: We studied 124 subtype B sequences available in the protease and reverse transcriptase regions. Sequences were isolated from HIV-1 diagnosed patients during 1989 and 2012 in Bulgaria. Patients reported MSM as transmission risk group. Maximum-likelihood phylogeny reconstruction with bootstrap evaluation was conducted in RAxML, using GTR as nucleotide substitution model and gamma (Γ) distribution of rate variability among sites. Specifically, phylogenetic analysis was performed on the 124 subtype B sequences from Bulgaria along with a random set (N=5,500) of globally sampled subtype B sequences available on Los Alamos HIV database. Phylogenetic analysis was performed in triplicate using a different reference dataset each time. Local transmission networks (LTNs) were monophyletic clusters including sequences from Bulgaria at proportions >75%. Phylodynamic analysis was performed by a Bayesian method using GTR+G, an uncorrelated lognormal relaxed clock model and a birth-death model (BDM) as implemented in BEAST v1.8.0. The MCMC convergence was checked by estimating the effective samples sizes (ESS>100).

Results: Phylogenetic analysis of subtype B sequences revealed several LTNs from Bulgaria (N=14 including 101 sequences), while the three largest included 25 (LTN1), 20 (LTN2), and 12 (LTN3) sequences. The most closely related sequences to LTN2 and LTN3 were from Poland and Caribbean, respectively. For the largest cluster the origin could not be identified in detail. LTNs were consistently found in all three replicates of phylogenetic analysis using different references. Molecular clock analysis revealed that the time of the most recent common ancestor (tMRCA) was in 2008 (95%HPD: 2006-2008) (LTN1), in 2004 (95%HPD: 1999-2005) (LTN2) and in 2009 (95%HPD: 1999-2010) (LTN3). The birth-death skyline suggested a rapid increase in number of infections for the largest LTN, lasting between 2009 and 2010. After then no additional new infections were detected. For the LTN2 the largest increase in number of new infections occurred between 2005 and 2006 and continued slowly after then. For the smallest LTN, a slow increase was observed the years between 2010 and 2013.

Conclusions: Our analysis suggests considerable regional dispersal among MSM in Bulgaria. The largest LTNs originated from different geographic areas. Notably the transmission dynamics and the origin of the three largest LTNs were diverse. The largest regional epidemic showed the most rapid increase that lasted for approximately one year, whilst for the two others epidemic growth occurred over a longer time period. Our results provide important findings about the epidemic dynamics in MSM and can be used to identify ongoing subepidemics.

No conflict of interest
Abstract: P_49

Viral Evolution & Genetic Diversity

Analysis of IDU-A HIV-1 variant tropism and its correlation with clinical and laboratory data

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Introduction: HIV penetration into cells is mediated by binding to the co-receptors CCR5 (R5-viruses), CXCR4 and CCR5 (R5X4 virus-) or CXCR4 (X4-viruses). Change of co-receptors used by the virus in the course of HIV-infection and the appearance of X4-variants in the later stages of the disease is a serious challenge for the use of entry inhibitors (maraviroc, etc.), which are ineffective against non-R5-tropic variants. The purpose of this study was to analyze the prevalence of R5-, X4/ R5- and X4-virused belonging to IDU-A HIV-1 variant in Russia and its correlation with clinical and laboratory data.

Materials and methods: We analyzed the HIV-1 DNA samples from 176 'naive' patients in Russia. 93 (52.8%) were infected due to intravenous drug usage, and 83 (47.2%) - as a result of heterosexual contacts. CD4-lymphocytes count and viral load ranged from 23 to 1450 cells/ml and from <50 to 10,000,000 RNA copies/ml, respectively. Additionally, the study included 318 sequences of the same HIV-1 genome region of Russian patients taken from GenBank (https://www.hiv.lanl.gov). All variants analyzed were obtained during the period from 1996 to 2012. Genotypic tropism determination was carried out using the geno2pheno tool (http://geno2pheno.org), FPR = 10%.

Results: It was found that 18.2% of the samples (32/176) are X4/R5X4-tropic, 81.8% (144/176) were R5-tropic. The presence of X4-viruses correlated negatively with CD4-lymphocytes count (r = -0.21; p <0.01), and positively - with viral load (r = 0.15; p = 0.04). There was no significant correlation between the risk group (r = 0.01; p = 0.78) and the frequency of detection of X4-viruses. The X4-viruses were first reported in 2003 (9.1%). In 2012 its proportion increased 2.7 times and accounted for 25.2% (p < 0.01).

Conclusion: The results demonstrated the link between the prevalence of X4/R5X4-tropic HIV-1 variants and the levels of CD4-lymphocytes and viral load. The viral tropism didn’t correlate with the route of HIV-1 acquisition. At the moment of the study, more than a quarter of patients in Russia, who may be potentially treated by maraviroc, have nonR5-tropic HIV-1 variants, which is consistent with the data obtained in Russia previously (A. Lopatukhin, 2013). The reason of the non-R5-tropic variants proportion increase may reflect the growth of HIV-infection late diagnostics, which has being observed in recent years.

No conflict of interest

Abstract: P_50

Viral Evolution & Genetic Diversity

Phylogenetic analyses highlight the role of transgender sex workers in the complexity of HIV-1 epidemic in Italy

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Background: HIV-1 sexual transmission has driven the epidemic and contributed to the spread of non B-subtypes at global level. Heterosexual males and men having sex with men (MSM) may acquire HIV infection from sex workers. Due to stigma and social exclusion, a high proportion of transgender people engage in sex work. Globally, it is estimated that around 20% of transgender women are living with HIV and are 49 times more likely to acquire HIV.
However, the role of transgender people in local HIV epidemics has been poorly investigated, especially in Europe. We aimed to study the molecular epidemiology of transgender women sex workers (TSW) to evaluate their contribution to the diffusion of new variants in Italy.

**Methods:** We enrolled patients whose gender identity is different to the social expectations and participated in sex work. We analyzed 135 HIV-1 pol sequences collected between 1999 and 2015 at the ‘L. Sacco’ Hospital of Milan. The HIV-1 subtype and recombination patterns were characterized using SimPlot and SplitsTree. References included 232 and 21 sequences from South America and Africa from Los Alamos database and 240 Italian strains of patients residing in the metropolitan area of Milan, equally distributed among risk categories. The phylogenetic analysis was conducted by MrBayes. Fluxes among risk groups and countries were analyzed by MacClade program.

**Results:** The majority of TSW were from South America (98.5%). Ninety eight patients (73%) carried a B subtype; among these, 57 (60%), 19 (20%), 10 (10.5%), 4 (4.2%), were from Brazil, Peru, Venezuela, Paraguay, while the remaining 5 (5.3%) were from Italy, Chile, Argentina and Bolivia. F1 subtype was detected in 15 subjects (11.1%; 10 Brazilian, 3 Paraguayan, 1 Peruvian and 1 Venezuelan). C subtype was present in 7 Brazilians (5.2%). Twelve patients (8.1%) carried BF recombinants (2 CRF12_BF, 1 CRF29_BF and 8 BF unique recombinants). CB unique recombinant, G subtype and CRF02_AG accounted for remaining patient variants. Separate phylogenetic analyses were conducted for B (n=331), F1 (n=188) and C (n=90) subtypes. The B phylogenetic tree showed one large cluster including 246 variants (74.3%), involving all TSW and the majority of Italian and South American references. Viral fluxes were observed from TSW to Italians (37.5%) belonging to all risk categories (33.3% to HE, 11.1% to both MSM and IDU). The F1 phylogenetic tree showed one large cluster including 174 sequences (92.5%), involving all TSW, Italian and South American references. Bidirectional viral fluxes involved TSW and Italians (20% and 40%), HE (13.3%) and MSM (6.7%). The C phylogenetic tree showed 6 small clusters involving TSW and Italian references. No fluxes were observed from TSW to Italians and between risk categories, likely due to the small sample size.

**Conclusions:** Sequence analysis revealed a high proportion of non-B variants carried by about 1/3 of patients and suggests that fluxes from TSW mainly involved Italian HE. Taken together, these data highlights how TSW contributes to the diversity and spread of the HIV-1 epidemic in Italy and strongly advocates that strategic plans for prevention should address transgender people as well as their clients.

No conflict of interest

**Abstract: P_51**

**Viral Evolution & Genetic Diversity**

**Increasing prevalence of non-B subtypes in Poland in the years 2010-2015.**

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**Background:** HIV-1 subtypes may exhibit differences in several properties including: rates of transmission, disease progression, antiretroviral treatment effectiveness, replication capacity, increased susceptibility to antiretroviral resistance due to naturally occurring polymorphisms and accuracy of diagnostic tests. To date, the HIV epidemic in Poland and the rest of the region has been largely dominated by subtype B infections; HIV-1 spread in Poland was strongly isolated from Western Europe's countries due to political limitations. Changes in the subtype patterns could have significant implications on HIV-1 epidemiology. The aim of the study was to assess the prevalence HIV-1 subtype patterns among patients diagnosed in 2010-2015 and to compare with those previously determined.

**Methods:** Plasma samples obtained from 2803 HIV infected patients undergoing routine diagnostics during the 2010 to 2015 years were analyzed using ViroSeq HIV-1 Genotyping Kit. The sequences of pol HIV-1 region were
analysed by ViroSeq software. The subtyping and DR mutation detection approaches were improved by using online tools provided by Stanford University HIV DR Database.

**Results:** As we previously presented, during the year 1999 study 100% of tested isolates in Poland were subtype B. In 2008y study subtype B was detected in 96% cases. In presented analysis we determined further changes in subtype pattern - 202 (89%) cases of subtype B in 2010y and 387 (79,8%) in the 2015 year. In the period 2010-2015 prevalence of A subtype increased from 1.4% to 11.7%; prevalence of CRF01_AE was similar and oscillates around 5% except 2010y, when reached 8.4%. CRF02_AG prevalence changes in range of 0.4%-1% during the entire study. Prevalence of C subtype changed from 0.9% (2010) to 2.9% (2015). The D, F, G, K subtypes were determined in single cases and are present in 1% cases.

**Conclusions:** The most common HIV-1 subtype in Poland in analyzed period was subtype B. Our present results document acceleration in HIV subtype diversity in a Polish population over the past five years and suggest that the prevalence of non-B subtypes will continue to increase disproportionately to B subtypes, with domination of A subtype. The most of the subtypes introduced are typical for neighbor East European countries. The circulation of non-B clades in Poland has been significantly increased in association with migration which is caused by economical reasons as well as by the military conflict on Ukraine territory. Thus, the HIV-1 landscape in Poland may in future be similar to this observed in the East European countries.

*No conflict of interest*
men who have sex with men (MSM) compared to subtype B (82.5% vs. 48.8%, p <0.001). At diagnosis, no differences with regard to Spanish origin (83.3% vs. 83%), AIDS defining illnesses (12.5% vs. 17.5%) or CD4 < 200 cells/µL (33% vs. 34.8%) were observed between subtype F and B infected patients, respectively. Mean HIV-RNA was higher in subtype F (5.32±0.8 vs. 5.01±0.7 log copies/mL, p<0.001). After diagnosis, 93.3% of subtype F and 83.8% of subtype B patients started ART (p=0.02) with no differences in ART regimen used (p=0.99). No differences in immunological restoration (CD4>200 or CD4 >500) were observed between individuals infected with the two different subtypes. However, poor virological response (HIV-RNA <50 copies/mL) was observed for subtype F (60.1% vs. 90% at 48 weeks, p<0.001) in Kaplan-Meier analysis. Subtype F was an independent factor for poor virological response (HR 0.423 (0.308-0.582), p<0.001) after adjusting by age, sex, baseline viral load, CD4 at ART initiation time and ART regimen in multivariate analysis. Importantly phylogenetic analysis confirmed that the majority of subtype F infected individuals were within a single monophyletic cluster.

Conclusions: Prevalence of subtype F is increasing in Northwest Spain. Virological suppression after ART initiation is significantly lower in subtype F patients at 48 weeks (60.1% vs 90%). These findings are relevant as it might hinder the clinical management of these patients, increasing the risk of HIV transmission even under ART and the comorbidities associated with an inadequate control of HIV infection.

No conflict of interest

Abstract: P_53

Viral Evolution & Genetic Diversity

Transmission Patterns of Concurrent HIV-1 Subtype Epidemics Circulating in Cyprus: A Phylodynamic Analysis

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Background: Cyprus has one of the lowest HIV-1 prevalence in Europe. According to ECDC, there were 794 cumulative reported cases in 2012, of which approximately 400 were individuals who reside or used to reside permanently in Cyprus. Since 2011, the number of newly diagnosed individuals has increased. Herein, we determined the prevalence and time trends of subtypes and the factors associated with their onward transmission in Cyprus.

Materials & Methods: 335 consenting study subjects were recruited from the AIDS Clinic of Larnaca General Hospital between 2003 and 2012. The genetic subtypes of the circulating HIV-1 strains were determined by REGAv3, COMETv1.0 and manual phylogenetic analysis. The HIV-1 sequence identified for each study subject was analyzed by BLAST and the 30 best-matched sequences were used as controls for the transmission cluster (TC) analysis. Maximum likelihood trees were constructed by RAxMLv8.2.1. TCs were identified using a genetic distance of <0.045 and a 98% bootstrap support as thresholds. Bayesian phylogenetic analyses (BEAST) were used to confirm TCs and to estimate the timing of specific clades. Statistical analyses were performed by R.
Results: 54% of the study subjects were infected with subtype B, 20% with A1, 9% with C, 6% with CRF02_AG and 6% with F1. There were no significant time trends in the distribution of subtypes in newly diagnosed individuals (n=204). 86% of the study subjects infected with subtype B and 57% of the study subjects with subtype A were Cypriots. However, only 48% of the Cypriot study subjects infected with subtype B and 61% of those infected with subtype A reported to have been infected in Cyprus. There were no significant differences in the distribution of HIV-1 subtypes between the cities of residence within Cyprus. Subtype B strains were more frequent (69%) among men who have sex with men (MSM) whereas non-B subtypes were more frequent (53%) among heterosexuals. TC analysis identified 47 TCs, 20 of which consisted of 3 or more patients. 83% (43/52) of study subjects were in subtype B TCs, compared to only 54% (13/24) in subtype A TCs (p<0.001). Additionally, 14 pairs for subtype B were identified, 6 pairs for A1, 4 for C, 1 for F and 3 for CRF02AG. Within the subgroup of TC ≥3, 13 clusters were subtype B, 4 were subtype A, 1 was subtype C and 1 was CRF02AG. However, 93% of the pairs and all the TC ≥3 originated before 2008. Within the later, 4 clusters originated in 2004 and included a large number of Cypriots (n=12), mainly subtype-B-infected MSM individuals. In univariate analysis, the epidemiological parameters of male gender, MSM and Cypriot origin were significantly associated with subtype B TCs. However, MSM was the only parameter associated with HIV transmission clusters according to the multivariate analysis. However, none of the parameters were significantly associated with transmission of subtype A.

Conclusions: Onward transmission of subtype B in Cyprus is associated with the MSM population, as in other European countries, whereas transmission of subtype A is not associated with any specific epidemiological parameters.

No conflict of interest

Abstract: P_54

Viral Evolution & Genetic Diversity

Dynamics of HIV-1 infections and protective effect of the CCR5 D32 allele within the HIV-1 transmission networks in Poland

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Background: Dated phylogeny allows to trace the dynamics of the HIV infection and model transmission times between the cases. In Poland, in the recent years 14-fold increase in the HIV infection frequency among MSM - from 2.5 new diagnoses per million of inhabitants in 2000 to 33.8 in 2011 and beyond, was observed. Additionally proportion of undiagnosed HIV among MSM was high (68.3%, 95%CI 53.9-76.1%). In this study we aimed to model the times to onward transmission among identified HIV-1 sequence clusters and to analyse the protective effect of the CCR5 D32 allele within these networks

Material and methods: In the dataset of 966 subtype B partial pol sequences from 9 Polish HIV treatment centres 321 clusters were identified by means of the maximum likelihood method. Clusters were defined using a genetic distance of 3% and aLRT values of 0.9. Subsequently, for clustered sequences dated Bayesian MCMC phylogeny under the coalescent constant prior of population growth with GTR nucleotide substitution model was used to reconstruct the time-scaled trees. To reflect the time between transmission events distances between mMRCA and nodes within the clusters of ≥ 3 sequences were calculated. Additionally, we have performed CCR5 D32/wt variant genotyping in 963 (99.7%) cases
Results: Median genetic distances for clusters/pairs were 0.0199 (IQR: 0.013-0.0237) and 0.023 (IQR: 0.0199-0.0276) for clusters ≥3 sequences. Periods between the estimated times of infection for the clusters, reflecting time to onward transmission, ranged from 0.05 to 6.08 years [median 1.38 (IQR: 0.59-2.52) years]. For clusters of ≥5 sequences the internode intervals were significantly shorter compared to the smaller clusters [median 1.1 (IQR: 0.43-1.9) years versus median 2.13 (IQR: 1.1-3.66) years, p=0.0026]. Distribution of the internode distances for the clusters indicated that 22.4% of clustered onward transmissions occurred within the first 6 months, while 37.6% within the first year from infection; Analyzing only MSM clusters, the model suggested that the 23.1% and 35.9% of clustered transmissions occurred within the first 6 months and one year from index case infection, respectively. This indicated that at least 3.9% (7.0% for MSM) of transmissions in the studied group occurred within 6 months and 6.6% (10.9% for MSM) within the first year of infection.

CCR5 D32/wt genotype was found in 146 (15.16%) individuals (no CCR5 D32/D32 homozygotes were observed in the group) and was notably less frequent among cases with clustered sequences (n=36, 11.25%) compared to the unclustered ones (n=110, 17.11%) [OR: 1.6 (95%CI:1.09-2.44), p=0.017].

Conclusions: In the setting of the expanding HIV epidemics in Poland modeled onward transmissions occur recently after infection of the index case, with 50% of clustered transmissions occurring within 16.5 months after infection (25% within 7 months and 75% within 30 months). This time is even shorter for the clusters ≥5 sequences. Presence of the CCR5 D32 allele may be protective in the setting of the clustered transmissions.

No conflict of interest
Abstract

Background: A correct HCV genotype/subtype assignment before treatment initiation is mandatory for the selection of the Direct Acting Antiviral Agents (DAAs) regimen. This study aimed at evaluating the concordance between commercial genotyping assays and HCV sequencing in the subtype/genotype assignment.

Methods: HCV sequencing of NS3-protease and/or NS5A and/or NS5B and/or 5'-UTR was performed by home-made protocols, specific for genotypes and subtypes. Phylogenetic analysis was performed to evaluate appropriate genotype allocation and concordance with previous genotype/subtype assignment.

Results: A total of 1007 HCV-infected patients with a genotypic resistance test pre or post therapy performed between 2011 and 2015 were analyzed to confirm the appropriate genotype allocation. All patients were previously genotyped by different commercial genotyping-assays. According to the genotypic resistant test requests, HCV sequencing was performed on NS3-protease (95.6% of samples), together with/in alternative to NS5A (53.3%) and/or NS5B (37.6%). Furthermore, for 33 patients, sequencing of 5'-UTR was also performed.

HCV sequencing and commercial assays were concordant in 91% of cases analysed. In the remaining 94/1007 (9%), HCV sequencing identified the following discordances: 16/1007 genotypes discordant with the assignment given by commercial assays (commercial/sequencing: 1b/2c [N=3]; 1a/3a [N=2]; 1b/4d [N=3]; and N=1 for 1/c2; 1a/2c; 1b/3a; 2a-2c/1b; 3a/1a; 3b/4d; 4/1b; 4/2c); 28/1007 discordant subtype GT1 cases (commercial/sequencing: 1a/1g [N=3]; 1a/1b [N=7]; 1b/1a [N=18]) (of interest, 5 cases discovered a discordant genotype/subtype, only at the time of performing a resistance test at a DAA regimen failure); 50/1007 patients (4.9%) with a previous result of mixed (N=22) or HCV-1 without subtype information (N=20) or indeterminate (N=7) or unknown (N=1) by commercial assays, were instead precisely resolved by HCV sequencing; in these latter cases, infection was always driven by a single genotype.

As a whole, 94/1007 (9%) patients achieved the correct genotyping assignment thanks to direct sequencing. When more than one genomic-region per patient was analysed (N=581), phylogenetic results were 100% each other concordant in NS3/NS5A/NS5B genes, confirming the specific genotype/subtype assignment. Conversely, the phylogenetic analysis using 5'-UTR sequences showed only 57.5% concordance with NS3/NS5A/NS5B results.

Conclusions: HCV sequencing (in at least one of NS3/NS5A/NS5B genes) allows precise subtype/genotype assignment, along with drug resistance information. Our results emphasize the importance of dedicating time and effort for a proper genotype/subtype assignment before starting therapy. The relatively low cost of sequencing (compared to a potentially wrong therapy) should encourage studies aimed at better defining the advantage of its use at therapy baseline in clinical practice.

No conflict of interest

Abstract: P_56

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Different prevalence of HCV resistance and HCV quantification within blood and liver samples (tumoral and non tumoral tissues) in HCC/ cirrhotic transplanted patients

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Background: HCV-reinfection is a constant event in liver transplant setting, suggesting existence of viral compartmentalization and/or putative HCV reservoirs affecting chances of cure. This study aimed to investigate variability of HCV-RNA, and prevalence of resistance
associated variants (RAVs), in plasma-(PL), hepatic tumoral-(TT), and not tumoral-(NT) tissue samples in patients undergoing liver transplant (LT) or hepatic resection (HR), mainly due to HCC and/or cirrhosis.

**Methods:** 18 HCV-infected patients (5 GT-1a, 8 GT-1b, 4 GT-3a, 1 GT-4d) undergoing LT or HR (N=15; 3) due to HCC without cirrhosis (N=5), HCC/cirrhosis (N=9), cirrhosis without HCC (N=3), or without HCC/cirrhosis (N=1) were analysed. HCV-RNA in all compartments (PL/NT/TT) was quantified by Abbott M2000 RealTime. Sanger-sequencing of NS3/NS5A/NS5B in PL and/or NT/TT was performed in all patients. Ultra-deep pyrosequencing (UDPS, cutoff >1%) of NS3/NS5A/NS5B was also performed in 3 pts. RAVs prevalence was evaluated.

**Results:** At the time of LT/HR, blood HCV-RNA was quantifiable in 17/18 patients (range: 2.6-6.3 LogIU/ml). Interestingly, one patient at LT with undetectable serum HCV-RNA (6 weeks of sofosbuvir+ribavirin pre-treatment), showed a consistent amount of HCV-RNA in the liver, higher in TT than in NT (51 vs 7 IU/µgRNA, respectively). Untreated HCC patients (with/without cirrhosis), differently, presented a trend of lower HCV-RNA in TT (median[IQR]=4.0[1.2-4.3] LogIU/µgRNA) compared to NT (median[IQR]=4.3[3.1-4.9] LogIU/µgRNA; Mann-Whitney test p=0.193).

Sanger-sequencing identified RAVs compartmentalization in 2/18 patients, both non-cirrhotic and GT-1b. Notably, in 1 patient, NS3-S122N/T/S were detected exclusively in PL and NT, both by Sanger-sequencing and UDPS, while NS5A-Y93H was found entirely in liver (TT/NT) by Sanger. UDPS confirmed the Y93H presence in tissues, but also in PL and revealed NS5B-S556G exclusively in TT. In the other patient, Sanger-sequencing detected NS5A-Y93H exclusively in NT. UDPS revealed the Y93H presence in NT and even in PL, but not in TT. Finally, exclusively by UDPS, additional RAVs compartmentalization was observed in 1 cirrhotic GT-1a patient: NS3-V36M in PL/TT, NS3-S122G in PL/NT, NS3-T54A only in NT, NS3-S122N exclusively in TT. All 3 patients analyzed by UDPS showed significant NS3/NS5A/NS5B-sequences genetic-distances (>0.02) between the 3 compartments. Phylogenetic-trees showed well-defined clusters among and within the same compartments, for all genes (more evident for NS5A and NS5B), exclusively in the 2 non-cirrhotic patients. Furthermore, these PL/NT-sequences were often within the same or in phylogenetically close clusters. Differently, in the cirrhotic GT-1a patient, frequent mixed clusters of NT/TT/PL-sequences were observed in all 3 genes, indicating lower levels of compartmentalization.

Finally, Shannon-entropy analysis in NS3/NS5A/NS5B Sanger-sequences identified only NS5A-Y93 as significant variable position (p=0.02), exclusively in NT.

**Conclusions:** NT and TT compartments showed different HCV-RNA amount and genetic variability, also as content of RAVs. In general, PL and NT showed more similar profiles in terms of RAVS and of phylogenetic distance if compared to TT. This scenario was less marked in cirrhotic patients, probably due to the tissue damage. These results, although preliminary, support the hypothesis of possible strain diversifications of HCV within the liver, and can explain why some patients have difficulties to be cured also in the era of new direct antivirals.

No conflict of interest

**Abstract: P_57**

**Novel Diagnostic Technologies & Approaches**

**HCV-RNA Quantification in Liver Biopic Samples of Transplanted Patients by using the ABBOTT m2000 RealTime System: a Real time Quantitative assay for HCV-RNA tissue Testing**

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**Background:** Advanced liver disease caused by hepatitis C virus (HCV) infection is the leading indication for liver transplantation worldwide. During post-transplant follow up, recurrence of hepatitis C is highly frequent, in...
the 95% graft cases. The quantification of HCV-RNA in both serum and liver might provide helpful and more accurate information regarding the HCV burden. We developed a rapid method to quantify the HCV-RNA in tissue, by using the Abbott m2000 RealTime-System.

**Material & Methods:** 18 HCV-infected patients (4 GT1a, 8 GT1b, 5 GT3a, 1 GT2) undergoing orthotopic liver transplantation (OLT) (N=16) or liver resection (LR) (N=2) were analyzed. 4/18 patients were treated with DAA (Direct Acting Antivirals) before OLT. HCV-RNA was quantified in 3-4 sections of the same liver-sample and the mean value was considered for the analysis. 13mg liver-biopic sample was homogenized in BufferRLT+β-Mercaptoethanol by TissueRuptor (QIAGEN) homogenizer. The sample was then used for HCV-RNA quantification by Abbott m2000 RealTime-System. Total RNA and DNA extractions from liver biopsies were performed by AllPrep DNA/RNA/Protein Mini Kit (QIAGEN) to allow normalization of HCV-RNA concentrations expressed in IU/µg of total RNA and IU/10⁶ liver-cells.

**Results:** When HCV-RNA concentration was normalized for IU/µgRNA, Non Tumoral (NT) samples varied from 0 to 5.5, with a median (IQR) value of 3.5 (1-4.6) logIU/µgRNA. In Tumoral Tissue (TT) samples varied from 0 to 5.3, with a median (IQR) value of 2.2 (0.9-4.1). Similar results were obtained when HCV-RNA was expressed in IU/10⁶ cells. Among non-treated patients, the median (IQR) HCV-RNA resulted always higher in NT respecting to TT: logIU/µgRNA=4.3 (3.1-4.9) in NT vs 4.0 (1.2-4.3) in TT (Mann-Whitney, p=0.193); and IU/10⁶ cells =6.4 (5.6-7.1) in NT vs 5.4 (3.8-6.2) in TT (Mann-Whitney, p=0.209). Among the 14 untreated patients, a positive and significant HCV-RNA correlation between serum and NT liver samples was observed (Pearson: ρ= 0.609, p=0.021), but not between serum and TT (ρ= 0.207, p=0.541). Moreover, the same correlation between serum and NT resulted significant in cirrhotic patients (N= 9/14) (ρ= 0.702, p=0.035) but not in non-cirrhotic (N=5/14) (ρ= 0.775, p=0.124). About the 4 DAA-treated patients, at OLT time, 3 were still in treatment, while 1 had completed therapy and showed a sustained-virological-response (SVR) at week-12. Notably, at the moment of OLT, all treated patients had undetectable serum HCV-RNA. However, the 3 patients who were still in treatment had still detectable HCV-RNA in liver tissues. The only patient who had undetectable HCV-RNA in both serum and all liver samples was the SVR patient.

**Conclusions:** The Abbott m2000 RealTime-System can be used to quantify HCV-RNA in liver tissue, providing quick and accurate results. In some cases, even in presence of undetectable serum HCV-RNA, HCV RNA was detectable in the liver. Different quantity of HCV-RNA was observed between NT and TT. Different correlations between serum and non cirrhotic/cirrhotic NT samples were found, probably due to tissue damage. In conclusion, these results suggest that this HCV-RNA quantitative assay can be used as a diagnostic tool for the measurement of the HCV burden in the liver.

**No conflict of interest**

**Abstract: P_58**

**Resistance to Antiviral Drugs (Hepatitis B, Hepatitis C and HIV)**

**Optimizing interferon-free therapy in genotype 1 HCV using NS5A resistance testing: Cost utility analysis from the perspective of the Italian NHS**

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**Background:** Patients with genotype 1 hepatitis C virus (HCV) infection who have either failed to respond to interferon-including antiviral therapy or who relapsed following treatment may well be considered for an interferon-free oral regimen incorporating a non-structural protein 5A (NS5A) inhibitor. Although reported sustained virologic response (SVR) rates with these regimens tend to be >90%, this can be reduced in patients with existing resistance to NS5A inhibitors. Latest EASL treatment guidelines recommend simprevir + sofosbuvir with or without ribavirin (SMV+SOF+RBV) for re-treating patients failing a NS5A inhibitor-containing regimen, as
response to SMV+SOF±RBV is not dependent on baseline NS5A resistance status. An alternative strategy would be to test for baseline NS5A resistance prior to treatment, with therapy choice optimized on the basis of the test results. This study investigates the cost-effectiveness of this strategy.

Materials & methods: An existing lifetime Markov model was used to estimate disease progression for treatment-experienced HCV genotype 1 patients with severe fibrosis or compensated cirrhosis (METAVIR F3/F4). NS5A resistance-testing prior to treatment and subsequent therapy with SMV+SOF±RBV or sofosbuvir + ledipasvir ± ribavirin (SOF+LDV±RBV) in patients with or without NS5A resistance, respectively, was compared to a ‘no testing’ scenario where all patients received SOF+LDV±RBV. Treatment duration in all cases was 12 weeks for F3 patients and 24 weeks for those with F4 disease. SVR data for SOF+LDV±RBV were derived from pooled analyses of phase II and III clinical trials (ION-1, ION-2, ION-3, LONESTAR, ELECTRON, ELECTRON-2, SIRIUS and 337-0113). SVR data for SMV+SOF±RBV were derived from the OPTIMIST-1 study for F3 patients and the COSMOS study for F4. Patient characteristics, HCV progression, mortality, resource utilization, (drug) unit costs and quality of life data were obtained from published sources. The analysis was carried out from the perspective of the Italian National Health Service and both costs and utilities were discounted at 3% per year.

Results: Testing for NS5A and optimizing therapy to SMV+SOF±RBV for patients showing resistance yielded 0.163 additional QALYs and increased costs of €2,789 per patient versus no testing. The incremental cost-effectiveness ratio (ICER) was €17,078/QALY. Deterministic sensitivity analysis identified the SVR attributable to each of the treatment regimens as the most sensitive determinant of ICER with results across a plausible range varying from €10,055/QALY to €43,501/QALY. Probabilistic sensitivity analysis demonstrated that, at a WTP threshold of €30,000/QALY, the probability that NS5A-directed treatment will be cost-effective is estimated at 81.4%.

Conclusions: NS5A resistance testing prior to treatment and subsequent optimizing therapy with SMV+SOF±RBV instead of SOF+LDV±RBV appeared to be cost-effective from the perspective of the Italian National Health Service, in treatment-experienced HCV patients with severe fibrosis or compensated cirrhosis.

Conflict of interest financial relationship(s): K Westerhout, W Bouwmeester, M Pliena and M Treur have received payment from Janssen for health economic consultancy. J Belsey has received payment from Janssen for medical writing. Duchesne, M Pisini, F Damele and B Gueron are employees of Janssen.

Abstract: P_59

Spread of Drug Resistance

Founder effect of NS3 variant Q80K in HCV1a infected patients in Italy


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Background: Italy is characterized by a high HCV burden, with higher prevalence rates in Southern and Insular areas compared to Central and Northern regions. Despite 90-95% success rates reported for all HCV genotypes with direct-acting antivirals (DAAs), eradication can be hampered by the presence of natural resistance-associated variants (RAVs), not only in treatment-naïve patients, but also in cases of retreatment after DAA failure. This study aimed to investigate HCV1a variability within Italy, as...
well as the association of clusters with epidemiological factors and NS3 RAVs.

**Materials and methods:** Between 2011 and 2015, 183 baseline NS3 sequences from plasma samples of HCV1a infected patients were obtained with Sanger sequencing. Individuals were followed in seven Italian regions (Abruzzo, Apulia, Emilia-Romagna, Lazio, Liguria, Lombardy and Sardinia). NS3 HCV1a control strains sequenced worldwide were gathered from Genbank. A total dataset of 1084 sequences was obtained after subtyping, quality control and alignment. Sequences were annotated with RAVs, year and country, and when available with gender, age, therapy status, transmission route and viral co-infection. Phylogenetic reconstruction was performed using RAxML, with GTR model and supported by 1000 bootstrap replicates. Clustering of sequences was identified with Cluster Picker, using 70% bootstrap support as threshold. Phylogenetic and temporal signal were determined with TreePuzzle and Path-O-Gen, after which clusters were confirmed using Bayesian phylogeny with BEAST.

**Results:** Median age of the 183 HCV1a positive patients was 55 years (IQR: 50-66), with the majority being male (80.9%) and DAA naïve (89.1%). Transmission route was available for 61/183 patients (33%) and only 12/183 patients (7%) were co-infected with HIV. Based on a maximum-likelihood and Bayesian phylogenetic tree, we identified two clusters, which included 3 patients from our cohort in total. The first cluster consisted of two DAA-naïve HCV mono-infected patients from Abruzzo (PP = 1). Both patients were male with one infected through intravenous drug use. For the second cluster (PP > 0.97), an HIV/HCV co-infected patient from Lazio clustered with an Italian control sequence, with for the latter lacking information for viral co-infections. Both sequences were derived from DAA-naïve patients with unknown transmission route. Remarkably, both clusters included patients all harbouring RAV Q80K. In depth-analysis showed a dispersion of the Italian sequences across the whole phylogenetic tree and a distinction of the tree into two major clades, with one consisting of all, except for one, sequences harbouring variant Q80K (n=96). Due to low support for the two separate clades, downsampling to 20% of the tree was performed multiple times, and this confirmed each time the existence of a founder effect for RAV Q80K.

**Conclusions:** Phylogeny of HCV1a NS3 sequences identified two clusters among DAA-naïve Italian patients each harbouring RAV Q80K. All HCV1a sequences could be assigned to one of two major clusters, one with and one without Q80K. This founder effect of Q80K has implications for treatment of HCV1a cirrhotic patients with simeprevir and sofosbuvir, since Q80K is associated with lower success rates. Phylogenetic cluster analysis may aid in predicting treatment response and assessing viral evolution under DAA selective pressure.

**No conflict of interest**

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**Abstract: P_60**

**Viral Evolution & Genetic Diversity**

**Evolution and determinants of prevalence of HCV infection and HCV genotype among HIV-infected patients between 1997-2015: data from cohort ICONA**

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**Background:** The evolution of HCV prevalence and of the HCV genotype distribution among HIV-infected patients in Italy is not known. Aim of this study is to analyze the variation of HCV prevalence and genotype distribution among persons living with HIV entering in care between 1997-2015 and their determinants.

**Materials & Methods:** All HIV-infected patients were analyzed who were tested for HCV-Ab in
ICONA, an Italian nation-wide cohort enrolling HIV+ pts naive for antiretrovirals, prospectively followed in 42 Italian centers. We analyzed HCV-Ab and HCV genotype prevalence over calendar period of enrollment and tested their association with epidemiological and demographic factors. Correlates of HCV-Ab prevalence and HCV genotype were tested by logistic regression.

Results: Of 12,135 HIV-infected patients, 3,407 (28%) were HCV-Ab+; 75% males, median age 36 years (IQR 31-43), median time from HIV diagnosis to enrollment 1 year (0-4), 15% had AIDS, 23% had a history of IDU, 6% were HBsAg+, 85% Italian-born, 54% were followed in Northern, 32% Central and 14% Southern Italy. Among 1,359 with known HCV genotype, 49.2% had genotype 1 (32.0% 1a, 12.7% 1b, 4.5% not specified), 35.9% 3, 10.7% 4, 3.4% 2 and 0.8% mixed. The prevalence of HCV infection decreased from 49.2% during 1997-2002 to 10.2% during 2009-2015. Meanwhile, the relative frequency of genotype 1 increased from 45.8% to 59.3%, while genotype 3 decreased from 38.5% to 27%. Independent predictors of HCV-Ab+ status were being female, IDU, cared in Northern Italy, Italian and enrolled in less recent calendar years. Factors independently associated with being infected with HCV genotype 1a were age (+10 years adjusted OR, AOR, 0.70, 95% CI 0.58-0.84), risk factor (being MSM vs IDU AOR 1.89, 1.21-2.94), geographic location (Central vs Northern Italy AOR 0.74, 0.55-1.00), calendar period (2009-2015 vs 1997-2002, AOR 2.33, 1.68-3.23). Factors associated with genotype 3 infection were risk factor (MSM vs IDU, AOR 0.45, 0.28-0.72) and calendar period (2009-2015 vs 1997-2002, AOR 0.67, 0.50-0.90).

Conclusions: Prevalence of HCV infection is significantly declining during more recent calendar years in HIV-infected patients in Italy, independently of risk factors, and is associated with female sex. Relative frequency of genotype 1a is increasing, and is associated with younger age and being MSM. Data on females and on MSM seem to suggest that sexual transmission is becoming the main driver of HCV epidemics. The abstract has been accepted as poster at EASL Conference, 13-17 April 2016, Barcelona, Spain and at IWHOD’S 20th international workshop on HIV observational databases in Budapest (Hungary) 7 - 9 April 2016.

No conflict of interest

Abstract: P_61

Viral Evolution & Genetic Diversity

A Phylogeographic Analysis of HCV Viral Sequences from the General Population and High-Risk Groups Including Intravenous Drug Users Attending Therapy Programs in Cyprus

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Background: HCV has been phylogenetically classified into 7 major genotypes (clades) and several subtypes (subclades). The distribution of HCV genotypes and subtypes differs according to the geographic origin and transmission risk group. Our previous molecular epidemiology studies suggested that among the Cypriot subjects, the predominant subtypes were 1b and 3a followed by 1a and 4a. In this study, we investigated the origin of HCV infection and determined HCV genotype-specific transmission patterns in Cyprus.

Methods: We analyzed HCV sequences encoding partial NS5B regions (N=21) from a prospective molecular epidemiology study (2011-2012) of intravenous drug-users (IVDUs) attending therapy in Cyprus and HCV sequences (N=89) from the general population and high-risk cohorts, from our previously published data. We assembled three global datasets for subtypes 1a, 1b and 3a, by including sequences from Cyprus and all HCV sequences available from online database repositories (Los Alamos HCV sequence database). Maximum-likelihood phylogeny reconstruction with bootstrap evaluation was conducted in RAxML, using GTR as nucleotide substitution model and gamma (Γ) distribution of rate variability among sites. The geographic origin of infections (viral mobility events) was implied from viral phylogenies by character reconstruction using parsimony. We also identified statistically significant viral dispersal
pathways between geographic regions from bootstrap trees estimated by ML.

**Results:** Phylogeographic analysis revealed that the geographic origin of subtype 1a infection in Cyprus originated from Switzerland, South East Asia, Australia and New Zealand. Interestingly United States of America (USA) was not revealed as a significant source for 1a epidemic in Cyprus. HCV subtype 3a, which was predominant among IVDUs, originated from Switzerland, France Greece, United Kingdom and Australia. The sources of origin of HCV subtype 1b, which was predominant in the general population, were Greece, Italy, the Netherlands, Russia, Spain, Switzerland, Australia and South East Asia. Notably, phylogenetic analysis using a globally sampled subtype 3a dataset revealed that all HCV sequences from incarcerated individuals (seven out of nine, 78%) fell within three monophyletic clusters including sequences from the general population and from IVDUs, forming local transmission networks (LTNs). Notably three sequences from the incarcerated individuals within the LTNs, were almost identical suggesting common source of infection. For subtype 1b two out of five sequences from the incarcerated individuals clustered with Cypriot isolates suggesting that the putative origin of infection was from Cyprus.

**Conclusions:** Our analysis suggests that the HCV epidemic in Cyprus originated from multiple sources. Although HCV subtypes 1a and 1b predominate in the USA, the major sources of these subtypes in Cyprus were Europe, South East Asia, Australia and New Zealand. HCV subtype 3a which is predominantly associated with IVDUs, originates from Switzerland, France Greece, United Kingdom and Australia and should be more effectively targeted by prevention policies.

*No conflict of interest*

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**Abstract: P_62**

**Treatment Strategies for HIV/ Hepatitis infected Patients**

**Molecular characterization of HIV-1 in HBV ± HDV ± HCV coinfected HIV-1 positive patients in Turkey**

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**Introduction:** Co-infection with either HBV ± HDV ± HCV in HIV-1 positive patients is not very common, but possible since all these viruses share transmission routes and geographical distribution. Interaction between these viruses generally amplifies liver damage, increasing the risk of developing end-stage liver disease and hepatocellular carcinoma. HIV/HCV co-infection is associated with poorer response to antiviral therapy. The objectives of this study were to determine the subtypes and the primary ART resistance mutations of HIV-1 in HBV ± HDV ± HCV co-infected HIV-1 positive Turkish patients.

**Material and Methods:** We have 106 co-infections from 35 infectious diseases clinics from 15 different provience of Turkey [ Gender, M/F n; 93/13, Age, median years (range); 38 (16 - 68), CD4+ T-cell count, median mm3 (range); 364 (3 - 1659), HIV-RNA load, median IU/ml (range); 7.38+E5 (6.4+E2 – 11.2+E6), HIV acquisition route, n (%); heterosexual contact; 58 (54.7), MSM; 36 (34), Bisexual contact; 4 (3.8), Injection drug use; 5 (4.7), Blood transfusion; 1 (0.9) and Medical/dental surgery; 2 (1.9), Co-infection status, n (%); HIV-1 + HBV;
Abstract

79 (74.5), HIV-1 + HBV + HDV; 3 (2.8), HIV-1 + HBV + HDV + HCV; 2 (1.9), HIV-1 + HCV; 22 (20.8)]. HIV-1 subtypes and CRFs were identified by phylogenetic analysis (neighbor-joining method) via sequencing of HIV-1 pol gene (CLC Sequence Viewer v7.5, Qiagen Aarhus A/S, Denmark). HIV-1 ART resistance mutations were analyzed according to criteria by the WHO 2009 list of surveillance drug resistance mutations.

Results: The molecular evidence in this study indicates subtype B (69%) and CRFs (23%) of HIV-1 are most prevalent subtypes. CRFs of HIV-1, that are described in HBV ± HDV ± HCV co-infected patients mainly caused from South-East Asia, East Asia and Central Africa (CRF 01_AE), West Africa, Central Africa and Middle East/North Africa (MENA) (CRF 02_AG), South America (CRF 12_BF) and Spain (CRF 14_BG), respectively. HIV-1 ART resistance mutations were detected in 20% and 20% HBV ± HDV ± HCV co-infected patients in HIV-1 positive Turkish patients, respectively. However, genotype D/subtype D1 (98%) in HBV and type 1b (93%) in HCV infected patients were predominant genotype.

Conclusions: HIV-1 molecular epidemiology studies in the HBV ± HDV ± HCV co-infected patients are important tools for tracking transmission patterns and the spread of CRF and monitoring of CRF subtypes of HIV-1 in globally scale may be important in vaccine development against HIV. However, the high prevalence of HIV-1 ART resistance mutations in such as patients suggested that the resistance testing must be an integral part of the management of HIV-1 infection and the choice of first-line therapy regime should be guided by the results of genotypic resistance in Turkey.

No conflict of interest

Abstract: P_63

Clinical case

Treatment of Patients with Hepatitis B or C May Reactivate Suppressed Hepatitis B or C Coinfection Risking Decompensation

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Hepatitis B and C coinfection is common because of similar modes of transmission. Usually, there is a dominant virus with the other coinfected virus remaining undetectable. We describe 3 patients who had reactivation of the suppressed virus after treatment for the active virus. In the first case, a 45-year-old man with HCV and HBV coinfection presented for treatment of HCV. Initially, his HCV PCR was 600,000 IU/mL and he had an undetectable HBV DNA. He was started on ledipasvir and sofosbuvir for 12 weeks. After 4 weeks, his HCV viral load became undetectable; however, his HBV DNA increased to 1939 IU/mL. At 12 weeks, the HCV viral load remained undetectable, and the HBV DNA was fortunately undetectable without requiring HBV treatment. In the second case, a 37-year-old man also with coinfection of HCV and HBV presented for treatment of HCV. Initially, his HCV PCR was 600,000 IU/mL and he had an undetectable HBV DNA. He was started on ledipasvir and sofosbuvir for 12 weeks. After 4 weeks, his HCV viral load became undetectable; however, his HBV DNA increased to 1939 IU/mL. At 12 weeks, the HCV viral load remained undetectable, and the HBV DNA was also fortunately undetectable, without requiring HBV treatment. In the second case, a 37-year-old man also with coinfection of HCV and HBV presented for treatment of HBV with tenofovir. This patient had acute HBV suppressing chronic HCV. His HBV viral load trended down over 16 weeks of treatment; however, HCV RNA which was previously undetectable, became positive just as HBV DNA was decreasing to undetectable levels at 12 weeks. At 16 weeks, HBV DNA was trending towards being undetectable, and the HCV RNA was fortunately undetectable without requiring HCV treatment. In the third case, a 21-year-old man with coinfection of HCV and HBV presented for treatment of HBV with tenofovir. His HBV DNA trended down over 32 weeks of treatment; however, HCV RNA which was previously undetectable, became positive just as HBV DNA was decreasing to undetectable levels at 32 weeks. He was having worsening liver enzymes which initially prompted testing for HCV viral load, and now may need initiation of

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treatment for HCV in the near future. New direct-acting antiviral-based therapy (DAAs) without the need for interferon are showing high treatment success with less toxicity and are becoming widely used. However, our cases show that patients with dual infection treated with DAAs for HCV can potentially reactivate HBV given the lack of anti-HBV activity with the new DAA regimens. Treatment of HBV can also result in reactivation of HCV. There are no current guidelines regarding monitoring of the reactivation of non-dominant virus in these patients undergoing treatment. Our patients fortunately have not yet required treatment of their reactivated virus, but patients with other medical comorbidities and cirrhosis may need early initiation of treatment to prevent acute decompensation. Close follow-up laboratory studies for viral reactivation and liver function may be a necessary addition to current guidelines as detecting a second active hepatitis virus can prevent hepatic decompensation by allowing for the timely initiation of another antiviral agent.


No conflict of interest

Abstract:

Viral Evolution & Genetic Diversity

HBV genetic evolution in HBsAg can constrain HDV replicative potential


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Introduction/Background: HDV genome encodes only HDV antigen (HDAG) that is present in viral capsid. This antigen interacts with the envelope glycoprotein (HBsAg) encoded by HBV genome, thus allowing HDV assembly and release. Although the interaction between HBsAg and HDAG is known to be critical for HDV morphogenesis, mechanisms underlying this process are not well defined. Here, we characterize the extent of genetic variability in HBsAg and HDAG in the setting of HBV+HDV infection.

Methods: Among 78 patients with chronic HBV+HDV infection, HBsAg genotype-D sequences (aa:1-226) are obtained for 31 patients and HDAG genotype-1 sequences (aa:1-214) for 47 patients. The 31 patients with HBsAg sequences are matched (1:2) with 62 HBV mono-infected patients according to sex, nationality, age (+5 years), HBV-DNA (+0.5 log
IU/ml), and anti-HBV drug received (for drug-treated patients).

Shannon Entropy is used to measure the extent of amino acid variability at each HBsAg and HDAg position. For HBsAg, Shannon Entropy is calculated in the group of HBV+HDV infected and HBV mono-infected patients, and stratifying HBV+HDV infected patients according to serum HDV-RNA (14 patients with HDV-RNA <3.5 log IU/ml and 17 with HDV-RNA >3.5 log IU/ml). Positions with Shannon Entropy = 0 are defined conserved.

**Results:** In HBV+HDV infected patients, median (IQR) HBV-DNA is 3.0 (1.8-3.4) log IU/ml and median (IQR) HDV-RNA is 3.7 (2.0-6.1) log IU/ml. By Shannon Entropy, the number of conserved positions in HBsAg is significantly higher in HBV+HDV infected patients than in HBV mono-infected patients (69.6% vs 52.6%, P=0.001). In particular, 19 positions in the major hydrophilic region and 12 positions in C-terminus are conserved only in HBV+HDV infected patients. Some of them (171/196/197/219), residing in C-terminus, are known to be critical for HDV assembly.

By stratifying patients according to serum HDV-RNA, mutations at positions 204 and 206 in HBsAg C-terminus are detected only in patients with log HDV-RNA >3.5 IU/ml (position 204: 5/14 vs 0/17, P=0.007; position 206: 3/14 vs 0/17, P=0.045). This suggests that mutations at these positions may affect HDV replicative potential.

In HDAg, a higher number of conserved positions is detected in the viral-assembly signal (VAS), known to interact with HBsAg and critical for HDV morphogenesis, than in other HDAg domains including nuclear localization signal (NLS), RNA-binding domains (RBD) and multimerization domain (MD) (70% [VAS] vs 52.4% [NLS], 31.3% [RBD] and 27.3% [MD], P=0.004).

**Conclusions:** HBV+HDV coinfected patients experience a higher degree of HBsAg conservation than HBV mono-infection. This suggests that the extent of genetic variability in HBsAg, mainly clustered in HBsAg C-terminus, can hamper HDV replicative potential. Conserved HBsAg and HDAg regions may pose the basis for design of innovative antiviral targets.

**No conflict of interest**
are compared in the two groups of patients by Mann-Whitney test. For each RT/HBsAg positions, dN/dS is calculated by PAML to estimate if a position is under positive selection (dN/dS>1.0) at a confidence level probability >0.97.

**Results:** Patients with HBV-R have comparable HBV-DNA and lower ALT than patients with AHB (median [IQR]: 5.9 [4.6-7.6] vs 6.0 [5.3-7.6] log IU/ml and 193 [48-568] vs 1777 [1292-2617] IU/L, p<0.001, respectively). By in silico prediction, the mean number of mutations in HBsAg HLA Class I and II epitopes is significantly higher in patients with HBV-R than those with AHB (mean±SD: 5.0±1.0 vs 0.8±1.0, p<0.0001 in class I and 7.4±1.9 vs 1.7±1.0, p<0.0001 in class II epitopes). In RT, the density of mutations within HLA class I and II epitopes is lower compared to that observed in HBsAg, with a significantly higher number of mutations in HBV-reactivated patients than those with AHB (mean±SD: 3.3±1.6 vs 1.2±1.1, p<0.0001 in class I and 4.7±1.6 vs 2.2±0.9, p<0.0001 in class II epitopes). By dN/dS analysis, 7 specific positions residing in HBsAg epitopes are under positive selection only in patients with HBV-R (Q30-Y100-L109-S114-G130-E164-P217). Among them, 3 positions (Y100-L109-P217) are located in epitopes presented by both HLA class I and II alleles, suggesting that in the setting of an altered immune-response, mutations at these positions could hamper HBsAg recognition by Th1-Th2 mediated immunity, thus sustaining HBV-R. Conversely, in RT, no positive selection at specific positions located within predicted RT class I and II epitopes is found among patients with HBV-R, endorsing the leading role of HBsAg (more than RT) mutations in driving HBV-R.

**Conclusions:** A high density of mutations specifically clustered within class I and II HLA HBsAg epitopes characterizes the patients with HBV-reactivation. This suggests that the weakening of immune system may favor the emergence of viral variants endowed with an altered class I and II HLA epitope recognition, supporting the role of immune-escape in HBV reactivation driven by iatrogenic immune-suppression.

No conflict of interest

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**Abstract:** P_66

**Clinical case**

**Loss of HBsAg in HIV/HBV Coinfected Patient with High HBV DNA Treated with Pegylated Interferon : A Case Report**

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**Background:** Worldwide, approximately 4 million people are HIV/HBV coinfected. The prevalence of chronic hepatitis B (CHB) infection in individuals with HIV varies between 5 and 20% in different parts of the world. Coinfection with HBV and HIV is accompanied by an increased risk for liver related morbidity and mortality compared with mono-infection if left untreated. Most of the guidelines advocate starting highly active antiretroviral therapy (HAART) including nucleos(t)ide as part of it if the patients meet the criteria for CHB treatment even in cases with high CD4 count. Pegylated interferon alfa treatment is favored in patients with CHB infection genotype A, low HBV DNA and high ALT levels.

**Case report:** We report the case of a 25-year-old homosexual man presenting with positive HIV-1 serology at first observation in March 2011. The laboratory tests showed HIV RNA 88.000 copy/ml, CD4 count 770/mm³, elevated liver enzymes with alanine aminotransferase (ALT) at 90 IU/ml and aspartate aminotransferase (AST) at 60 IU/ml. He was positive for HBsAg, HBeAg, anti-HBc and negative for anti-HBe, anti-HBs, anti-HDV. HBV DNA was 500.000.000 IU/ml. Anti-HAV total was positive and anti-HCV negative. The patient was followed for 96 weeks and didn't receive any HAART. The HBV DNA level reached 1.500.000.000 IU/ml. Liver biopsy was performed and it revealed Ishak fibrosis score:2 with histology activity index:7. HIV RNA level was 76.000 copy/ml and CD4 count 730/mm³. Pegylated interferon alfa 2a 180 mcg weekly was initiated for 48 weeks. At week 4, 12 and 24 HBV DNA levels dropped by 0.5,1 and 3 logs
respectively. At week 48 HBV DNA level was below the detectable level 20 IU/ml with HBsAg loss without any seroconversion. ALT was 23 IU/ml and AST 27. The HIV RNA was 883 copy/ml. Twenty six months after the end of the treatment the patient still had undetectable HBV DNA, negative HBsAg, HBeAg and normal transaminases.

Discussion: The lower probability of spontaneous loss of HBeAg and HBsAg in HIV/HBV coinfected patients is due to impaired host innate and adaptive immunity. In this patient who had high CD4 counts a sustained virological response was achieved. These findings suggest that pegylated interferons might be a good choice especially in HIV/HBV coinfected very slow progressors, elite controllers and in patients not suitable for antiretroviral therapy.

No conflict of interest

Abstract: P_67

Clinical Implications of Antiviral Drug Resistance (Hepatitis B, Hepatitis C and HIV)

Resistance in PBMCs and insufficient previous suppression predict virological rebound after therapy switch in patients with undetectable HIV-RNA

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Background: The clinical relevance of resistance detected in peripheral blood mononuclear cell (PBMC) compartment is still debated. Thus, we evaluated the impact of baseline resistance detected in PBMC genotypic-resistance-test (GRT) on maintaining virological suppression after therapy switching in combined-antiretroviral-therapy (cART) treated patients with undetectable HIV-RNA.

Materials & Methods: cART-experienced patients switching therapy with a GRT from PBMCs available before therapy change (median [Interquartile-range,IQR]: 1[1-4] months) were included. The prevalence of resistance was evaluated using IAS/Stanford resistance lists, and genotypic-susceptibility-score (GSS) was calculated according to HIVdb ver.7.1 algorithm. Survival analysis was used to assess probability of virological rebound (VR: two consecutive viremia >50 copies/mL or one viremia >1000 copies/mL after switching) according to baseline PBMC GSS. Multivariable Cox-regression was performed according to age, gender, CD4 nadir, virological suppression duration before switching, number of previous viral blips (transient HIV-1 RNA ≤1000 copies/mL before switching), previous treatments, number and type of antiretrovirals administered at switch.

Results: Overall, 136 cART-treated patients, with virological suppression lasting from a median (IQR) of 3.8 (1.1-6.9) years before switching were analyzed. During this period, 29.4% of patients showed at least one viral blip. Patients were on ART since a median (IQR) time of 10 (4-16) years, with a CD4 nadir of 164 (66-300) cells/mm3. Patients had a median (IQR) number of previous regimens of 5 (2-8); of them, 50.6% experienced ≥3 antiretroviral classes. 3.7% and 29.4% of patients switched to darunavir-monotherapy and dual therapy, respectively. At baseline, 47.8% of patients showed at least 1 resistance mutation (PI:16.2%; NRTI:33.1%; NNRTI: 16.9%); 83.8%, 14.0% and 2.2% of patients showed fully susceptible, intermediate resistant and fully resistant GSS, respectively.

Twenty-four months after therapy switching, the overall probability of VR was 18%. Patients showing intermediate or full resistance to at least one drug used after PBMC GRT had a higher probability of experiencing VR compared to those carrying a fully susceptible virus (31.1% vs. 14.8%, p=0.017). A higher probability of VR was found in patients having ≤1 year of suppressed viremia before switching compared to those with a longer time of undetectability (≤1 years: 40.7%; unknown time:
By Multivariable Cox-regression, a higher adjusted hazard (AHR) of experiencing VR was observed in patients with intermediate or full resistant GSS compared to those with fully susceptible GSS (AHR: 2.0, 95% CI:1.1-3.93, p=0.033), in patients with ≤1 year of previous undetectable HIV-RNA (AHR: 20.7, 95% CI: 4.2-101.2, p<0.001) and in patients showing ≥1 previous viral blip (AHR: 11.0 95%, CI: 2.2- 55.1, p=0.004).

Conclusions: In clinical practice, treatment switch with long-term undetectable HIV-RNA warrants a high rate of maintaining virological suppression. However, patients with GSS intermediate or fully resistant in PBMCs and with a short/intermittent previous virological control have a higher risk of rebound after therapy switching. In patients under virological suppression, PBMC genotyping might be a useful tool for tailoring ART switch.

No conflict of interest
regardless therapy change (ITT approach), of: i) VS (first viremia <50 copies/mL from therapy starting); ii) IR (a CD4 cell count gain from therapy initiation ≥150 cells/mm³). The following variables were evaluated as potential confounders: gender, age, subtype, year of therapy starting, treatment, pre-therapy plasma HIV-1 RNA and CD4 cell count.

**Results:** Patients were mainly male (350, 79%), with a median (interquartile range, IQR) age of 41 (34-48) years. INIs were used in 67 patients (15.1%), and their usage significant increased by increasing pre-therapy viremia ranges (500,001-1,000,000: 10.1%; >1,000,000: 22.7%; p=0.001). Remaining patients were treated with protease inhibitors (PIs) (263, 59.4%) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) (113, 25.5%). On the overall population, the median time [95% Confidence Interval, CI] of achieving VS was 7.3 [6.6-7.9] months and the probability of VS by 12 months of therapy was 76%. Patients receiving PIs showed the longest median time to achieving VS (median [95%CI] months, PIs: 8.2 [7.4-9.2]; NNRTIs: 6.3 [5.5-7.6]; INIs: 5.7 [4.7-7.7]), and the lowest probability of VS at 12 months (PIs: 73%; INIs: 77%; NNRTIs: 81%) (p<0.0001). By multivariable Cox regression, a higher adjusted hazard ratio (AHR) of VS was associated with INI- and NNRTI-usage compared to PI-usage (AHR [95%CI], INIs: 1.43 [1.06-1.94], p=0.021; NNRTIs: 1.37 [1.07-1.77], p=0.013), pre-therapy CD4 cell count >200 cells/mm³ compared to CD4 cell count ≤200 cells/mm³ (AHR [95%CI]: 1.34 [1.07-1.69], p=0.012), and a more recent year of starting therapy (AHR [95%CI] per 1 year increase: 1.11 [1.04-1.19], p=0.003).

By 12 months of therapy, the probability of IR was 76%, and was similar in patients treated with NNRTIs (74%), INIs (75%) or PIs (76%) (p=0.813). Cox multivariable regression confirmed that no significant differences in the AHR of IR were found in INI- and NNRTI-usage compared to PI-usage (AHR [95%CI], INIs: 1.04 [0.75-1.45], p=0.820; NNRTIs: 0.91 [0.70-1.18], p=0.469).

**Conclusions:** In naïve HIV-1 infected patients with plasma HIV-1 RNA >500,000 copies/mL starting a first-line regimen containing INIs or NNRTIs yielded a faster reduction of viremia compared to PIs; conversely, the immune recovery was not affected by the class of antiretrovirals used. In about 25% of patients the virus was still detectable at 12 months, and this suggests the need of personalized regimens for naïve patients with a very high pre-therapy viremia to improve virologic success.

No conflict of interest

**Abstract: P_69**

**Novel Diagnostic Technologies & Approaches**

**geno2pheno[coreceptor-hiv2]: a computational tool for the prediction of HIV-2 coreceptor usage**

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The drug maraviroc is a coreceptor antagonist that prevents the entry of HIV into CD4+ cells by blocking the CCR5-coreceptor. Before initiating treatment with maraviroc, viral coreceptor usage should be determined to ensure that the viral population of a patient uses only the CCR5-coreceptor (R5-tropic) and cannot evade the drug by using the CXCR4 coreceptor (X4-capable). Although maraviroc can also be a treatment option for individuals infected with HIV-2, no online tool for the genotypic identification of HIV-2 coreceptor usage was available until now. Therefore, we developed the geno2pheno[coreceptor-hiv2] web service that predicts HIV-2 coreceptor usage from the V3 loop.

geno2pheno[coreceptor-hiv2] is based on a support vector machine (SVM), which was trained on a data set of 73 R5 and 52 X4-capable V3 loop sequences with known phenotypic coreceptor usage. For training the models, we considered multiple kernel functions, such as the linear, polynomial, RBF, and edit kernel. To select a model, predictive performance was evaluated using 10 runs of 10-fold cross validation and the final classifier...
performance was evaluated using 10-fold nested cross validation.

The predictive performance of SVMs with different kernel functions were similar. Still, a linear SVM achieved the largest mean area under the ROC curve (0.95) during the 10 cross-validation runs. During nested cross validation, the SVMs achieved a sensitivity of 86.5% and a specificity of 96.5%, which is a predictive performance not significantly different from that of the rules-based approach developed by Visseaux et al. [1] (McNemar's test: p-value 0.617). The model not only reproduced the previously established major markers of CXCR4-usage, but also identified novel markers of X4-capability. For example, insertions at position 22 of the V3 loop were 100% specific for X4-capable viruses and the mutation Q27K was significantly associated with X4-capability (Fisher's exact test: p-value 0.0004). An easily interpretable visualization of the weights associated with an input sequence is available on the website of the tool at http://coreceptor-hiv2.geno2pheno.org.

In this study, we developed the first online tool for the prediction of coreceptor usage for HIV-2. Treatment options for HIV-2 are limited compared to HIV-1 and this tool can aid physicians in deciding whether or not to prescribe coreceptor antagonists to HIV-2-infected patients and also allows for broader epidemiological studies on HIV-2 coreceptor usage. By evaluating the weights of the SVM, it was possible to determine the impact of certain V3 loop mutations on viral coreceptor usage. This enabled us to confirm previously established markers of X4-capability, but also to determine novel features associated with CXCR4-usage.


No conflict of interest

Abstract: P_70

Novel Diagnostic Technologies & Approaches

Added value of HIV-1 DNA load analysis in routine patient monitoring

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Introduction/Background: Currently the majority of HIV+ patients are successfully treated, resulting in sustained undetectable viral load. Determination of the total HIV-1 DNA load may be an interesting supplementary assay for long term follow-up of treated patients. We investigated the possibility to perform HIV-1 DNA load analysis on the stored buffy coat fraction from samples collected for viral load determination and considered the added value of this marker for routine monitoring of patients on ART.

Material & Methods: HIV-1 DNA load was determined on the last available buffy coat sample of 249 patients on ART with an in-house qPCR amplifying part of the HIV-1 LTR region using primers, a hydrolysis probe and the QuantiTect multiplex PCR kit (Qiagen). The amplification was run on a LightCycler 480 (Roche). The HIV-1 DNA copies were normalized to the white blood cell number by simultaneous amplification of the human albumin gene. 8E5 cells, containing one copy of the HIV genome, were used as standard. Based on the results of the qPCR, patients were classified as high DNA load (> 150 copies/10^6 cells) or low DNA load (≤ 150 copies/10^6 cells) and both groups were compared for sex, age, infection route, origin, CD4+ cell count nadir, peak VL, viral load rebound, duration of known infection, duration of ART and duration of present viral suppression. Additionally, for 4 patients, evolution of the HIV-1 DNA load was followed over time by analyzing 7 to 14 longitudinal samples collected over a period of 45 to 69 months. χ² and Mann-Whitney U were used for statistical analysis.

Results: The mean HIV-1 DNA load for the 249 cross sectional samples was 219 HIV-1 DNA copies/10^6 cells (IQR: 69 – 252 copies/10^6 cells;
Abstract

126 had a high HIV-1 DNA load (> 150 copies/10⁶ cells). A CD4 cell count nadir below 200 cells/µl (p = 0.011) and a peak pretreatment viral load of > 5 log₁₀ copies/ml (p = 0.034) were the only characteristics significantly associated with higher HIV-1 DNA load. In the 4 patients followed longitudinally, a reduction in HIV-1 DNA load was observed after therapy initiation, but the magnitude of the decline was low (0.67, 0.85, 0.89 and 0.99 log₁₀ over a period of 29, 15, 16 and 23 months).

Conclusions: HIV-1 DNA load determination can be performed on the stored buffy coat fraction of samples collected for viral load analysis. In cross sectional samples from patients on ART the total HIV-1 DNA load is primarily associated with the pre-treatment CD4⁺ cell count nadir and peak VL. The differences in DNA load between individual patients were rather small (maximum 580-fold difference) and, although the DNA load declines after treatment initiation, the magnitude of this decline is limited. These observations suggest that frequent DNA load analysis in treated patients may have limited added value.

No conflict of interest

Abstract: P_71

Spread of Drug Resistance

Recent trends for transmitted drug resistance and non-B subtypes in new HIV infections - results from the national molecular surveillance, Germany 2013-2015

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Background: The German molecular HIV surveillance system provides information on currently transmitted drug resistance (TDR) and circulating subtypes by HIV-1 genotyping of recent infections among newly diagnosed cases.

The aim of the present study was to assess the current trends in TDR for all HIV subtypes with special regard to subtype A which is the most frequent non-B subtype in Germany.

Methods: Diagnostic laboratories provide dried serum spots (DSS) for ~60% of all reported new HIV-diagnoses. DSS serologically classified as 'recently acquired infections' (<155 days; BED-CEIA, Sedia) are genotyped in the HIV-pol-region to identify TDR according the WHO SDRM list and to determine the HIV-1 subtype. The results are then linked to the statutory notification data. For subtype A, sequences from recently infected patients (n=87) were aligned with 56 sequences from the German seroconverter cohort and 560 closely related sequences retrieved by a BLAST search of the Los Alamos HIV database. Bayesian phylogenetic analysis was performed to detect putative transmission clusters (TC, posterior probability =1) and the discrete asymmetric diffusion model was used to infer epidemiological linkage with notification data and TDR.

Results: In 2013-2015 2,627 of 7,896 DSS originated from recent infections. Of these, 1,197 were successfully sequenced and analysed. Total TDR was 10.6%, comprising 3.7% with mono resistance to nucleotide reverse transcriptase inhibitors (NRTI), 2.7% to non-NRTIs, 3.0% to protease inhibitors and 0.6%/0.3% with dual/triple class resistances, respectively. These proportions remained stable over time and no preferred transmission route was identified. 90% of NRTI mutations were thymidine-analogue mutations (TAM) and K103N accounted for 60% of the NNRTI mutations. Because a significantly increasing trend (p(trend) = 0.01) was observed for subtype A infections (n= 87; 7.2 %), in particular in the group of German men having sex with men (MSM), phylogenetic reconstructions were used to identify and characterize transmissions clusters within this subtype and with respect to TDR. Among recently subtype A infected individuals, two distinct clades with ongoing transmission of PI resistance were identified: one clade (n=5) with M46I in the protease and one clade with a minor protease resistance mutation I85V (n=4). Both are recent endemic clades consisting exclusively of HIV-sequences from German MSM and clustering with sequences from Eastern Europe, especially from the Russian Federation.
Conclusion: TDR prevalence in recent HIV infections among notified newly diagnosed HIV patients in Germany remained at a stable high level (>10%) in 2013-2015 and is comparable to that in other European countries. While the frequently observed TAMs have less impact on the treatment success of current first-line regimens that no longer comprise thymidine-analogues, the high prevalence of K103N might still be associated with treatment failure of two NNRTI, Nevirapine and Efavirence. Our data also demonstrate that subtype A is becoming established in the German population and is spreading among MSM. Moreover, ongoing spread of PI resistance conferring resistance to all PI but Darunavir and Saquinavir (M46I) and contributing to the reduction of the activity of Atazanavir (I85V) was identified in two recent subtype A transmission clusters.

No conflict of interest

Abstract: P_72

Spread of Drug Resistance

Absence of routine baseline resistance testing can result in an alarming increase of transmitted drug resistance to first-line regimen

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Background: The Caribbean has the second highest HIV prevalence in the world (1.1%), following sub-Saharan Africa. Data on transmitted HIV drug resistance (TDR) from this region is limited. Genotypic resistance tests generated for clinical practice in Aruba showed a worrying increase of mutation K103N in RT, which generates high-level resistance to their current first-line NNRTI regimen. We investigated whether these patients were truly naïve and studied transmission dynamics.

Methods: HIV-1 pol genotypic population sequencing was performed for 133 patients of the Oduber Hospital in 2010-2015. The resistance test was performed before exposure to antiretroviral therapy (ART) (=baseline) in 104 patients; for 29 patients resistance testing was only done during therapy failure. TDR was determined using the WHO-list. Phylogenetic analysis included 147 subtype-B sequences from Aruba (100 baseline, 47 failure), subtype-B baseline sequences from the Netherlands (n=220) and the most similar sequences selected via BLAST (n=145). A maximum-likelihood phylogenetic tree was constructed (GTR-model, 1000 replicates). Clinical and virological data were retrieved from patient records. In a subset of patients additional next-generation sequencing (NGS) using Illumina was performed on the same samples.

Results: The number of newly diagnosed patients in the country that was offered baseline resistance testing increased from 26% (6/23) in 2010 to 69% (31/45) in 2015. The patients tested were mostly male, median 40 years old and originating from Aruba. Most patients indicated to be infected in Aruba through sexual contact. 20% presented with an AIDS-defining illness at diagnosis. TDR to NRTIs (n=2) or PIs (n=2) was low, but the prevalence of K103N at baseline was 30.8% and increased over time to 41.9% in 2015 (13/31). K103N was also detected in 55% (16/29) of patients who were only tested during therapy failure. Phylogenetic analysis revealed that K103N variants were transmitted to therapy-naïve patients via at least 6 distinct introductions. One introduction resulted in a large cluster of 36 men. Within this cluster 73% of therapy-naïve patients had K103N, of whom 8 had a recent infection based on negative serology. NGS did not show minority K103N variants in patients with drug sensitive virus at population sequencing (n=68). In patients with K103N at baseline (n=7), NGS confirmed in all samples persistence of K103N at high frequency (>98%). Three of these patients had low CD4 counts at diagnosis (23, 66 and 161 cells/mm³) suggestive of long-term infection.

Conclusion: This study demonstrates that the prevalence of resistance can increase to alarming levels in a setting without routine baseline resistance testing. The alarming 103N prevalence in naïve patients could only partly
explained by clustered transmission, since multiple introductions are observed. We previously showed that K103N has limited impact on viral fitness, which explains the absence of evidence of reversion and the persistence at high frequencies despite long-term infection in our population. This suggests that population sequencing would be sufficient to detect transmission of K103N. In a setting with such high rates of transmitted NNRTI resistance, it should be considered to implement baseline population resistance testing routinely or to adapt the choice of first-line regimen.

No conflict of interest

Abstract: P_73

Treatment Strategies for HIV/ Hepatitis infected Patients

TAF Does Not Deplete Mitochondrial DNA in Human T-Cell Lines at Intracellular Concentrations Exceeding Clinically Relevant Drug Exposures

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Background: Tenofovir alafenamide (TAF) and tenofovir disoproxil fumarate (TDF) are prodrugs of the HIV-1 nucleotide reverse transcriptase inhibitor (NRTI) tenofovir (TFV). Although TAF and TDF both generate the active metabolite, tenofovir diphosphate (TFV-DP), TAF achieves higher intracellular levels of TFV- DP in peripheral blood mononuclear cells (PBMCs), resulting in more robust clinical antiretroviral efficacy than TDF at lower doses. Some HIV-infected patients treated with NRTIs, particularly didoxycytidine (ddC), have experienced a range of clinical symptoms due to mitochondrial toxicity. These older NRTIs deplete mitochondrial DNA (mtDNA) by inhibiting mitochondrial DNA polymerase Y. Although previous in vitro studies have demonstrated that TFV has minimal effect on mtDNA synthesis, this study was designed to address whether relevant exposures to TAF that delivered increased intracellular levels of TFV-DP could affect mtDNA content in human lymphocytes.

Methods: Activated or resting PBMCs, MT-2 and Jurkat cells were used for the in vitro studies. For 10 consecutive days, cells were either untreated, continuously treated with ddC, or pulse treated with TAF or TDF for 2 hours or 20 minutes, respectively, at 24-hour intervals to mimic pharmacologically relevant drug exposures. Following the treatment period, a quantitative real-time PCR method was used to assess the effect of each intervention on mtDNA content in treated cells. Statistical significance relative to untreated cells was determined by paired, two-tailed Student’s t-test.

Results: Treatment of activated and resting PBMCs with up to 20.0 μM ddC had no effect on mtDNA content, indicating that PBMCs exhibit low sensitivity to NRTI-mediated mtDNA depletion in vitro. In contrast, treatment of rapidly dividing human T-cell lines, such as MT-2 and Jurkat, with 0.002 to 20.0 μM ddC resulted in a dose-dependent decrease in mtDNA. Therefore, MT-2 and Jurkat cells were selected as models suitable for evaluating the effect of TAF and TDF on mtDNA content. Treatment of MT-2 and Jurkat cells with a pulse of 3.3 μM TAF for 10 days resulted in 3-7 fold greater intracellular TFV-DP levels than observed clinically at steady state drug exposure. At these concentrations, no significant reduction in mtDNA content was observed compared to untreated cells (106.7% ± 15.9 and 84.1% ± 5.7; N=3; p = 0.77 and 0.12, respectively). Similarly, no significant reduction in mtDNA content was observed in MT-2 and Jurkat cells treated with a 1.1 μM TDF pulse for 10 days (100.6% ± 20.1 and 91.0% ± 15.4; N=3; p = 0.91 and 0.37, respectively).

Conclusions: Neither TAF nor TDF inhibited mtDNA synthesis in human T-cell lines at suprapharmacological drug exposures in vitro. These data are consistent with an established lack of inhibition of mitochondrial DNA polymerase Y by the active metabolite TFV-DP, and indicate that despite delivering higher intracellular levels of TFV-DP than TDF, TAF has low potential for inhibiting mtDNA synthesis in T-cells of HIV-infected and TAF-treated patients.

Conflict of interest

financial relationship(s): I am an employee of Gilead Sciences
Abstract: P_74

Treatment Strategies for HIV/ Hepatitis infected Patients

Therapy-driven control of CXCR4-tropic HIV-1– A way towards selective virus eradication?

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Background: In the absence of therapy CXCR4-tropic HIV increases over time, associated with an accelerated disease progression. In most successfully treated patients the situation is quite different: The majority harbors CCR5-tropic variants in the circulation. As antiretroviral therapy itself may be responsible for reducing CXCR4-tropic HIV-1 this study aimed at monitoring the abundance of CXCR4-tropic viral sequences in infected cells during suppressive antiretroviral therapy.

Methods: For a group of patients in the Swiss HIV Cohort Study with documented suppressive ART, all carrying subtype B virus, the relative frequencies of CXCR4-tropic proviral HIV-1 variants in circulating PBMC were assessed by next-generation sequencing and interpretation by Geno2Pheno (FPR 3.5%, R5=X4<2%) before and after treatment initiation. Aside from a continuous viral suppression a steady CD4 T cell recovery under cART were was documented for all patients. Viral phylogenetics and evolution were analyzed.

Results: In 28 of the 35 patients (80%) we observed that frequencies of CXCR4-tropic provirus decreased or in fewer remained stable under therapy. This is in contrast to the situation in untreated patients. In the 7 other individuals (20%) the frequency of CXCR4-tropic provirus increased. In all these latter cases a single viral variant emerged, which was already detectable at time points before therapy initiation. Although a certain proviral sequence evolution was demonstrable in >50% of all patients under therapy this growing diversity was not associated with a similar frequency increase of CXCR4-tropic proviruses.

Conclusion: Our study demonstrates that under successful therapy particularly those cells that are infected with CXCR4-tropic HIV decline in most patients, leading to an overrepresentation of CCR5-tropic provirus. This indicates a preferential pressure on cells harboring CXCR4-tropic envelopes. Such a progressive proviral reduction in the immune-competent host could eventually lead to the selective depletion of the pool of CXCR4-tropic HIV in successfully treated individuals, paralleling the improving immune response. A better understanding of the underlying mechanism and of the involved cell types may provide crucial information for the new strategies towards HIV eradication. Moreover, the gradual loss of CXCR4-tropism further supports the recently implemented WHO-strategies of early therapy initiation and may advocate for the early use of co-receptor antagonists in these therapy concepts for the most effective suppression of the remaining CCR5-tropic virus.

No conflict of interest
Abstract: P _75

Viral Evolution & Genetic Diversity

Dating regional subtype B subepidemics: evidence for earliest introduction in Western Europe and Caribbean


Abstract

Viral Evolution & Genetic Diversity

Dating regional subtype B subepidemics: evidence for earliest introduction in Western Europe and Caribbean


Introduction/Background:
HIV-1 subtype B is the predominant clade in Western/Central Europe and the rest of the Western world. In a previous study we investigated the subtype B pattern of dispersal on a global scale by analyzing a global dataset of 8,370 sequences. Our previous analysis revealed the existence of 32 regional monophyletic clusters including sequences from a single area at proportions > 75% (local transmission clusters, LTNs). Our aim was to estimate the time of the most common recent common ancestor (tMRCA) of the subtype regional epidemics that corresponds approximately to the date of the introduction of subtype B in each area by means of phylodynamic analysis.

Material and Methods: We studied the temporal characteristics of 32 subtype B LTNs identified globally. Specifically, we analyzed 23 clusters from Europe, 5 from Caribbean and 4 from Asia. Clusters ranged between 8 - 1.737 sequences. Phylogenetic analysis was performed by using a Bayesian method as implemented in BEAST v1.8.0, using the GTR as nucleotide substitution model with gamma (Γ) heterogeneity model, an uncorrelated lognormal relaxed clock model and a Bayesian skyline non-parametric plot demographic model with 10 groups. Non-informative priors were used for the MCMC runs. The MCMC was run for 30-80x10^6 generations and convergence was checked by estimating the effective samples sizes (ESS>100). The MCMC was run for the largest LTN in replicates of 50 and 100 sequences after random subsampling.

Results: Molecular clock analysis revealed that the tMRCA for the European regional subepidemics ranged between 1964 and 2001 (median estimates). By classifying the European clusters geometrically, we found that the estimated time of the tMRCA for Central LTNs ranged between 1987 and 2001 (median estimates) and for Western LTNs from 1964 - 1991 (median estimates). The largest European cluster (N=1,737) was estimated to be the earliest with estimated tMRCA in 1964 (median value; 95%HPD: 1950-1975). The latter LTN
included sequences mostly from Western Europe. Finally for Caribbean and Asian LTNs the estimated time of the tMRCA ranged between 1984-1991 and 1987-1994 (median estimates), respectively.

Conclusions: Our study provides evidence for the first time that regional dispersal of the subtype B epidemic occurred at different time points across the globe. Specifically, the origin of regional subepidemics was earlier in Western than Central Europe. Importantly, the largest regional epidemic in Western Europe was introduced much earlier (upper limit of tMRCA: 1975) than the identification of HIV-1. For Caribbean although the earliest sampled sequences were not available in pol, the tMRCA of at least one subepidemic was between late-seventies and early-eighties. The earliest introduction of B strains spreading locally in Western Europe and Caribbean was probably due to the tighter links of these areas with the Americas.

No conflict of interest

Abstract: P_76

Novel Diagnostic Technologies & Approaches

Evaluation of the Aptima HIV-1 Quant Dx assay in comparison to the Cobas Ampliprep/Cobas Taqman HIV-1 v2 assay

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Background: The Aptima HIV-1 Quant Dx assay (Hologic) is a new method for detection and quantification of HIV-1 RNA viral load (VL) in plasma samples from HIV-1 infected individuals. The objective of this study was to compare the performances of the Aptima HIV-1 Quant Dx assay (Aptima) used on the Panther automate to those of the Cobas Ampliprep/Cobas Taqman (CAPCTM) HIV-1 v2 assay used on the Cobas Ampliprep-CobasTaqman docked 96 platform for measuring plasmatic HIV-1 RNA VL.

Methods: 115 unselected fresh plasma samples and 252 selected frozen plasma samples with a mean VL of 5.18 log and a known sub-type (157 B, 40 CRF02-AG, 55 others) were tested in parallel on both systems according to the manufacturer's recommandations. Correlation between the 2 methods was evaluated using linear regression and Bland-Altman plotting. Linearity was assessed for B and CRF02-AG subtypes by testing 3 to 6 replicates of plasma dilutions with 50,000, 5,000, 500 et 50 copies/ml. Intra-assay reproducibility was assessed for A, B and CRF02-AG subtypes by testing 20 replicates of plasma dilutions of 200, 100 et 50 copies/ml, and inter-assay reproducibility was assessed with the Aptima assay only by testing an aliquote of the same dilutions on 15 successive days.

Results: Among 110 fresh samples with a valid result on both systems, 74 gave concordant results with both assays, with a global coefficient of correlation (GCC) of 0.9420 for the quantifiable plasmas and only minor discrepancies, all of them around the lower limit of quantification of both assays. Among 227 frozen plasmas with a valid result on both systems (142 B, 36 CRF02-AG, 49 others), 170 were measurable with both assays, with a GCC of 0.9660. The mean difference between results (Aptima – CAPCTM) was -0.09 log. Thirty-four discrepancies over 0.5 log were obtained, with no link to any specific sub-type. Linearity of the 2 assays was similar with R² coefficients > 0.97 for both B and CRF02-AG subtypes.

Intra-assay reproducibility of both assays was comparable for the 3 tested sub-types in terms of standard deviations (SD), all below 0.2 log, and of coefficients of variation (CV) for the 200 et 100 copies/ml levels. The CV obtained with Aptima were however lower for the 50 copies/ml level for the 3 subtypes tested. Inter-assay reproducibility fot the Aptima assay was excellent with SD below 0.2 log and low CV for the 3 subtypes and the 3 VL levels tested.

Conclusion: Aptima HIV-1 Quant Dx performances were similar to those of CAPCTM HIV-1 with a very good correlation study on a diverse HIV-1 subtypes population and with an
excellent intra and inter-assay reproductibility, even for the lowest VL levels. Aptima HIV-1 Quant Dx appears therefore as a reliable alternative for HIV-1 RNA viral load measurement for the follow up of HIV-1 infected patients.

*These data were previously presented in French at the RICAI meeting (Paris, December 14-15th 2015)*

No conflict of interest

**Abstract: P _77**

**Novel Diagnostic Technologies & Approaches**

**High sensitivity of the new Roche cobas® HIV-1 test for use on the cobas®4800 system for quantification of viral load in HIV-1 B and non-B subtypes**

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**Background:** Quantification of plasma virus load (VL) is critical in clinical practice to monitor antiretroviral therapy efficacy. We evaluated the technical performance of the new Roche cobas® HIV-1 Test for use on the cobas® 4800 system (cobas® 4800 HIV-1).

**Material and Methods:** cobas® 4800 HIV-1 test results were compared to those obtained from Roche's COBAS® AmpliPrep/COBAS® TaqMan® Test v2.0 (CAP/CTM) and Abbott's RealTime HIV-1 Assay (ABT) (243 and 224 clinical samples, respectively). Primary tube equivalency was evaluated on 102 matched clinical samples collected in Becton Dickinson EDTA Lavender Top Tubes (LTT) and in Vacutainer® Plasma Preparation Tubes (PPT). Clinical samples were processed in duplicate on all 3 platforms, and represented subtypes A, B, C, D, F, G, H, CRF01-AE, CRF02-AG and Group O strains. Viral load of the samples spanned the linear range (i.e., from Roche LLoQ of 20 cp/mL to 100,000 cp/mL). Sensitivity of the cobas® 4800 HIV-1 test was determined on the 243 clinical samples used for the correlation analysis. Bland-Altman and Deming regression analyses were used to evaluate agreement and correlation between the two Roche assays.

**Results:** 240 valid results were obtained from 243 samples tested with the cobas® 4800 HIV-1 test and 239/240 results were assigned positive giving a 99.58% sensitivity. All of the 50 HIV negative plasma samples returned a negative result for both LTT and PPT primary collection tubes and a mean log 10 difference of -0.04 was obtained (CI95%, -0.08 to 0.01) for 52 positive B and non-B subtype samples covering the entire measuring range indicating a very good equivalency between the two EDTA primary tubes. A mean log difference of 0.28 (0.25-0.30 95% confidence interval, CI95%) was observed between the two Roche assays indicating higher quantification of the new cobas® 4800 HIV-1 test relative to CAP/CTM. Deming regression analysis revealed a high correlation over a wide dynamic range (R²=0.98). Additionally, Bland-Altman analysis revealed high agreement between the cobas® 4800 HIV-1 test and the ABT assay, demonstrated by a mean difference of just 0.01 log (CI95%, -0.02 to 0.05) and indicates minimal overall VL quantification differences between platforms within the tests’ dynamic range. This was supported by a good linear correlation (R²=0.96).

For samples at or above the medical decision point of 50 cp/mL, 98% agreement was found between the two Roche assays.

**Conclusions:** In this evaluation the new cobas® 4800 HIV-1 test shows comparable sensitivity to CAP/CTM and good linear correlation to both CAP/CTM and the ABT assay.

*No conflict of interest*
Abstract: P_78

Novel Diagnostic Technologies & Approaches

Impact of shortening V3-information on genotypic HIV-1 tropism prediction

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Background: Knowledge of the host cell tropism of HIV is critical for initiating a therapy with a coreceptor antagonist and may be of importance for judging the clinical course. Genotypic methods are today available that are mainly based on the variable loop 3 (V3) in the viral env gene, either on the plain sequence (Geno2Pheno) or by analyzing duplex properties (XTrack). Since the V3 loop is flanked by sequences with a higher degree of conservation, amplification typically utilizes these regions for PCR amplification. Thereby commonly used primes may reach slightly into the region, and this may potentially introduce a bias, as the primer-provided nucleotides cannot be taken into account for interpretation.

Methods: To overcome this bias we now designed shorter primers that, through 2’- modifications, are able to retain comparable binding affinity despite the shortening. These primers now do not reach into the V3 loop and allow full representation of patient-derived V3 sequences. The amplified sequences were in parallel analyzed by duplex-tracking (XTrack) and using the genotypic Geno2Pheno (G2P) assay, which is based on tropism prediction using the amino acid sequence of the V3 loop. In silico G2P analysis of V3 sequences differing only in the affected primer region was also performed with consensus sequences from various subtypes. Replicative phenotyping were used as gold standard in order to clarify contradictory results.

Results: A comparison of sets of sequences from subtype AE and –C viruses revealed that for most of them there is no principal difference (CXCR4- vs. CCR5-tropism), and no significant differences in the False Positive Rates (FPR) between the two V3 sequence lengths for XTrack or the G2P assays. Surprisingly even the V3-sequence of consensus HIV-2 does not yield a principally different interpretation. Complete omission of the respective flanking sequences renders interpretation difficult – as suggested by the conserved nature of this region among virus isolates.

Conclusion: This study illustrates the importance to consider the entire sequence of the V3 loop for tropism determination. On the other side our data clearly show the limited contribution from mutations and variation in the V3-flank to a given viral tropism. Of note, the biggest changes in the FPR and thereby shift towards a tropism change was seen for isolates of the –AE subtype CRF_01. Larger data sets are still needed to gain a better validity for clinical utility of our findings.

No conflict of interest

Abstract: P_79

Novel Diagnostic Technologies & Approaches

Comparison of the Veris DxN Quantification Assay and Realtime in Quantifying HIV-positive Plasma Samples

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Background: Viral load testing is the gold standard for therapy monitoring. The recently released VERIS DxN system (Beckman Coulter) offers a fully automated platform for the quantitative analysis of molecular targets like HIV, HCV and HBV with less hands-on-time. The aim of this study was to assess the variation concerning sensitivity and precision between the VERIS DxN and the Abbott Realtime assay.

Material/methods: In precision analysis, five HIV quality controls were diluted to nominal concentrations (0 Log IU/ml; 2.08 Log IU/mL; 3.26 Log IU/mL, 5.26 Log IU/mL and 7.00 Log IU/mL) and tested on the VERIS system in duplicate analysis for 20 days. 100 specimens from patients infected with HIV and viral loads from 40 to million copies/mL were retested with
both systems. Additionally, 80 archived EDTA-plasma specimens derived from 20 pts undergoing HIV-therapy within a time period of approximately 3 years were reanalyzed with both systems. For linearity analysis, one high viremic sample (> 10,000,000 copies/mL) was diluted to several concentrations to determine linearity.

**Results:** For precision analysis, coefficients of variation (CV) for logarithmic VERIS results increased slightly from 0.46% for the highest to 4.17% for the lowest nominal concentration, respectively. The linearity was validated for 2.19 to 5.67 log copies/mL and showed a correlation of $R^2=0.99$ for clinical samples. The VERIS system showed a tendency towards a slightly higher quantification of samples above 3 log copies/mL as compared to Realtime. In Bland Altman analysis both assays showed a mean difference of overall +0.49 log (Veris minus Realtime). With an analytical sensitivity of 19.5 copies/mL (CI95%: 15.9-27.5 copies/mL) both assays were comparable.

**Conclusions:** In this analysis, the VERIS DxN system showed a good correlation and agreement with the Realtime assay at the lower end of the linear range. However, from 3 log up to 6 log copies/mL the discrepancies between both systems appeared to be measurable higher. The VERIS system demonstrated high sensitivity and comparable precision to Realtime with a less time-consuming full-automated sample handling approach.

No conflict of interest
Abstract

Spread of Drug Resistance

Sustained transmission of drug resistance mutations in newly diagnosed HIV-1 subtype B patients in Denmark between 2011 and 2015.

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Introduction/Background: Transmission of HIV-1 resistance mutations among treatment naïve patients impairs the efficiency of antiretroviral therapy (ART). Therefore, genotypic resistance testing of newly diagnosed patients is recommended, as this both allow for the selection of the correct ART regimen at baseline and to monitor the spread of transmitted drug resistance mutations (TDRM) among newly diagnosed HIV-1 patients. In Denmark, the occurrence of TDRM in newly diagnosed HIV-1 patients is monitored through the SERO project. Here we report on recent changes observed for TDRM and phylogenetic analysis of sequences to investigate if sustained transmission clusters existed among newly diagnosed HIV-1 patients in Denmark.

Material & Methods: Samples from newly diagnosed HIV-1 patients were sent along with epidemiological information to the Virological Surveillance and Research group at Statens Serum Institut. HIV-1 RNA extraction, RT-PCR and Sanger sequencing of the pol gene was performed using an in-house assay. The sequences were analyzed using BioNumerics v. 6.6 and manually checked for the presence of mixed mutations. Sequences were analysed for mutations using both the Calibrated Population Resistance (CPR) tool (SDRM 2009 list) and the HIVDB 7.0 algorithm implemented at the Stanford database and classified as either TDRM or resistance associated polymorphisms (RAP) in accordance with the IAS-USA 2015 chart. Sequence alignments was performed in

Results: Totally, 660 sequences were studied: 228 belonged to 2005-2009 group and 432 – to 2010-2015 group. 32/660 of sequences studied harbored at least one SDRM mutation with K103N/S in RT region being the most frequent (40.63% of samples). We found that prevalence of mutations increased from 2005 to 2015. In 2005-2009 group the SDRMs prevalence was 2.63% (6/228; 95% CI, 0.97-5.73%), in 2010-2015 group – 6.02% (26/432; 95% CI, 3.93-8.82%), p=0.05. It should be noted that the distribution of SDRMs prevalence among the Russian regions studied in 2010-2015 was unequal. The drug resistance prevalence was maximal in Central (9.17%) and Far Eastern (9.43%) Federal Districts, the lowest prevalence was in Siberian Federal District (1.87%).

Conclusions: The trend of drug resistance prevalence increasing in ART-naïve patients in Russia in the past 10 years was revealed (2.63% compared to 6.02%, p=0.05). In some Federal Districts the SDRMs prevalence was higher than 5%. It is important to increase the efforts of anti-HIV measures and drug resistance spreading prevention at the country and regional levels.

No conflict of interest
Mafft and phylogenetic analysis was performed using Mega 6.0. TDRM and RAP clusters were identified on the phylogenetic tree and their reproducibility was assessed by bootstrap analysis and by performing phylogenetic analysis both with and without the mutation codons.

**Results:** In Denmark, an increase in the proportions of both TDRM and RAP among newly diagnosed HIV-1 patients was observed in 2013. Phylogenetic analysis showed that most of the TDRM’s and RAP’s did not occur in clusters, indicating either non-sustained or low level of transmission. However, two subtype B clusters consisting of the TDRMs E138A and M41L respectively and one subtype B cluster consisting of the RAP I85V were identified. These three clusters were active through the 2011-2015 study period and consisted of ART naïve patients.

**Conclusions:** Our observations show that sustained transmission of both TDRM’s and RAP’s occurred among newly diagnosed Danish HIV-1 subtype B patients. This emphasizes the importance of a national surveillance program in order to discover changes in TDRM occurrences and transmission.

**No conflict of interest**

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**Abstract: P_82**

**Therapeutic Challenges in Resource-limited settings**

**Comorbidities and Polypharmacy Among HIV-Positive Patients Aged 50 Years and Over: A Case-Control Study**

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**Background:** Due to the antiretroviral therapy (ART) use, prevalence of people worldwide older than 50 years old infected with human immunodeficiency virus (HIV) has increased recently, as well as comorbidities and use of drugs associated with age. The objective of this study was to determine the prevalence of polypharmacy and comorbidities comparing patients older than 50 with patients younger than 50 years old.

**Material and methods:** A case-control study of patients >16 years infected with HIV-1 and with at least 6 months of treatment with ART attending at the Hospital de Infectologia, ‘La Raza’ National Medical Center, between October 2013 and March 2015 was conducted. Polypharmacy (5 or more drugs use at the moment of interview) and comorbidities were collected: systemic arterial hypertension, dyslipidemia, diabetes mellitus, osteoarthritis, acid peptic disease, chronic kidney failure, heart disease, chronic obstructive pulmonary disease, liver failure, and obesity. Risk factors were evaluated with bivariate analysis by chi square test for comorbidities and polypharmacy. To adjust for the effects of potential confounders, we used logistic regression model.

**Results:** There were recruited 125 patients in the study; 60 (48%) patients were older than 50 years old. The median age in the older patients group was 55.5 years [interquartile range (IQR): 52–59.7], and in the younger group was 31
years old (IQR: 26-36). In both groups, proportion of women was less than men, 16% for the older patients group and 8% for the younger patients group.

Outpatients with at least 6 months of treatment in the HIV clinic were included. Median CD4+ cells count in patients with at least six months as outpatients in the medical center was 509 cells/µL (IQR: 324 -730) for the older patients group and 384 cells/µL (IQR: 262-562); P= 0.021 for the younger patients group. The percentage of patients virally suppressed was significant statistically between older and younger patients 63% vs 80%; p= 0.037.

The most prevalent drugs used by older patients were antihypertensive 21 (35%) vs 2 (3.1%) for the younger group OR 11.37 [(2.75 – 46.6); p<0.001], hypolipidemic drugs 17 (28.3%) vs 2 (3.1%) OR 9.2 [(95% CI: 2.22 – 38.19); p<0.001], non-steroid anti-inflammatory drugs (NSAID’s) 14 (23.3%) vs 4 (6.2%) OR 3.79 [(95% CI: 1.32 – 10.88); p=0.006] and anticonvulsants/anxiolytics 14 (23.3%) vs 4 (6.2%) OR 3.79 [(95% CI: 1.32 – 10.88); p=0.009].

The number of comorbidities was significantly higher in the older patients group with a median of 2 (IQR: 2 - 3) vs 1 (IQR: 0 - 1); p = <0.001. Among the comorbidities studied after adjustment in a logistic regression model for the older group systemic arterial hypertension OR 6.23 (95% CI: 1.01-38.13); p=0.048, osteoarthritis OR 10.85 (95% CI: 2.37 – 49.76); p=0.002, Diabetes mellitus OR 11.9 (95% CI: 1.5 – 89.5); p=0.002 and hyperlipidemia OR 2.80 (95% CI: 1.0 – 7.7); p=0.048 and polypharmacy OR 2.90 (95% CI: 1.08 – 7.77); p=0.033 remain significant.

Conclusion: Polypharmacy and the number of comorbidities were significantly higher for the older patients group. Osteoarthritis, systemic arterial hypertension, diabetes mellitus and dyslipidemia were more frequent in elderly patients.

No conflict of interest
TDF/3TC/EFV, the most common ART regimen currently being used in Nepal, demonstrated 12.5% virological failure and 37.17% immunological failures rates. It was slightly higher than the failure rates of other common combinations in the country. Virological failure rate was higher among children < 15 years (14.5%) ($p = 0.03$), however no association was observed between ART outcomes and gender.

**Conclusion:** In a nutshell, despite the success of Highly Active ART (HAART) there is still some chances of virological and immunological failure. Monitoring for HIV drug resistance and early management would greatly increase the overall effectiveness of the ART program.

**No conflict of interest**

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**Abstract: P_84**

**Therapeutic Challenges in Resource-limited settings**

**Effectiveness and risk factors for virological outcome of raltegravir-based therapy for treatment-experienced HIV-infected patients**

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**Background:** Raltegravir (RAL) has shown to be generally safe and well tolerated, with potent antiretroviral activity in treatment-experienced patients. In real life a few studies have been developed to evaluate this drug. We evaluated the effectiveness of RAL-containing regimen plus an optimized background regimen in HIV-1 highly treatment-experienced patients.

**Material and Methods:** We conducted a retrospective multi-center cohort. Adults >16 years old with virological treatment failure starting therapy with a RAL-containing regimen were included. Effectiveness was evaluated as the percentage of patients with an undetectable HIV-1 RNA viral load (<50 and <200 copies/mL) after 48 weeks, and changes in CD4+ cell counts. We evaluated the risk factors associated with treatment failure. Clinical histories were reviewed regarding antiretroviral (ARV) regimens, CD4+ cells count, HIV-1 RNA viral load, and serum laboratory parameters at the beginning of the therapy with RAL (baseline) and 48 weeks later. The presence of resistance was defined according to the Stanford HIVdb sensitivity score (SS). Each ARV drug was assigned a score according to the five-level Stanford HIVdb interpretation. The sum of individual scores for specific drugs provided the total GSS of that treatment. We also classified the total GSS score in the following categories: 0–1, 1–2, or ≥2.

**Results:** We included 107 patients with HIV; of them 86% were men with a median age of 45 years (IQR 40–52). They had a median of six previous regimens (IQR 4–7). After 48 weeks of treatment, 73% (IQR 63%-80%) of patients (n=78) had an HIV-1 RNA viral load of <50 copies/mL and 85% (IQR 77%-90%) (n=91) had <200 copies/mL. The CD4+ cell count increased by 383 cells/μL (258–564 cells/μL) at 48 weeks vs. baseline, $P < 0.001$. The median GSS in the OBR for all patients was 1.25 (IQR 1.0–2.0); 29 patients (27%) had a GSS ≥2. DRV-containing regimen was used in 102 (95.3%) of patients, 3 (2.8%) use a TPV-containing regimen and 2 (1.8%) patients used a regimen free of PIs. The most frequent RAMs for DRV were I84V (35.8%), L33F (34.9%), I47V (10.4%) and V32I (7.5%). After a logistic regression model, risk factors associated with virological outcome in HIV-1 RNA <200 copies/mL were age >40 years [odds ratio, OR 5.61 (95 % CI 1.61- 18.84); $P = 0.006$], and use of Tenofovir in the regimen [OR 0.16 (95 % CI 0.03- 0.80); $P = 0.026$]. Total cholesterol showed a significant increase ($P=0.003$) from a baseline of 166 mg/dL (137-200 mg/dL) to 179 mg/dL (161–214 mg/dL) at week 24, and it remained significant at 48 weeks of 197 mg/dL (157-213 mg/dL; $P<0.001$). Tryglicerides showed no significant increase.
from a baseline of 193 mg/dL (137-264 mg/dL) to 193 mg/dL (156-271 mg/dL) at week 24, and it remained not significant at 48 weeks of 216 mg/dL (169-303 mg/dL).

Conclusion: In this Mexican cohort, Raltegravir was metabolically safe, well tolerated and achieved high rates of virological suppression and a significant increase in CD4+ cells count in highly treatment-experienced patients infected with HIV-1.

No conflict of interest

Abstract: P_85

Therapeutic Challenges in Resource-limited settings

HIV-1 subtype diversity, epidemiological, immunological and clinical profile of HIV/HCV co-infected patients, Southern Brazil

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Background: Currently, a new era in the treatment of chronic HCV infection began with the approval of DAA oral medications in interferon-free regimens. However, in the context of HIV/HCV co-infection, the combination PEG-IFN and ribavirin remains recommended. Also, host genetic factors play a vital role in the clearance of HCV infection, as genetic variation in the IL28B gene. As well as, the ITPA gene polymorphisms and the risk of ribavirin-induced hematologic toxicity. There are few data in HIV / HCV co-infected patients on HCV therapy and the impact of host IL28B and ITPA SNPs on treatment response and toxicity in Brazilian patients, also little is known about the HIV genetic diversity in this population. We report the HIV genetic variability, epidemiological, immunological and clinical profile of a cohort of HIV/HCV co-infected patients in follow up in a tertiary care academic hospital in Southern Brazil.

Material & Methods: In this cross-sectional study, data were evaluated by medical records review and patient interviews. Periodic blood draws were taken to determine HIV-1 genetic variability and host genetic patterns.

Results: A total of 38 patients were included. The median age was 49 years (IQR 44-74), and 68.4% were male. HIV and HCV median diagnosis time was 14.5 years (IQR 11 - 24) and 9 years (IQR 5 - 17), respectively. Liver biopsies were performed in 22 (57.9%) patients, of these 18 (81.8%) had fibrosis, 18 (54.5%) steatosis, 6 (27.3%) cirrhosis and only one (4.5%) showed normal results. HIV genotypes were identified in 28 (73.7%) patients, of which 14 (50%) were i subtype C, 9 (32.1%) B, 4 (14.3%) BC, and 1 (3.6%) F. With respect to HCV genotypes, 22 (57.1%) were 1, 1 (2.6%) 2, 8 (21.1%) 3, 1 (2.6%) 4, and 6 (15.8%) were not genotyped. Regarding to IL28B rs12979860 genotyping, 22 (56.4%) patients were CC, 1 (2.6%) 3, 1 (2.6%) TT, and 4 (10.3%) were not genotyped. For the ITPA gene, the frequencies of two SNPs (rs7270101 and rs1127354) were analyzed. For the rs7270101, 22 (57.9%) presented AA, 6 (15.8%) AC, and 10 (26.3%) were not genotyped. For the rs1127354, 25 (65.8%) patients had CC, 1 (2.6%) AC, and 12 (31.6%) were not genotyped. Eighteen patients (47.4%) underwent treatment for HCV with RBV/PEG-INF, of these, 8 (44.4%) had SVR, 9 (50%) null response and 1 (5.6%) had recurrence. 55% of patients who received treatment for HCV had anemia, and all patients had genotype ITPA favorable for this event (rs7270101 genotyping AA or AC and rs1127354 CC).

Conclusion: Studies about the SNP related to the response and toxicity to treatment in Brazil is recent. Similar to previous studies, they should be recommended in the pre-treatment period, when the results could benefit not only the patients but also the public health system, guiding the rational use of drugs in situations where response rates to treatment are particularly low and adverse effects are high.

No conflict of interest
Abstract: P_86

Therapeutic Challenges in Resource-limited settings

Evaluation of HIV-1 drug-resistance and tropism in Cameroonoan children according to PMTCT exposure by using next generation sequencing

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Background: In resource-limited settings (RLS), major therapeutic challenges in HIV/AIDS include the scarcity of treatment options (i.e. NNRTI- and PI/r-based regimens), especially for children. Moreover, antiretroviral exposure, in the context of prevention of mother-to-child transmission (PMTCT), chiefly widens the extent of paediatric HIV-1 drug-resistance (HIVDR) in infected children. Therefore, our objectives were to ascertain majority and minority HIVDR mutations and viral tropism among vertically infected children following the mother’s experience with PMTCT in Cameroon, a RLS with generalised HIV epidemiology.

Materials and Methods: Using a comparatively designed approach, we investigated HIVDR and viral tropism among 18 children vertically infected with HIV-1 (seven born to mothers exposed to antiretroviral for PMTCT, and eleven born to mothers never exposed to antiretrovirals). Ultra-deep 454-pyrosequencing (UDPS), with a variant threshold accuracy of 1%, and Sanger-based sequencing were performed. Phylogenetic analysis was performed for subtype assignation with MEGAv.6. HIVDR in protease/reverse-transcriptase (RT) and viral tropism were assessed respectively with the 2015 Stanford HIVdb list and geno2pheno.v2.5 (classifying as non-CCR5 tropic viruses [non-R5] by UDPS when ≥2% viral species had a false positive rate [FPR] ≤3.5%, or by Sanger-based sequencing when FPR was ≤10%). Using the statistical open source environment R.v.3.1.1, comparisons of paediatric HIVDR mutations and viral tropism were done between the two children groups (PMTCT exposed vs. non-exposed), and HIV-1 tropism was stratified following age and CD4-count; with p-value <0.05 considered statistically significant.

Results: Children enrolled had a median [interquartile range, IQR] age of 6 [4-10] years. Median [IQR] plasma viral load and CD4-count were 5.5 [4.9-6.0] log₈ copies/ml, and 526 [282-645] cells/mm³, respectively. Molecular epidemiology revealed the following subtypes: CRF02_AG (50%), F (33.3%), CRF01_AE (11.1%) and CRF11.cpx (5.6%). Based on Sanger-based sequencing, all children appeared infected with wild-type viruses in both PMTCT-groups. Based on UDPS, minority variants were detected in only a single infant (presence of K103N with a frequency of 8.31%), born to a mother treated with RT inhibitors and presenting resistant mutations L74V (2.54%) and Y181C (96.67%). Complete sequencing data on viral tropism were available for 15/18 children. Based on UDPS, 33.3% of them (5/15) were infected with a non-R5 tropic-virus, including two cases not similarly reported by Sanger-based sequencing (UDPS frequencies of 3.9% and 36.2%). Interestingly, children ≤5 years were all reported solely with R5 compared to older ones (100% [6/6] vs. 44.4% [4/9], p=0.044). This underscores a high likelihood of paediatric infection with R5 and potential effectiveness of CCR5 antagonists for therapeutic management at early-age. Likewise, R5 were highly frequent among children with higher immune status: 9/11 [87.8%] for CD4>200 cells/mm³ versus 25% (1/4) for CD4<200 cells/mm³, p=0.077. This underscores a possible switch to non-R5 variants following decreasing immunity.

Conclusions: For an optimal therapeutic management of HIV-1 infected children, exposed to PMTCT in RLS, our findings support...
using NNRTI-sparing regimens for initiating paediatric antiretroviral therapy. Of note, CCR5-based regimens might be convenient alternatives for initiating antiretroviral therapy at early-age, thus preserving the currently effective PI/r-based regimens (since no PI/r resistance found) to handle long-term treatment challenges in RLS.

No conflict of interest

Abstract: P_87

Therapeutic Challenges in Resource-limited settings

Problems and challenges of management of patients with HIV-HBV and/or HIV-HCV co-infection in Ukraine.

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Introduction/Background: The current state of HIV in Ukraine remains a serious and complex. HIV-associated diseases and co-infections have a significant impact on the rate of mortality of patients with HIV in Ukraine. Recently co-infections of HIV-HBV and/or HIV-HCV have become a serious problem for public health in Ukraine especially in regions with a high prevalence of HIV. Purpose of the study to evaluate the strengths and weakness of management of patients with of HIV-HBV and/or HIV-HCV co-infection to optimize the model of care and improve its efficiency.

Material& Methods: We conducted an analysis of the regular reports of the Regional AIDS Center for 2014-2015 with a focus on dynamics and prevalence of co-infection of HIV-HBV and/or HIV-HCV in a cohort, access to screening, prevention and treatment, the impact of these co-infection on the progress of HIV and mortality. We use SWOT analysis to identify the strengths and weaknesses of the management to indicate the main areas for reform.

Results: 24 160 patients with HIV are currently registered in Regional AIDS Center. Heterosexual route of transmission -49.6%; parenteral-33.8%; vertical-16.0%. Only 34% of patients were receiving ART. Serological testing for HBV and HCV markers was conducted in 75% of all registered patients, but virological monitoring by HBV DNA testing is rarely used. The study revealed a predominance of HCV (26%) on HBV-(11%). 2.6% of patients had a mixed infection (HCV + HBV). Access to HCV therapy was also incomplete with only 12% of patients, receiving therapy. 45% of patients with HBV were treated with tenofovir containing ART regimes. Vaccination against HBV only got 1.3% of patients. The mortality rate among HIV patients remains high (28.2 per 100 000 population), 56% of them were injecting drug users. The highest mortality rate was among those aged 40-49 years old, 29% of them - women. 53.6% of all deaths were due to co-infection with tuberculosis, 29% of patients – due to multiple infections. The frequency of progressive liver diseases, HBV and HCV ranged from 20% in 2014 to 18% in 2015. Conducted SWOT analysis showed that the main weaknesses of Health system program for patients with co-infection HIV/hepatitis are the following: lack of financial support, concentration of all resources management and care facilities in AIDS centers, weakness of interdisciplinary communications, poor patients adherence to prevention, diagnosis and treatment of HCV and HBV.

Conclusions: Co-infections of HIV-HBV and/or HIV-HCV is becoming increasingly important, due to the prevalence of both hepatitis and their impact on the mortality rate. Lack of timely diagnosis and prevention, low treatment coverage contribute to liver disease progression and deaths. Analysis of the existing gaps in the provision care for HIV patients provides a tool to focus on the possible prospective areas: the vulnerable groups of patients, primary care assistance, strengthening of the interdisciplinary cooperation.

No conflict of interest
Abstract: P_88

Novel Diagnostic Technologies & Approaches

Ultra-deep sequencing and bioinformatics approach to detect natural resistance-associated variants in DAA-naive HCV positive patients.

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Background: Resistance-associated variants (RAVs) occur naturally in HCV viral population at low frequency, reducing treatment response of Direct acting Antiviral Agents (DAAs). Next Generation Sequencing (NGS) technologies and a bioinformatics approach were used to disclose genetic variations at hot spot positions in HCV NS3/4A protease. We investigated presence of natural RAVs in baseline sequences from a cohort of patients using a novel combination of dedicated NGS data analysis methods.

Materials and methods: In 2014, serum samples from eight HCV chronic infected patients were collected. HCV RNA viral load was measured using a routine diagnostic method. HCV NS5B region was sequenced by Sanger technique. HCV genotyping/subtyping was performed by phylogenetic analysis of NS5B region (100 aa) using RAxML, and by the use of two HCV subtyping tools: COMET and Oxford HCV. Ultra-deep sequencing of NS3 region (181 aa) using the Ion Personal Genome Machine (PGM) Sequencer was performed. Sample-specific NS3 consensus sequences were generated by an in-house developed script. Briefly, after quality control of raw data using FastQC and pre-processing with the Pollux error correction software, a de novo assembly was performed using software packages VICUNA and V-FAT. Resistance mutation positions for NS5B Sanger and NS3 NGS sequences were then analyzed using Geno2pheno.

Results: All patients were DAA-naïve, not co-infected with HIV or HBV, and infected by HCV 1b. Seven patients were experienced to interferon/ribavirin (PEG-IFN/RBV) and only one was naïve to this combination. Treatment included triple therapy with PEG-IFN/RBV and telaprevir (TVR) in 7 patients or boceprevir (BOC) in one patient. Median age was 55 years (range 44-68), 6/8 were males. Surgery and/or cohabitation with HCV-Ab positive individuals were the most frequent risk factors in 6/8 patients, followed by surgery/tattoo in 1 individual. At baseline, median HCV RNA was 6.32 log_{10} IU/ml. All patients achieved undetectable HCV RNA levels at the end of therapy. The bioinformatics pipeline, developed to analyze short reads obtained by PGM, allowed us to identify protease resistant mutations. Variant V36L, conferring resistance to BOC and possibly to TVR or simeprevir, was detected in PEG-IFN/RBV naïve patient's HCV isolate. In 4/8 patients, we identified variant I132V associated with resistance to TVR. C316N mutation for the NS5B polymerase inhibitor dasabuvir (DSV) was found in two patients' isolates. Moreover, in all HCV isolates, V338A and D30E+I170V aminoacidic substitutions, not drug resistance related, were detected in NS5B and NS3 regions, respectively.

Conclusions: PGM and our in-house developed analysis pipeline are a useful method to analyse HCV genetic variability and variants present in the NS3 coding region. Indeed, free software packages allow detection of non-synonymous mutations, generating an in silico specific consensus sequence for each sample through de novo assembly. This allows to investigate which substitutions have a potential impact on treatment outcome. Interestingly, five patients carried RAVs associated to TVR resistance, suggesting a risk of therapy failure. Also, despite the fact that only a part of the NS5B region was analysed using Sanger sequencing, C316N variant was still detected in two patients, for which treatment with DSV would be contraindicated.

No conflict of interest
Abstract

**Abstract: P_89**

Novel Diagnostic Technologies & Approaches

**Viral Kinetics in HCV-infected Patients during DAA-based therapy: impact to monitor treatment response and duration**

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**Introduction/Background:** The introduction of the new direct antiviral agents (AADs) for the treatment of viral hepatitis C infection (HCV), has allowed shorten treatment duration to 12 weeks in most patients. Currently, it is evaluating the possibility to shorten even more (i.e. 8 weeks). In this context, to know the viral kinetics in HCV-infected patients under treatment with DAA-based therapies can be useful to optimize the monitorization of the virological response and treatment duration. In this study, the HCV kinetics was evaluated in HCV-infected patients under DAA-based therapies.

**Material & Methods:** This is a prospective study in patients with chronic HCV infection in clinical follow-up in a reference hospital of the Northwest of Spain who began treatment for HCV infection since March 2014. Epidemiological, clinical and virological data of patients were collected. HCV viral load was determined at different times: days 0, 3, 5, 7, 10, 12, 14 and weeks 4, 8 and 12 after treatment initiation. HCV-RNA was quantified using the Abbott RealTime HCV assay (low limit of detection of 12 IU/mL).

**Results:** A total of 13 patients with chronic HCV infection who started a DAA-based regimen were selected. The 61.5% were men, with an average age of 57±12 years and 2 patients were co-infected with HIV. Median liver stiffness was 16.6 [12–25.6] kPa and 76.9% had cirrhosis (>12.5 kPa). HCV genotype 1b was the most prevalent (69%), 1a (15.3%) and 3&4 (7.7%, respectively). Most were interferon-free treatments (84.6%) being the most common combination sofosbuvir+simeprevir (30.8%), and sofosbuvir+daclatasvir (23.1%). All the treatments included ribavirin. The median viral load (HCV RNA log IU/mL) at day 0 was 5.57 [5.4 to 6.1] log IU/mL. The HCV-RNA decline between baseline and day 3, week 4, 8 and 12 were 2.7 [2.2 to 3.7], 4.98 [4.2 to 5.9], 5.2 [4.4 to 5.7] and 5.71 [5.2 to 6.1] log IU/mL, respectively.

**Conclusions:** The mean HCV-RNA declines in HCV-infected patients who started DAA-based therapies is high and fast (2.7 log IU/mL at day 3) even being genotype 1 (84.6%) the most prevalent and most of them cirrhotic (76.9%) patients. Moreover, although the proportion of patients with undetectable viral load at week 4 and 8 was 46.1% and 61.5%, respectively most of them (84.6%) achieved undetectable viremia at week 12. These results suggest that times to monitor HCV viral load and determined the treatment response during DAA therapies need to be re-evaluated and optimized.

No conflict of interest
Abstract: P_90

**Novel Diagnostic Technologies & Approaches**

**Hepatitis C virus genotype and subtype determination by NS3/NS5A/NS5B sequencing in comparison with commercially available assays**

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**Background:** A correct determination of hepatitis C virus (HCV) genotype in the era of direct-acting antiviral agents (DAA) combination therapies is crucial to select regimen options and to prevent non-response to very expensive treatment.

Some commercial assays may fail to identify genotype or subtype and genotype prediction based on sequencing and phylogenetic analysis is considered to be more accurate method.

In this study we compared baseline genotypes obtained by commercial Real Time PCR /reverse hybridization based assays with HCV sequencing performed on NS3 and/or NS5A and/or NS5B gene regions in serum samples from patients with chronic HCV infection.

**Materials & Methods:** HCV genotype was determined by direct sequencing in consecutive serum samples from patients whose genotype was obtained by commercial assays.

Genomic regions were amplified from HCV-RNA using reverse transcriptase PCR followed by a nested PCR. Amino acids 1-181 of HCV NS3 protease, amino acids 1-213 of NS5A and amino acids 219-347 of NS5B were included. Purified PCR products were sequenced using 3130-Avant Genetic Analyzer (Life Technologies, NY, USA); sequences were aligned by SeqScape Ver. 3.3 Software (Life Technologies, NY, USA).

For genotype determination, consensus sequences were submitted to the web based tool Geno2pheno. (http://www.geno2pheno.org/)

**Results:** Genotype assignment by sequencing was performed in 196 patients previously genotyped using commercial assays. HCV sequencing and commercial assays were in agreement for 182/196 (92.8%) samples. HCV sequencing reassigned 4 (2.0%) and 6 (3.1%) genotypes and subtypes, respectively, discordant with the previous genotype determination performed by Versant LiPA 1.0 assay (N=2), Versant LiPA 2.0 assay (N=2) and unknown methodology (N=6). Four HCV infected patients showed a mixed genotype by commercial assays: in one sample sequencing detected a single infection. In 3 samples the genotype determination remains unresolved and further testing is required.

**Conclusions:** With the increasingly therapeutic options, more patients will be eligible for therapy, and correct determination of the HCV genotype and subtype is of fundamental importance prior to treatment decision to ensure that the most appropriate regimen is applied for these patients.

Some of the data were presented at HELISA, Genoa 16-Sep-2015

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