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Abstracts

Oral Presentations
Abstract 01

HIV-1 B subtype genetically diverges over the years 2003-2016 and changes its pathogenic potential

Alteri C1, Fabeni L2, Scutari R1, Bemo G3, Di Carlo D1, Gori C2, Bertoli A3, Fedele V2, Carta S4, Vergori A2, Mastrorosa F, Bellagamba R3, Mussini C3, Colafigli M4, Montella F5, Pennica A6, Mastroianni C7, Girardi E2, Andreoni M8, Antinori A2, Ceccherini-Silberstein F1, Svicher V1, Perno C1,2, Santoro M1

1University of Rome Tor Vergata, 2Lazzaro Spallanzani Hospital IRCCS, 3University Hospital of Modena, 4San Gallicano Hospital, 5San Giovanni Hospital, 6Sant’Andrea Hospital, 7Santa Maria Goretti Hospital, 8University Hospital of Rome Tor Vergata

Background: We aimed at defining the genetic divergence of HIV-1 B subtype in Italy over the years 2003-2016 and its impact on CTL escape and on pathogenic potential.

Methods: A total of 3,328 HIV-1 B subtypes pol sequences were collected from HIV-1 newly diagnosed patients in Italy between 2003 and 2016 (one sequence per patient within 18 months from diagnosis). Sequences were analyzed for genetic divergence from consensus B (by genetic-distance, Tajima-Nei model) and non-synonymous and synonymous rates (dN and dS calculation). Shannon Entropy analysis was used to estimate the genetic-variability at each amino acidic position. Prevalence of CTL escape mutations (documented in the ‘CTL/CD8+ Epitope Variants and Escape Mutations’ HIV Los Alamos table) was also evaluated. Kruskal-Wallis and Chi-squared test for trend were used to estimate significant changes in genetic divergence, and in CTL escapes number over 4 time-windows defined according to the year of sample collection (2003-2006, 2007-2009, 2010-2012, 2013-2016).

Results: Most patients were males (84.6%) and Italian (78.2%), with a median (Interquartile-range [IQR]) age of 37 (30-45) years. Recent infections were 210 (21.1%, percentage based on available avidity-index). Median (IQR) viral-load, CD4+T cells and CD8+T cells were 4.9 (4.3-5.4) log10copies/ml, 333 (130-521) and 725 (460-1085) cells/mm3, respectively. Genetic-distance (mean ± standard-deviation [SD] 0.043±0.012 in the overall population) increased over time in both new diagnoses and recent infections (2003-2006: 0.037±0.008 and 0.039±0.008; 2013-2016: 0.048±0.009 and 0.049±0.009; p-values<0.001). Similar results were obtained for dN and dS. Entropy analysis showed that genetic-variability increased over time at 23 pol amino acid positions (5 of them associated to CTL escape variants, such as positions 71 in protease, and 135, 162, 177, 211 in reverse-transcriptase) and decreased at 14 amino acidic positions (only one, at protease position 82, associated to CTL escape). Sequences with >2 CTL escapes (55.3% in the overall population) progressively increased in both new diagnoses and recent infections (2003-2006: 45.8% and 37.3%; 2013-2016: 63.4% and 62.5%; p-values:<0.001 and 0.05), and were mainly characterized by the combination of CTL escapes at positions 162+177, and 135+162 (phi: 0.11 and 0.15, p-values<0.001). Interestingly, patients with >2 CTL escapes had higher viral-load, lower CD4+T cell count, and lower CD4/CD8 ratio than patients without CTL escapes at HIV-1 diagnosis (4.92 [4.37-5.43] vs. 4.84 [4.22-5.3] log10copies/ml, p-value=0.012; 312 [113-506] vs. 378 [188-577] cells/mm3, p-value=0.002; 0.37 [0.16-0.64] vs. 0.43 [0.18-0.74], p-value=0.005). By analyzing a subgroup of 153 patients with at least two pol sequences collected before therapy initiation (time-window between sequences within 18 months), the evolutionary rate per site per year (95% confidence-interval) was significantly lower in patients diagnosed after 2009 and with >2 CTL escapes than in patients diagnosed before 2009 and with ≤2 CTL escapes (0.0032 [0.003-0.004] vs. 0.0045 [0.004-0.005], p-value[LR]:<0.001[12.76]), suggesting a more suitable host adaptation for most recent viruses, driving a higher number of CTL escapes.

Conclusion: Our data revealed that HIV-1 B subtype is undergoing a deep genetic modification that involves CTL escape positions. In particular, viruses driving several CTL escapes increase over the years, and show higher pathogenic potential and best host adaptation.
Abstract 02

Integrase Inhibitors based regimens limit HIV-1 tropism evolution

Alteri C1, Scutari R2, Bertoli A1, Armenia D1, Gori C2, Fabbri G2, Mastroianni C1, Cerva C1, Cristaudo A3, Andreoni M4, Antonini A2, Svicher V3, Ceccherini-Silberstein F1, Perno C1,2, Santoro M1

1University of Rome Tor Vergata, 2Lazzaro Spallanzani Hospital IRCCS, 3Santa Maria Goretti Hospital, 4University Hospital of Rome Tor Vergata, 5San Gallicano Hospital

Background: Integrase-Inhibitors (INIs) are known to rapidly reduce HIV-1 viral-load, replication cycles, and new viral integrations, thus potentially limiting viral evolution. In this context, we assessed the role of INIs on HIV-1 tropism evolution in a cohort of HIV-1 infected patients followed in clinical practice.

Methods: 74 HIV-1 infected patients, starting an INI- (N=32) or a non-INI-based regimen (N=42), with 2 plasma V3 genotypic tests available at current-therapy initiation and at virological failure (VF, defined as at least one viral-load >50copies/ml), were included. Geno2pheno algorithm was used to evaluate the false-positive-rate (FPR) and infer HIV-tropism (using FPR<10% as cut-off for X4-tropism definition). FPRs between the V3-sequences obtained at VF and at baseline were compared to define a delta-FPR. Association between a delta-FPR<0 and INI- or non-INI-based regimen was assessed by Fisher’s exact test and multivariable logistic regression analysis. Impact of INI- or non-INI-based regimen on V3 evolution was also evaluated by genetic-distance calculation and by testing positive-selection (dN/dS).

Results: Patients were mainly male (68.9%), Italians (75.7%), and infected by B subtype (70.3%). Median (interquartile-range, IQR) viral-load and CD4+T cell count at baseline were 4.9 log10copies (3.7;5.6) and 193 cells/mm3 (65;431), respectively. Median (IQR) FPR at baseline was 33.8 (8.6;68.5) with a prevalence of X4 of 27.0%. At VF, median FPR was 22.2 (7.2;73.6) and the prevalence of X4 remained stable (28.4%). The median value (IQR) of delta-FPR was 0.0 (-5.4;+2.3), and 34 patients (45.9%) showed a delta-FPR<0. By considering antiretroviral-treatment, the prevalence of individuals with a delta-FPR<0 was lower in INI-treated respect to non-INI treated patients (31.3% vs. 57.1%, P=0.023). In line with this, the genetic-distance between V3 at VF and at baseline was significantly lower in INI-treated than in non-INI treated patients (median [IQR]: 0.05 [0.01;0.04] vs. 0.05 [0.03;0.09], P=0.013). dN/dS ratio between V3 sequences obtained at VF and at baseline showed a quite limited positive selection in INI-treated compared to that in non-INI treated patients (median [IQR]: 0.05 [0.0;0.59] vs. 0.26 [0.03;1.10], respectively). The dN/dS ratio at each V3 amino acid position showed an increased positive selection at only one position (aa:19) in V3 belonging to INI-treated patients and at 3 positions (aa:19;23;27) in V3 of non-INI treated patients. Of note, all these positions are known to favour CTL-escape variants and X4-switch. Multivariable logistic regression confirmed the independent correlation of INI-treatment with a lower probability of delta-FPR<0 (odds-ratio 0.16 [Confidence-Intervals:0.03-0.75] P=0.02), after adjusting for CD4+T cells and viral-load at baseline, subtype, and therapy-backbone.

Conclusion: This proof-of-concept study shows that, in real clinical practice, INI-based regimen may limit HIV-1 tropism evolution. This also results in a decrease of positive selection, in a lower risk of immune-escape variants development, and thus in a faster CD4+T cells gain.
Abstract 03

Virological failure to Protease inhibitors in Monotherapy is linked to the presence of signature mutations in Gag without changes in HIV-1 replication

Blanch-lombarte O1, Santos J2, Peña R3, Ruiz A4, Jimenez-Moyano E5, Neogi U2, Paredes R1,3, Clotet B1,2, G Prado J5

1AIDS Research Institute IrsiCaixa, Hospital Universitario Germans Trias i Pujol, 2Lluita contra la SIDA Foundation, Hospital Universitario Germans Trias i Pujol, 3Division of Clinical Microbiology, Karolinska

Background: Patients who fail to monotherapy with boosted Protease Inhibitors (PIs) containing regimens often have a virus that lacks protease associated mutations. The identification of HIV-1 genetic determinants outside of the protease involved in virological failure is an unresolved question that affects the clinical use of PIs. Here, we aimed to identify HIV-1 mutational patterns involved in virological failure through the characterization of Gag-protease genes from 9 HIV-1 infected patients failing Lopinavir/Ritonavir or Darunavir/Ritonavir monotherapy.

Methods: We performed a retrospective analysis of 520 HIV-1 infected patients that initiated Lopinavir/Ritonavir or Darunavir/Ritonavir monotherapy. Out of 520 patients, we found 9 patients that experienced virological failure. We amplified the Gag-protease coding regions by RT-PCR from plasma samples at the time of virological failure. We identified variations in the Gag-protease region by direct comparison with the HXB2 reference sequence and we performed Viral Epidemiology Signature Pattern Analysis (VESPA) to define HIV-1 signature mutations associated with virological failure. Moreover, 11 recombinant virus from 4 patients were generated by electroporation of Gag-protease derived plasmids in MT4 cells. The replication capacities of Gag-protease recombinant virus were assayed in Jurkat cells.

Results: We found mutational changes in HIV-1 Gag in the absence of protease associated mutation in all patients failing to Lopinavir/ritonavir or Darunavir/ritonavir monotherapy. We identified mutations associated to resistance or exposure of PIs in Gag cleavage sites at positions K436R (1%), I437V (1%) and S451N (1%) and in Gag at positions at higher frequencies R76K (55%), I389T (44%), E12K (33%), V370A (33%) and T81A (11%). VESPA analysis provided a signature pattern of mutations in Gag including residues K95R, E203D, V215M, R286K and R490K by comparison with 2000 subtype B HIV-1 Gag sequences from naïve patients (all p<0.01). We obtained 11 Gag-protease proviral clones from 4 out of 9 patients, one of which importantly revealed I54V and V82A protease associated mutations. Replicative capacity experiments demonstrated that Gag-protease recombinant virus replicated as well as the wild type in 63.6% of the cases. Meanwhile, the 36.4% of the virus showed a reduction in virus replication.

Conclusions: Our data identify mutations in HIV-1 Gag involved in the development of virological failure to PIs in monotherapy. These mutations are preferentially distributed in Gag structural proteins without affecting the virus replicative capacity in most of the patients. Additional experiments of susceptibility to PIs would be needed to address the direct role of these mutations in the development of virological failure to PIs.
Abstract 04

Frequency of occurrence of HIV-1 superinfection in MSM

Hebberecht L1, Vancoillie L1, Schauvliege M1, Dauwe K1, Mortier V1, Verhofstede C1

1AIDS Reference Laboratory UZ Ghent

Background: HIV-1 superinfection occurs when a HIV-positive individual gets re-infected with a different HIV strain after seroconversion. It distinguishes itself from co-infection being a dual infection before development of an immune response. Reports on superinfection are limited and mostly restricted to sequential infections with viruses of different subtypes. The scarceness of information on superinfection with more closely related viruses is partly due to the fact that its detection is technically challenging. Using deep sequencing we defined the extent of HIV-1 superinfection in a cohort of men who have sex with men (MSM) diagnosed with HIV-1 subtype B infection.

Material and methods: 74 patients were selected based on the following criteria: MSM, diagnosed between 2008 and 2013 with HIV-1 subtype B, at least 2 stored plasma samples available, collected before therapy initiation with an interval of > 6 months. Viral RNA was extracted from both plasma samples and subjected to deep sequencing of the env V3 region using Roche 454 technology. A minimum of 500 sequencing reads were analyzed per patient. They were edited, manually corrected for homopolymer errors and pooled based on homology. All reads with a coverage of 4 or more were aligned and a maximum likelihood phylogenetic tree was constructed. Visualization in iTol allowed inspection for indications of co- or superinfection, defined as the presence of two or more monophyletic clusters with ≥ 90% bootstrap support and separated by sequences of at least one other patient. Mean pairwise distances were calculated for each sample. For patients with evidence of co- or superinfection additional intermediate samples were deep sequenced subject to availability.

Results: 4 patients showed clear evidence of re-infection with a heterologous virus (5.4%). In all 4, the second strain was not seen in the first and oldest sample, indicating superinfection. The re-infection resulted in an increase in intrapatient pairwise genetic distance of 7, 10, 12 and 17 %. Subsequent analysis of intermediate samples confirmed the presence of a second virus strain, thereby excluding contamination. These longitudinal analyses also allowed more precise timing of the superinfection. In 2 of the 4 patients an increase in viral load of 0.94 and 2.19 log was observed shortly after the estimated time of superinfection.

Conclusions: Clear indications for superinfection were found in 5.4 % of subtype B infected MSM, confirming the hypothesis that this phenomenon is not rare in this population. Moreover, the observed frequency of superinfection is probably an underestimation due to the use of highly stringent criteria to define superinfection and some technical limitations such as relying on deep sequencing of a single small coding region (V3) and the use of leftover samples from routine blood collections which may not be adequately timed to detect the superinfection. In 2 of the 4 patients superinfection was associated with a considerable rise in viral load. Further analysis of the dynamics of the superinfecting strains and the potential formation of recombinants may provide new insights in immune pressure after infection and help the design of future vaccines.
Abstract 05

Molecular epidemiology and patterns of Transmitted Drug Resistance in HIV-1 infected African migrants followed-up in Portugal

Pingarilho M1, Pineda-Peña A1,2, Gomes P3,4, Libin P5,6, Theys K6, Martins M1, Dias S1, Vandamme A1,6, Camacho R6, Abecasis A1

1Global Health and Tropical Medicine (GHTM), Instituto de Higiene e Medicina Tropical/Universidade Nova de Lisboa (IHMT/UNL), Lisboa, Portugal, 2Molecular Biology and Immunology Department, Fundación Instituto de Inmunología de Colombia (FIDIC), Basic Sciences Department, Universidad del Rosario, 3Laboratório de Biologia Molecular (LMCBM, SPC, CHLO-HEM), 4Centro de Investigação Interdisciplinar Egas Moniz (CiEM), Instituto Superior de Ciências da Saúde Egas Moniz, 5Artificial Intelligence lab, Department of computer science, Vrije Universiteit Brussel, 6Clinical and Epidemiological Virology, Department of Microbiology and Immunology, Rega Institute for Medical Research, KU Leuven, University of Leuven

Introduction: The surveillance and characterization of the HIV transmission patterns, with or without resistance to antiretroviral ARVs (TDR), is of paramount importance for public health. In Portugal, the prevalence of HIV-1 is less than 1% in the general population. 24.1% of new diagnoses occurred in migrants, contributing to the disproportionate number of new infections acquired heterosexually (1).

Objectives: To analyze the prevalence of TDR in HIV-1 infected patients from the migrant population in Portugal.

Methods: A dataset of HIV-1 positive migrants from Angola, Mozambique, Cape-Verde and Guinea-Bissau - clinically followed in Portuguese hospitals between 2001 and 2014 - was analyzed. Data collected include clinical and social characteristics and the viral genomic sequences, obtained from the first drug resistance test, before the beginning of ARVs therapy.

Results: 858 HIV-1 positive patients were included (52% female and 47% male). Transmission route is unknown in 85% of the cases. 25.3% were infected with CRF02_AG, 15.6% with subtype C and 14.9% with subtype G. In migrants from Angola and Mozambique the major prevalent subtype is C presenting 21.6% and 71.8%, respectively, from Cape-Verde is G (29.7%) and from Guinea-Bissau is CRF02_AG (62.9%). Between 2001 and 2014, 7.8% (IC-95%, 5.8-10.4) of the patients presented primary resistance (PR) to ARVs which increases to 9.3% (IC-95%, 7.0-13.0) if we consider only patients who have undergone the resistance test in the last 4 years. The PR presented in Guinea-Bissau is worrying because it presents the highest value (10%), increasing to 12% in the last 4 years (2010-2014). PR to Non-Nucleoside Reverse Transcriptase Inhibitors is the highest, with 5.0% of patients presenting resistance to this class.

Conclusion: This study allows us to have an overview about the molecular epidemiology of the HIV-1 epidemic in the immigrant population in Portugal, in order to help Public Health entities to design prevention policies.

References:

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Abstract 06

High levels of resistance among HIV-1 treatment naive patients in Greece, a nationwide study: Evidence for country and regional level transmission networks

Paraskevis D1, Kostaki E1, Magiorkinis G1, Gargalianos P2, Xylomenos G2, Lazanas M3, Chini M4, Metallidis S5, Magiorkinis E1, Skoutelas A6, Papastamopoulos V6, Mouzaki A6, Antoniadou A1, Papadopoulos A7, Psichogiou M8, Daikos G9, Paraskeva D1, Piladas D10, Chrysos G11, Paparizos V12, Koukounti S12, Chatzidimitriou D13, Sambatakou H14, Sipsas N15, Lada M16, Panagopoulos P17, Maltezos E17, Drimis S11, Gogos C6, Hatzakis A1, Skoura L4

1Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, 21st Department of Internal Medicine, G. Genimatas GH, 33rd Internal Medicine Department-Infectious Diseases, Red Cross Hospital, 4National AIDS Reference Centre of Northern Greece, School of Medicine, Aristotle University of Thessaloniki, 5th Department of Medicine and Infectious Diseases, Evaggelismos GH, 6Department of Internal Medicine, Medical School, University of Patras, 7th Department of Medicine, Attikon University GH, National and Kapodistrian University of Athens, 8th Department of Medicine, Laikon GH, National and Kapodistrian University of Athens, 9School of Medicine, Aristotle University of Thessaloniki, 10Department of Internal Medicine, Tzaneio GH, 11HIV/AIDS Unit, 2nd Department of Internal Medicine, Hippokration GH, Medical School, National and Kapodistrian University of Athens, 12Pathophysiology, Laikon GH, National and Kapodistrian University of Athens, 132nd Department of Internal Medicine, Sismanogleio GH, 14Department of Internal Medicine, University GH, Democritus University of Thrace

Background: Transmitted HIV drug resistance (TDR) among patients unexposed to antiretrovirals remains a serious concern since it is associated with increased risk of virologic failure after therapy initiation. Our aim was to investigate the prevalence of resistance among drug-naive HIV-infected individuals in Greece, and also to investigate potential transmission networking among those carrying resistant strains using nationwide design.

Material and methods: We analyzed protease (PR) and partial reverse transcriptase (RT) sequences from 4,020 treatment naïve patients obtained between 2006 and middle-2015 sampled in different areas (Southern/Central Greece: 3,055; Northern Greece: 931; and Western Greece: 34). We included the earliest sampled sequences for all individuals. Antiretroviral drug resistance was estimated using the HIVdb interpretation algorithm. The within country dispersal patterns of resistance viruses were estimated by phylogenetic analysis using as references globally sampled sequences. Phylogenetic trees were inferred by maximum likelihood method as implemented in RAxML using the GTR+G as nucleotide substitution model with bootstrapping.

Results: PR and partial RT sequences scored as non-sensitive in HIVdb program were considered resistant. The prevalence of resistance to PIs, N(t)RTIs and NNRTIs was in: Southern/Central Greece, 4.8% (N=146), 2.5% (N=76), 17.3% (N=531), ii) Northern Greece, 2.2% (N=20), 10.9% (N=101), 23.9% (N=222) and iii) Western Greece 2.9% (N=1), 2.9% (N=1), 14.7% (N=5), respectively. The overall prevalence to NNRTIs was the highest across the country. Northern Greece was the area with the highest levels of resistance including N(t)RTIs and NNRTIs. The dominant resistance mutations were E138A, and K103N for NNRTIs and A62V and T215 revertants for N(t)RTIs. Y181C (n=65, 7%) and E138Q (n=137, 4.5%) were detected at high levels in Northern and Southern/Central Greece, respectively. Phylogenetic analyses showed that the most prevalent resistant mutations (E138A, K103N for NNRTIs and A62V and T215 revertants for N(t)RTIs. Y181C (n=65, 7%) and E138Q (n=137, 4.5%) were detected at high levels in Northern and Southern/Central Greece, respectively. Phylogenetic analyses showed that the most prevalent resistant mutations (E138A, K103N, E138Q and Y181C) were mostly associated with transmission within phylogenetic clusters from Greece (local transmission networks, LTNs). For subtype A we found that most sequences with E138A and K103N resistance mutations from Southern/Central Greece clustered within four (148 out of 179, 82.7%) and one LTNs (48 out of 56, 85.7%), respectively, while for Northern Greece most sequences with E138A, K103N and Y181C formed a single cluster (n=143) consisting of three mutation-specific sub-networks. The origin of the resistance cluster in Northern Greece was from...
Southern/Central Greece. For subtype B most sequences with different mutations (K103N, E138A, T181C, T215 revertants and V179D) formed a single cluster in contrast to sequences from Southern/Central Greece which clustered across subtype B phylogeny.

Conclusions: We found high levels of NNRTI resistance across Greece with the highest prevalence to be in Northern Greece. Our study provides evidence that the majority of the resistant viruses (Southern/Central Greece: n=348, 80.2% and Northern Greece: n=172, 96.6%) were transmitted within regional transmission networks. Notably, the existence of regional clusters for both subtype A and B resistant viruses in Northern Greece suggest high transmission networking of the population in this area; a finding that might explain the higher prevalence of transmitted drug resistance (TDR) in Northern Greece.

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Abstract 07

Trends for transmitted drug resistance and prevalence of non-B subtypes in recent HIV infections – results from the national molecular surveillance, Germany 2013 - June 2016

Hauser A1, Hofmann A2, Hanke K1, Bremer V2, Bartmeyer B2, Bannert N1

1Robert Koch Institute - HIV and other Retroviruses, 2Robert Koch Institute - HIV/AIDS, STI and Blood-borne Infections

Background: The molecular HIV surveillance in Germany provides information on current trends on transmitted drug resistance (TDR) and of HIV-1 subtypes in recent infections of newly diagnosed cases. Between January 2013 and June 2015 the proportion of TDR was 10.6% while subtype non-B infections, in particular subtype A, was increasing. The aim of the present study was to provide an update on these trends by adding data from the most recent HIV-1 infections diagnosed between July 2015 to June 2016 in Germany.

Materials and methods: Dried serum spots (DSS) of newly diagnosed HIV-cases are received together with the statutory notification data. Samples classified as "recently acquired infections" (<155 days) by a "Recent Infection Test Algorithm" (BED-CEIA ODn values ≥0.8 and CD4 cell counts >200 cells/ml) are genotyped in the HIV-pol-region to identify (i) TDR in the protease (PR) and reverse transcriptase (RT) genomic region according to WHO SDRM list (ii) mutations in the integrase (INT) genomic region associated with resistance to integrase inhibitors according to IAS-USA list 2016 and (ii) the HIV-1 subtype according to the REGA subtyping tool.

Results: Between January 2013 and June 2016 3,247 of 10,312 DSS were classified as recent infections. Of these, 1,644 (50.3%) were successfully analysed in HIV-1 PR-RT and 655 (39.8%) additionally in INT. The prevalence of TDR was 10.6%, with a slightly decreasing tendency for nucleotide RT inhibitors (NRTI)
Levels of TDR against non-NRTIs were slightly increasing (3.9% (11/279) in 2013 to 5.5% (16/289) in 2016; \( p \text{ trend}=0.13 \)), in particular due to the K103N (3.2% (9/279) in 2013 to 4.5% (13/289) in 2016; \( p \text{ trend}=0.15 \)). Integrase resistance mutations were present in 2.1% (13/655) of the sequences and consisted of the L74M and T97A substitutions (n=5 and n=7, respectively and 1x both) conferring only minimal reduction in susceptibility to INT inhibitors. The previously observed increase of subtype A infections to 11% (67/219) in 2015 is followed by a decline to 8% (24/268) in 2016. In contrast, circulation of subtype C gained momentum with a prevalence of 7.7% (22/286) in 2016. These subtype C infections in 2016 occurred mainly in Germans (16/22), transmitted by heterosexual contact (4/16), MSM (4/16), intravenous drug use (6/16) or by an unknown route (2/16).

Conclusions: TDR prevalence in recent HIV infections among notified newly diagnosed HIV cases in Germany between January 2013 and June 2016 remained stably high at 10.6%. However, a seemingly increasing tendency of the NNRTI mutation K103N as well as the frequency of INT inhibitor resistance mutations should be monitored due to its impact on many current first-line regimens. Our data also demonstrate that subtype C is circulating in the German population. In particular the recent accumulation in German intravenous drug users should be observed.

### Abstract 08

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


**Background:** Doravirine (DOR) is a novel HIV-1 non-nucleoside reverse transcriptase (NNRTI) that is currently in clinical development. It has been recently shown that DOR in combination therapy has non-inferior efficacy to darunavir/r (800/100 mg) in treatment-naive patients. DOR has an in vitro resistance profile that is distinct from other NNRTIs retaining activity against viruses containing the most frequently transmitted NNRTI mutations, K103N, Y181C and G190A. DOR selects for distinct mutations in vitro; including mutations at positions 106, 108, 221 and 227 with multiple mutations required for significant levels of resistance. The aim of this study was to examine the prevalence of DOR-associated mutations in HIV-1-infected treatment-naive patients in Europe.

**Materials and methods:** Resistance genotypic tests were performed at five reference laboratories, 2 in Paris (Pitié-Salpêtrière and Bichat Claude Bernard hospitals) and 3 in Italy (University of Rome Tor Vergata, INMI Spallanzani-IRCCS, Modena Hospital). A total a 7004 reverse transcriptase sequences obtained between 2010 and 2016 from HIV-1 treatment-naive patients in routine clinical care were analyzed. DOR-associated mutations identified in

Results: Among the 7004 sequences, 3355 were performed between 2010-2012 and 3649 between 2013-2016. The distribution of subtypes was: 53.7% B, 18% CRF02, 4.1% A1, 3.8% C, 3.3% F1 and 17% other various non-B. There was an increase of non-B subtypes between 2010-2012 and 2013-2016 (41% versus 48%, p < 0.001). The overall prevalence of sequences with at least 1 DOR-associated mutation was 1.3% (n = 91). This was significantly lower than the prevalence of sequences with at least 1 EFV-associated mutation (4.3%, n = 304) or with at least 1 RPV-associated mutation (6.7%, n = 472), (p < 0.001). Among the DOR-associated mutations, the most frequent mutations were V106A/M 0.1% (7), V108I 0.6% (45), H221Y 0.2% (16), F227C/L/V 0.1% (7), M230I 0.05% (3), L234I 0.01% (1), P236L 0% and Y318F 0.3% (22).

There was no significant increase over time and no relationship with any HIV-1 subtype for any of these mutations. In comparison, the prevalences of common NNRTI mutations K103N/S, E138A/G/K/Q/R, Y188C/H/L and G190A/E/S were 2.4% (171), 5.3% (369), 0.3% (20) and 0.6% (41), respectively. Between 2010-2012 and 2013-2016, there was a significant increase in K103N/S (1.8% versus 3%, p = 0.002) and in G190A/E/S (0.3% versus 0.8%, p = 0.003).

Conclusions: These results suggest that the prevalence of DOR-associated mutations in HIV-1-infected treatment-naive patients is very low and significantly lower than EFV or RPV-associated mutations. In addition, the prevalence of DOR-associated mutations in this population of patients was stable over time and there was no relationship between the presence of DOR-associated mutation and HIV-1 subtype.

Abstract 09

The extent of genetic variability in HBsAg C-terminus profoundly affects HBsAg levels in eAg-negative chronic HBV genotype D infection

Salpini R1, Battisti A1, Carioti L1, Di Carlo D1, Anastasiou O2, Gill U3, Colaggiosi L1, Bertoli A1, Fabeni L4, Fini V5, Piermatteo L1, Iuvara A6, Malagnino V7, Cerva C8, Lichtner M9, Mastroianni C9, De Sanctis G9, Paoloni M9, Marignani M9, PasquaZZi C10, Iapare N11, Parutti S12, Vecchiet I13, Samati L14, Andreoni M9, Angelico M15, Greliti S16, Kennedy P3, Verheyen J2, Perno C1, Svicher V1

1Tor Vergata University, Department of Experimental Medicine and Surgery, 2Institute of Virology, University-Hospital, University Duisburg-Essen, 3Hepatology, Centre for Immunobiology, Blizard Institute, Barts and The London School of Medicine & Dentistry, QMUL, 4National Institute for Infectious Diseases, L. Spallanzani, IRCCS, Antiretroviral drug monitoring laboratory, 5Tor Vergata University Hospital, Microbiology and Virology Unit, 6Tor Vergata University Hospital, Infectious Diseases Unit, 7 “Sapienza” University, Department of Public Health and Infectious Disease, 8 Umberto I University Hospital, 9 S.S. Filippo e Nicola” Hospital, Infectious Disease Unit, 10 S. Andrea Hospital, Department of Gastroenterology, 11 “San Salvatore Hospital”, 12 Nuovo Regina Margherita” Hospital, 13 Infectious Disease Unit, Pescara General Hospital, 14 Clinic of Infectious Diseases, Department of Medicine and Science of Aging, University “G. d’Annunzio” Chieti-Pescara, 15 Tor Vergata University Hospital, Hepatology Unit

Background: HBsAg levels have been proposed as a marker of the intrahepatic HBV reservoir. However, a recent in vitro study showed variation in HBsAg production in different HBV genotypes. Paucity of information is so far available on HBsAg levels in patients infected with different HBV genotypes in HBeAg-negative chronic HBV infection, and on factors underlying such differences.

Methods: This study includes 301 consecutive patients with HBeAg-negative chronic HBV infection, drug-naive, and monitored for >1 year: 126 inactive carriers with persistent serum HBV-DNA <2,000IU/ml and normal transaminases (defined as group A), and 175 with persistent
serum HBV-DNA >2,000IU/ml (defined as group B). HBV genotype is assessed by phylogeny. Degree of HBsAg variability is measured by calculating mean genetic distance. In patients infected with HBV genotype D, the correlation of mutations with HBsAg levels was determined by Fisher exact test. I-Tasser is used to predict three-dimensional HBsAg structures (aa:1-226) and their stability (∆∆G[wt-mutated]<0 indicating reduced stability in presence of mutation based on Quan,2016).

Results: Median (IQR) serum HBV-DNA is 2.8(2.3-2.9) and 4.1(3.7-5.2)IU/ml, while median (IQR) ALT is 28(21-38) and 34(25-55)U/L in group A and B, respectively. HBV-genotypes are: D=72.2%, A=15.9%, E=11.9% in group A, and D=78.3%, A=14.3%, E=7.4% in group B. In group A, median (IQR) HBsAg is significantly lower in genotype-D than in genotype-A and E (730[204-2,932] vs 5,741[3,526-14,290] and 10,288[7,172-13408]IU/ml, P<0.001). A similar result is observed in group B (3,436[1,466-8,126] vs 7,992[5,069-21,221] and 10,825[6,544-18,216]IU/ml, P<0.01). Moreover, in group A, HBsAg levels <1,000IU/ml (proposed to define HBV genotype-D patients as inactive carriers) are observed in 57.1% of genotype-D, 15.0% of genotype-A and 0% of genotype-E (P<0.001). In genotype-D, the degree of HBsAg C-terminus genetic variability is significantly higher in patients with HBsAg<1,000IU/ml than in patients with HBsAg>1,000IU/ml (0.041+0.024 vs 0.022+0.017, P<0.001). Interestingly, in genotype D, specific mutations in HBsAg C-terminus (known to be critical for HBsAg secretion) significantly correlated with HBsAg <1,000IU/ml (V190A: 13.7% vs 0%, P=0.02; Y206C: 29.4% vs 0%, P<0.001; and S204N: 43.1% vs 10.2%, P=0.001). By structural analysis, these mutations strongly decrease the stability and profoundly affect the conformation of HBsAg C-terminus. This suggests an impaired HBsAg C-terminus stability in presence of these mutations that may lead to an impaired secretion.

Conclusions: HBsAg levels in HBV-genotype D are significantly lower than in genotype-A and E in different phases of HBeAg-negative chronic HBV infection, including the inactive carrier status. In genotype-D infected patients, the extent of genetic variability in HBsAg C-terminus specifically correlates with lower HBsAg levels and profoundly affect the structure of HBsAg C-terminus, suggesting an impaired HBsAg secretion. In this setting, HBsAg levels may not directly reflect the transcriptional activity of intrahepatic reservoir, supporting the role of HBV genotyping to better characterize patients with HBeAg-negative chronic HBV infection.
Abstract 10

Positively charged mutations in HBsAg C-terminus are tightly correlated with HBV-induced hepatocellular carcinoma by altering the structural folding of this domain

Salpini R1, Carioti L1, Aragri M1, Di Carlo D1, Colagrossi L1, Battisti A1, Piermatteo L1, Bertoli A1, Fabeni L1, Ricciardi A2, Longo R3, Romano S3, Cappiello G3, Spanò A3, Trimoulet P4, Fleury H4, Vecchiet J5, Lapadre N5, Barfattani A1, Mari T6, Pasquazzi C2, Lenci I7, Francioso S8, Parruti G1, Sarmati L2, Andreoni M9, Angelico M10, Ceccherini-Silibrstein F1, Perno C1, Svicher V1

1Tor Vergata University, Department Of Experimental Medicine And Surgery, Rome, Italy, 2Tor Vergata University Hospital, Infectious Diseases Unit, 3Microbiology and Virology Unit, S. Pertini Hospital, 4Hospital Pellegrin tripode, Laboratoire de Virologie, 5Clinic of Infectious Diseases, Department of Medicine and Science of Aging, University “G. d’Annunzio” Chieti-Pescara, 6San Salvatore Hospital, 7Hepatology Unit, S. Giacomo Hospital, 8Nuovo Regina Margherita Hospital, 9S.Andrea Hospital, Department of Gastroenterology, 10Tor Vergata University Hospital, Hepatology Unit, 11Infectious Disease Unit, Ospedale Civile, 12National Institute for Infectious Diseases L. Spallanzani-IRCCS

Background: Acquisition of positively charged amino acids (aa) can affect the folding of a transmembrane protein domain. HBsAg C-terminus is a hydrophobic transmembrane domain, composed by alpha-helices, critical for proper HBsAg secretion. Altered HBsAg folding in endoplasmic reticulum (ER) membrane can affect HBsAg secretion and in turn promote HBV-induced hepatocellular carcinoma (HCC). The role of mutations associated with gain of charged aa in HBsAg C-terminus on HBV-induced HCC onset is not known.

Methods: This study includes 807 HBV chronically infected patients from routine clinical practice: 28 with HCC (78.6% D; 21.4% A), and 779 patients without HCC (79.8% D; 20.2% A). Mutations associated with gain of charged aa in HBsAg C-terminus (aa189-226) are analyzed. Association of mutations with HCC is assessed by multivariable logistic regression model. Hydrophobicity profiles of HBsAg C-terminus are constructed to predict stability of a domain in a membrane (Black, 1991). I-Tasser is used to predict three-dimensional HBsAg structures (aa:1-226) and their stability (ΔΔG[wt-mutated]<0 indicating reduced stability in presence of mutation based on Quan, 2016).

Results: The gain of >1 positively charged aa at HBsAg C-terminus positions 204, 207, and 210 tightly correlates with HCC (71.4% with HCC vs 30.2% without HCC, P<0.001). Multivariable analysis confirms this correlation correcting for patients’ demographics, HBV genotype, serum HBV-DNA and anti-HBV drugs use (OR[95%CI]:6.3[2.6-15.3], P<0.001). The gain of positively charged aa derives from mutations S204R, S207R and S210R present in 14.3%, 28.6% and 28.6% of HCC-patients. S204R, S207R and S210R determine a reduction in hydrophobicity index of HBsAg C-terminus compared to wt (S204R:16.0, S207R:16.0, S210R:16.2 vs wt:16.4), and in ΔΔG values (ΔΔG[S204R-wt]=0.27; ΔΔG[S207R-wt]=0.11; ΔΔG[S210R-wt]=0.14). Moreover, S207R and S210R determine a shortening of membrane-spanning alpha-helix motif compared to wt (predicted alpha-helix length: aa209-224 for S207R and S210R vs 205-225 for wt). Overall, this suggests an impaired HBsAg C-terminus stability in presence of these mutations.

Conclusions: Gain of positively charged aa at specific HBsAg C-terminus positions tightly correlates with HCC, by altering the proper folding of this domain in ER membrane. These mutations might affect HBsAg secretion and in turn contribute to the initiation of HBV-related tumorigenesis. Their role in identifying patients at higher HCC-risk deserves further investigation.
Abstract 11

Analysis of the association of HIV-1 low level viremia and treatment failure to antiretroviral therapy

Lübke N1, Pironti A2, Knops E3, Jensen B4, Oette M5, Esser S6, Lengauer T2, Timm J1, Kaiser R3

1Institute of Virology, Heinrich-Heine-University Düsseldorf, 2The Computational Biology and Applied Algorithmics Department, Max Planck Institute for Informatics, 3Institute of Virology, University of Cologne, 4Department of Gastroenterology, Hepatology and Infectiology, Heinrich-Heine-University, University Hospital, 5Department of Gastroenterologie, Augustinerinnen Hospital, 6Department of Dermatology, University Hospital Essen

Background: The German-Austrian guidelines for the treatment of HIV infection define therapeutic success as the reduction of the HIV-1 viral load (VL) below 50 copies/ml. Low level viremia (LLV) is defined as a repeated detection of HIV-1 RNA below a defined threshold. Unfortunately, the threshold at which LLV becomes predictive of disease progression varies between studies. Nevertheless, LLV is a constant problem in the management of HIV therapy, as it has been previously been shown to be predictive to virological failure (VF).

Here, we provide an independent analysis of the association of LLV and subsequent VF to antiretroviral therapy (ART).

Materials and methods: The AREVIR database comprises clinical and virological data of therapy-naïve and -experienced HIV-1-infected patients in Germany, including the data of the RESINA cohort. We queried the AREVIR database for patients who attained confirmed therapeutic success under ART and who experienced confirmed LLV thereafter. We constrained our query to therapies in which the VL was measured at least once every 24 weeks. In our study, LLV is defined as repeated VL measurements between 50 and 200 copies/ml after initial therapeutic success. We defined VF as a confirmed VL greater than 200 copies/ml following therapeutic success. p-values were calculated with Fishers’ exact and Wilcoxon rank sum test.

Results: The database query resulted in 2,485 first-line and 3,657 further-line therapies. LLV occurred in 294 (4.8%) of these therapies, specifically in 47 (1.9%) first-line and in 247 (6.8%) further-line therapies. The mean time to LLV was 27 months (σ = 20.7), with no significant differences between first- or further-line therapies (p=0.4597). The majority of patients showing LLV were treated with PI-based therapies (165/294; 56%), followed by NNRTI-based regimens (76/294; 26%). 56 out of 294 (19%) patients experienced VF after LLV with a median VL at failure of 472 copies/ml (range 203-116590 cop/ml) after a mean LLV episode of 77.4 weeks (σ=68.0). The failure rate was increased in therapy-experienced patients (48/247; 19.4%), as compared to therapy-naïve patients (5/47; 10.4%); (p=0.2129). Although the most risk for LLV was detected with PI-based and NNRTI-based therapies (165/294; 56.1% and 76/294; 25.9%), the risk of VF subsequent to LLV was comparable between the different therapies regardless of the backbone (33/165; 20% and 13/76; 17.1%, respectively; p=0.6049). Among all drug classes, VF was never related to entry inhibitors, integrase inhibitors or the more recently approved compounds DRV, TPV, and RPV (45/204 vs. 0/83, respectively; p<0.0001).

Conclusions: The prevalence of LLV in patients on suppressive ART is low (4.8%). Nevertheless, 19% of patients with LLV experienced VF thereafter. The strongest predictor for VF after LLV was a treatment regimen based on drugs approved before 2005. Therefore, episodes of LLV in patients treated with drugs with high potency and a high barrier to resistance are not predictive of VF.
Abstract 12

Effectiveness of protease inhibitor-based second-line antiretroviral therapy for the treatment of HIV-1 infection in sub-Saharan Africa: systematic review and meta-analysis

Geretti A1, SSA Second-Line ART Study Group

1University Of Liverpool

Background: The need for second-line ART is projected to increase in the next decade in sub-Saharan Africa in keeping with the expansion of treatment provision. A recent meta-analysis of studies reporting the effectiveness of first-line ART indicated that 67% of participants showed virological suppression at 12 months, 65% at 24 months, and 62% at 48 months by intention-to-treat (ITT) analysis. Systematically collated data on outcomes of second-line ART are needed in order to inform policy and access to third-line therapy. As a number of recent trials and observational studies have examined the outcomes of second-line ART in sub-Saharan Africa, the aim of this study was to present pooled estimates of the effectiveness of second-line ART in the region.

Methods: We performed a systematic review and meta-analysis of studies reporting the virological outcomes of protease inhibitor (PI)-based second-line ART in sub-Saharan Africa. The primary outcome was virological suppression (HIV-1 RNA <400 copies/ml) after 48 and 96 weeks of treatment. The secondary outcome was the proportion of patients with major protease resistance mutations at failure. Pooled aggregate data were analysed using a DerSimonian-Laird random effects model.

Results: By ITT analysis, virological suppression occurred in 70.0% (95% CI 56.7, 81.7) at week 48 (3408 participants, 13 studies), and in 61.5% (47.2, 74.9) at week 96 (2145 participants, 8 studies). The rate of emergence of major protease resistance mutations at failure (12 studies) was 0-7% for the total at-risk population, and 0-25% for the virological failure population. The likelihood of virological suppression at week 48 was lower (OR 0.3 [95% CI 0.10, 0.90]; p=0.032) among participants lacking evidence of pre-existing NRTI resistance (5 studies). NRTI resistance mutations were predominantly M184V (prevalence range 67.0-84.6% of participants) and thymidine analogue mutations (12.5-56.2%). Rates of virological suppression were significantly higher among participants of RCTs compared to observational cohorts at both week 48 (85.9% [95% CI 80.8, 90.3] vs. 58.1% [46.0, 69.7]; p<0.001) and week 96 (76.5% [72.8, 80.4] vs. 55.7 [43.1, 67.8]; p<0.001).

Conclusions: In the first two years of treatment, PI-based second-line ART (with continued NRTI use) achieves virological suppression in most patients in sub-Saharan Africa and carries a relatively low risk of PI resistance. Long-term data are needed. As one third of patients did not achieve virological suppression, optimal strategies for the management of second-line ART failure represent an urgent research priority.
Abstract 13

Impact of M184V resistance mutation on virological efficacy of lamivudine-based maintenance dual therapies: an Italian ARCA cohort study

Gagliardini R1, Ciccullo A1, Borghetti A1, Maggiolo F2, Bartolozzi D3, Borghi V4, Pecorari M6, Di Biagio A6, Callegaro A7, Bruzzone B8, Saladini F9, Paolucci S10, Bellazzi L11, Di Giambenedetto S12, De Luca A12

1Institute of Infectious Diseases, Catholic University Of Sacred Heart, 2Division of Infectious Diseases, ASST Papa Giovanni XXIII, 3Clinic of Infectious and Tropical Diseases, Azienda Ospedaliera Universitaria Careggi, 4Clinica Malattie Infettive e Tropicali, Azienda Ospedaliero Universitaria di Modena, 5Microbiology and Virology Unit, Azienda Ospedaliero Universitaria di Modena, 6Infectious Diseases, IRCCS AOU San Martino-IST, 7Microbiology and Virology Unit, ASST Papa Giovanni XXIII, 8Hygiene Unit, IRCCS AOU San Martino-IST, 9Department of Medical Biotechnologies, University of Siena, 10Virologia Molecolare, Fondazione IRCCS Policlinico San Matteo, 11Infectious Diseases Clinic, S. Matteo Hospital, 12Infectious Diseases Unit, Siena University Hospital

Background: The M184V resistance mutation is associated with reduced susceptibility to lamivudine (3TC) but also with reduced HIV replication. A previous study showed high efficacy of dual therapy (DT) with a protease inhibitor + ritonavir (PI/r) + 3TC despite the presence of M184V in a large proportion of patients (pts). Nonetheless, a direct comparison of DT efficacy with or without of M184V has not been performed. Aim of this study was to compare virological efficacy of DT in pts with M184V+ and without (M184V-) a history of M184V detection. As an additional control group, we used patients treated with PI/r or dolutegravir (DTG) monotherapies.

Methods: The ARCA (Antiviral Response Cohort Analysis) database, containing data on HIV resistance and antiretroviral therapy (ART) from around 40,000 pts in Italy was queried to retrieve pts with HIV-RNA <200 copies/mL switching to DT (3TC+ PI/r or DTG) and with at least one previous genotype. Pts were followed from baseline (BL, starting of DT) until discontinuation or virological failure. The primary endpoints were time to virological failure (VF, HIV-RNA >50 copies/mL in 2 consecutive determinations or a single determination >1000 copies/mL) and to treatment failure (TF, defined as VF or treatment discontinuation).

Results: 454 pts were included: 317 (70%) males, 57 (13%) with previous AIDS events, 92 (20%) HCV-positive, median age was 48 years (yrs) (IQR 41-55), duration of ART 8 yrs (4-15), CD4+ at BL 626 cells/µL (450-802), CD4+ at nadir 205 cells/µL (72-300), 89 were M184V+ in the last available genotype prior to BL. 47 (10%) switched to 3TC with lopinavir/r, 108 (24%) with atazanavir/r, 166 (37%) with darunavir/r and 128 (28%) with dolutegravir. The groups M184V+ and M184V- differed in term of sex, CD4+ at nadir, age, duration of ART. Median follow-up was 1.7 yrs (0.8-2.9).

VF occurred in 13/365 M184V- pts (1.6 per 100 PYFU) and in 4/89 M184V+ pts (1.9 per 100 PYFU). At survival analysis, the 3-yr probability of remaining free from VF was 94.7% (95% CI 91.6;97.8) in M184V- and 93.7% (87.6;99.8) in M184V+ while the respective probability of remaining free from TF were 40.5%(33.3;47.7) and 43.9% (28.0;59.8) with no impact of M184V. However, the probability of remaining free from TF was significantly greater in the overall DT group (p<0.001) and even in M184V+ DT group (p=0.007) when compared with the monotherapy group (n=232).

At multivariate Cox regression analysis, only sex (male vs female, aHR=0.29, p=0.012) and zenith viral load (aHR 2.73 per 1-log higher, p=0.005) were independently associated with VF while M184V was not a predictor of either VF or TF. Virological blips occurred in 34/318 (11%) M184V- pts and 19/85 (22%) M184V+ pts (p=0.001). A statistically higher mean change of CD4+ at 1 year in the M184V- group (+73 cells/µL versus +29, p=0.027) was observed, but this was not confirmed at 2 and 3 yrs.

Conclusion: The presence of M184V at BL did not seem to affect the virological efficacy of 3TC-based maintenance DT, although it was associated with a larger number of virological blips and lower short-term CD4+ gain.
Abstract 14

**A low genetic barrier and reactivation of pre-existing proviruses harboring a single INSTI-RAM explains virological failure during dolutegravir maintenance monotherapy**

Wijting I², Rijnders B², van der Ende M², Pas S¹, Lungu C¹, Boers P¹, Gruters R¹, Boucher C¹, van Kampen J¹

¹Department of Viroscience, Erasmus MC, ²Department of Internal Medicine, Erasmus MC

**Introduction:** Given the high genetic barrier observed in vitro for dolutegravir (DTG), an integrase strand-transfer inhibitor (INSTI), we hypothesized that DTG could be used as maintenance monotherapy in HIV-1 infected patients suppressed on cART. However, we recently conducted a randomized controlled trial (NCT02401828) and showed that DTG maintenance monotherapy in these patients leads to higher virological failures rates (8/95) than in patients continuing with cART (3/152) [Wijting et al., CROI, 2017]. Here, we describe the dynamics of resistance in HIV-1 infected individuals with virological failure (VF) during DTG maintenance monotherapy.

**Methods:** A total of 13 patients with VF were studied: 8 patients from the NCT02401828 study (CD4 nadir ≥ 200 cells/µL, HIV-1 RNA zenith < 100,000 cop/mL), 2 from our DTG maintenance monotherapy pilot study (CD4 nadir <200 cells/µL, HIV-1 RNA zenith < 100,000 cop/mL, 2/4 patients had VF), and 3 patients from our clinic who were treated with DTG maintenance monotherapy on the discretion of the treating physician. VF was defined as a confirmed plasma HIV-1 RNA > 200 copies/mL for the patients participating in clinical trials or a single plasma HIV-1 RNA > 200 copies/mL for the remaining patients. Sanger sequence of the integrase gene was determined before start with DTG monotherapy and at time of VF. The Stanford HIV drug resistance database (updated 02-03-2017) was used to define INSTI resistance associated mutations (RAMs). For the 10 patients participating in clinical trials, self-reported compliance was > 95%, and DTG plasma concentrations at the moment of VF were determined.

**Results:** Patients included in this study had a median CD4 nadir of 230 cells/µL (IQR 85-310) and a median HIV-1 RNA zenith of 56.300 cop/mL (IQR 19.300-99.635). The median time of a suppressed HIV-1 RNA in plasma by cART before switch to DTG maintenance monotherapy was 71 months (IQR 34-119). Twelve patients were infected with HIV-1 subtype B and 1 patient with CRF02_AG. At baseline, the T97A INSTI-RAM was detected in one patient, while no INSTI-RAMs were detected in the remaining patients (one pending). During VF, INSTI-RAMs were detected in 7/13 patients, in 4 patients no mutations were detected and in another 2 plasma HIV-1 RNA levels were below the limit of detection for sequencing. In most cases single INSTI-RAMs compared to baseline were detected at failure: S230R (in two individuals), E263K, N155H, E92Q+N155H, A97A and G163R. Interestingly, there was a large spread in time to failure ranging from 4, 10, 12, 24, 26, 30, 30, 36, 42, 43, 48, 60 and 72 weeks. The DTG plasma concentrations were adequate in 10/10 patients.

**Discussion:** As single INSTI-RAMs were sufficient to cause VF, the genetic barrier to resistance of DTG when given as maintenance monotherapy seems low. The large variation in time to failure indicates that stochastic reactivation of a single cell harboring a provirus with pre-existing INSTI-RAM as the mechanism for failure. The absence of known INSTI-RAMs in 4/13 patients points to unknown INSTI-RAMs in the integrase gene or a novel DTG resistance pathway.
Abstract 15

Sensitivity of a novel translocation defective reverse transcriptase inhibitor, EFdA in comparison to second generation NNRTIs, Etravirine and Rilpivirine on diverse HIV-1 subtypes

Njenda D1,2, Rogers L3, Aralaggupe S1, Rahman S1, Singh K1, Sarafianos S3, Sonnerborg A: Neogi U1

1Karolinska Institutet, 2Stellenbosch University, 3University of Missouri

Background: EFdA (4'-Ethnyl-2'-Fluoro-2' deoxyadenosine) is a novel translocation-defective reverse transcriptase inhibitor (TDRTI). In contrast to other approved NRTIs, EFdA contains 3'OH group that improves the phosphorylation potential of this drug in vivo, making it a strong competitor with natural (dATP) substrate during HIV-1 cDNA synthesis. EFdA has been shown to have low toxicity higher in vitro potency to therapy naïve viruses. Our study investigates the virological and biochemical inhibitory potentials of EFdA against a broad spectrum of subtype-specific viruses and a panel of known reverse transcriptase inhibitor (RTI) and protease inhibitor resistant strains and compare the data with first and second generation non-nucleoside reverse transcriptase inhibitors (NNRTI).

Materials and Methods: The gag-pol (n=24) from treatment naïve individuals was cloned into pNL4.3Δgag_pol to prepare recombinant replication competent viruses. A panel of six each protease inhibitors (PIs) and reverse transcriptase inhibitor (RTI) resistant strains (obtained from the NIH AIDS reagent and reference program) together with the NL43 strain (as control) were used to investigate comparative potency of RTI drugs. Phenotypic drug sensitivity assay (DSA) was performed in TZM_bl cell lines against different drugs- EFdA, Rilpivirine (RPV), Etravirine- (ETV), Nevirapine- (NVP) and Efavirenz (EFV) respectively. Fold-change in EC50 was calculated against NL43 viruses. Patient derived RTs from HIV-1B, HIV-1C, 01_AE and 02_AG subtypes were cloned, expressed and purified. In vitro inhibition and binding affinity assays were performed using patient derived RTs against all the above mentioned drugs.

Results: While comparing the recombinant viruses from treatment naïve viruses without any drug resistant mutations (DRMs), EFdA displayed better in vitro potency (mean EC50 = 3.067nM) compared to Rilpivirine (mean EC50 =6.89nM), Etravirine (mean EC50 = 12.27nM), Nevirapine (mean EC50 = 127.64nM) and Efavirenz (mean EC50 = 31.17nM). EFdA inhibited viruses of all subtype to comparable efficacy. Fold change analysis among the groups identified that EFdA had significantly higher potency across all subtypes and including RTI resistance strains (p – value = 0.024). The EC50 value for EFdA against the RTI strains were below 20nM. In vitro biochemical assays using patient derived RTs identified subtype specific difference in binding affinity among the 1st and 2nd generations NNRTIs. The binding affinity of NVP was significantly low compared to other drugs, while EFdA has higher binding efficacy.

Conclusion: Our combined in vitro virological and biochemical data suggests that EFdA inhibits both wild type and RTI-resistant viruses efficiently in subtype independent manner. No subtype specific effect was observed in in vitro DSA despite difference in in vitro binding affinity on RTs without any DRM was observed across subtypes with different classes of RTIs. Therefore, EFdA can make a strong choice drug for clinical trials involving both therapy naïve and therapy failure individuals.
Abstract 16

Resistance-associated and fitness-associated substitutions on NS3, NS5A and NS5B genes in real-life HCV positive patient failed to 3D therapy

Marascio N1, Di Salvo S2, Pavia G1, Zicca E1, Marano V1, Barreca G1, Fabiani F3, De Siena M4, Giancotti F2, Gravina T2, Torti C4, Liberto M1, Focà A1

1Department of Health Sciences, Institute of Microbiology, School of Medicine, University of "Magna Graecia", Viale Europa, Germaneto, 88100 Catanzaro, Italy, 2Unit of Hepatology, “Mater Domini” University Hospital, Viale Europa, Germaneto, 88100 Catanzaro, Italy, 3Unit of Medical Genetics, “Mater Domini” University Hospital, Viale Europa, Germaneto, 88100 Catanzaro, Italy, 4Department of Medical and Surgical Sciences, Unit of Infectious and Tropical Diseases, School of Medicine, University of "Magna Graecia", Viale Europa, Germaneto, 88100 Catanzaro, Italy

Background: Ombitasvir/paritaprevir/ritonavir, dasabuvir ± ribavirin (3D± R) is an effective and well-tolerated regimen with SVR rates at 12 weeks >95% in pegylated-interferon/ribavirin treatment-naïve patients. We report a case whose baseline and follow-up HCV RNA displayed Resistance Associated Substitutions (RAS) by population sequencing with a 15% cut-off.

Case report: A 65-year-old man, naïve to all HCV treatments, not co-infected with HIV or HBV, with type 2 diabetes, without cryoglobulinemia, was admitted at “Mater Domini” University Hospital in April 2016. The patient underwent transient elastography (FibroScan®) and liver stiffness was 10.5 kPa (F3). Patient was infected by HCV1b and treated with 3D without ribavirin for 12 weeks as scheduled by European Association for the Study of the Liver (EASL)/Italian Association for the Study of the Liver (AISF) guidelines. No adverse events were reported. HCV RNA was measured using a routine diagnostic method. HCV RNA at baseline was 7,430,000 IU/mL, at week 4 and at the end of treatment HCV RNA was undetectable, while it was 5,370,000 IU/mL at week 12 post treatment. Serum samples were collected and HCV NS3, NS5A and NS5B regions were sequenced by Sanger technique at baseline and at week 12. Also, HCV typing was performed by phylogenetic analysis of all of three regions using PhyML and by COMET and Oxford HCV subtyping tools. Additionally, to investigate on reappearance of viral strain at week 12 after treatment, sequences at two time points were analysed using the maximum likelihood tree. Both sequences of three genes from the patient closely clustered with each other (bootstrap support > 97%) and not with reference sequences, clearly identifying a viral relapse rather than a reinfection. RASs on NS3, NS5A and NS5B genes were analysed aligning full genome reference sequences. The baseline isolate showed G substitution on NS3_122 hot spot position related to simeprevir resistance (122R) and NS5B_159F sofosbuvir RAS + NS5B_316N fitness-associated substitution. By contrast, the isolate at week 12 post treatment carried the same baseline substitutions, plus NS5A_93H daclatasvir, elbasvir, ledipasvir, ombitasvir and velpatasvir RAS. Furthermore, polymorphisms “not yet related” to drug resistance, were found only in NS3 region at baseline (89S) and only in NS5B region at week 12 (300T). The 6R, 34V, 44R, 61V, 78R, 138L were carried in NS5A region of both isolates.

Discussion: Nucleotide sequence analysis of three genomic regions, carrying the acquired NS5A_93H RAS and natural NS5B_159F RAS + NS5B_316N fitness-associated substitution + NS3_122G substitution may explain treatment failure. This case report underlines the importance to perform resistance testing at failure, as well as at baseline on the three eligible genes for a personalised treatment. Also, it is suggested the importance of amino acid changes co-evolving during Interferon-free therapy especially without ribavirin; as in our case report, they could be related to fitness-associated effect promoting further accumulation of RASs. Lastly, phylogenetic analysis is important to differentiate whether a breakthrough is indicative of a failure rather than of a re-infection event.
Abstract 17

Real-life efficacy of HCV-retreatment after DAA-failure: the role of NS5A-resistance

Cento V1, Barbaliscia S1, Di Maio V.C1, Masetti C2, Minichini C1, Rossetti B4, Marenco S6, Troshina Y6, Bagnuera C7, Zazzi M8, Dentone C9, Landonio S10, Nicolini L.A.11, Caudai C8, Melis M12, Schiavini M13, Bruzzese B14, Calvaruso V6, Micheli V15, Paolucci S16, Aragri M17, Bertoli A18, Lenci I19, Ruggiero T20, Messina V19, Boglione L21, Buonomo A.R.22, Morisco F22, Barbarini G23, Craxì A14, Babudieri S12, Ciampi A24, Rizzardini G10, De Luca A4, Coppola N3, Angelico M2, Perno C.F.1, Ceccherini-Silberstein F1 on behalf of VIRONET-C

1Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, 2Hepatology Unit, Policlinic Foundation of Rome Tor Vergata, 3Infectious Diseases, Department of Mental and Physical Health and Preventive Medicine, Second University of Naples, 4Infectious Diseases, Siena University Hospital, 5Division of Hepatology, University of Genoa-AOU IRCCS San Martino-IST, 6Gastroenterology, Department of Medical Sciences, City of Health and Science of Turin, University of Turin, 7Infectious Diseases, Hospital Niguarda Ca’Granda, 8Virology, Siena University Hospital, 9Infectious Diseases, Sanremo Hospital, 101st Division of Infectious Diseases, ASST Fatebenefratelli Sacco, 11Infectious Diseases, Department of Health Sciences (DISSAL), University of Genoa-AOU IRCCS San Martino-IST, 12Infectious Diseases Unit, Department of Clinical and Experimental Medicine, University of Sassari, 13Hygiene Unit, University of Genoa-AOU IRCCS San Martino-IST, 14Gastroenterology, “P. Giaccone” University Hospital, 15Clinical Microbiology, Virology and Bioemergencies, ASST Fatebenefratelli Sacco, 16Molecular Virology, Policlinic Foundation San Matteo, 17Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, ASL Città di Torino, 18Infectious Diseases Unit, A.O. S. Anna e S. Sebastiano, 19Unit of Infectious Diseases, University of Turin, 20Infectious Diseases, University «Federico II», 21Department of Gastroenterology, Scientific Institute for Digestive Disease “Saverio de Bellis” Hospital, 22Department of Clinical Medicine and Surgery, University «Federico II», 23Division of Infectious and Tropical Diseases, Policlinic Foundation San Matteo

Background: In Italy, >70,000 HCV-infected patients have so far received an IFN-free direct-acting antiviral (DAA)-regimen. Even with a failure rate as low as 5-10%, around 3,500-4,000 patients need retreatment. Drug-class switch is generally recommended by international guidelines, along with baseline (BL) genotypic-resistance-testing (GRT), if available. Few real-life data are available on GRT performance in this setting, and on the efficacy of retreatment according to BL presence of resistance associated substitutions (RASs).

Materials and methods: Within the Italian collaborative network VIRONET-C, we analyzed the efficacy of retreatment after failure of IFN-free regimens containing a NS5A-inhibitor (NS5Ai)±ribavirin (N=13), a protease-inhibitor (PI)+sofosbuvir±ribavirin (N=23), or only sofosbuvir±ribavirin (N=62). GRT was performed by population-sequencing at clinicians’ discretion.

Results: Ninety-eight patients infected with HCV GT1a/1b (N=47), 2c (N=5), 3a (N=35), and 4d (N=11), 85.7% with cirrhosis, were included. 84/98 (85.7%) patients performed a BL-GRT, that disclosed RASs in 21/31 (67.7%) PI-failing, 11/13 (84.6%) NS5Ai-failing, and 13/74 (17.6%) sofosbuvir-failing patients. The overall prevalence of NS5A-RASs before retreatment was 32.1% (27/84). 89 patients were retreated with NS5Ai (daclatasvir, N=47; ledipasvir, N=38; ombitasvir, N=4), including 5 NS5A-experienced patients; 8 patients were retreated with simeprevir + sofosbuvir after NS5A-failure. One GT-4d received sofosbuvir+ribavirin after simeprevir+daclatasvir failure with multiclass-RASs. Interim results on 77 patients showed a sustained 12-week virological response (SVR12) in 61 (79.2%), including 54/67 (80.6%) treated with NS5Ai+sofosbuvir±ribavirin, 5/6 (83.3%) with simeprevir+sofosbuvir±ribavirin, and 2/3 (66.7%) with paritaprevir/ombitasvir + dasabuvir + ribavirin. Overall, 52/66 (78.8%) cirrhotic patients, and 22/26 (84.6%) GT-3 infected-patients achieved SVR12. SVR12 rate was reduced in patients treated for 12-weeks (62.5% [10/16] vs. 83.6% [51/61] for 24-weeks; p=0.08) and/or without ribavirin (66.7% [12/18] vs. 83.1% [49/59] with ribavirin; p=0.18). Patients whose retreatment strategy did not follow current guidelines nor BL-GRT achieved SVR12 in 46.2% of cases (6/13), vs. 86.4% (36/44) of those who followed guidelines (with N=34) or without [N=10] baseline NS5A-GRT , and 85.0% (17/20) of
those who primarily followed resistance information by BL-GRT (p=0.01 by Fisher exact-test). The majority of patients (70/77) were retreated with NS5Ai, with a SVR12 rate of 80.0% (56/70). NS5Ai-retreatment efficacy was reduced for short (12-weeks) ribavirin-free regimens (28.6% [2/7] vs. 80.9% [38/47] of 24-weeks regimens with ribavirin; p=0.01), in patients with prior NS5Ai-experience (40.0% [2/5] vs. 83.1% [54/65] in NS5A-naïve; p=0.05), and in patients with baseline NS5A-RASs. Indeed, SVR12 was achieved in 36/41 (87.8%) patients without BL NS5A-RASs, vs. 7/14 (50.0%) patients with >1 NS5A-RASs (p=0.01). Only 3/7 patients with Y93H/C NS5A-RASs reached SVR.

Sixteen patients (14 with cirrhosis) failed retreatment. 10/15 tested at BL already presented RASs, in 90% of cases on both NS3 and NS5A. Retreatment failure led to emergence of new NS5A-RASs in 4/7 patients retested, further complicating subsequent treatment.

Conclusion: SVR rates of second-line DAA-regimens are reduced in short and/or ribavirin-free regimens and/or in presence of BL NS5A-RASs (especially at the Y93 residue). In this setting, GRT may help to optimize retreatment strategy, and to select patients that, having particularly complex RAS patterns, are natural candidates for future generations of DAAs with higher genetic barrier and/or distinct resistance profile.

Abstract 18
Retreatment of DAA failures with approved regimens and resistance information in a real life setting: data form the HepcResp GEHEP004 cohort

Perez A1, Chueca N1, Fernández-Caballero J1, García-delToro M2, Rivero-Juarez A3, Pineda J4, Tellez F5, Simón M6, Merino D7, Aldamiz-Echevarria T8, Collado A9, Pascasio J10, Vivancos M11, García F1, on behalf of HepcResp GEHEP004 Cohort

1Hospital Universitario San Cecilio, 2Infectious Diseases Unit, Hospital General, 3Infectious Diseases Unit, Hospital Reina Sofia, 4Infectious Diseases Unit, Hospital de Valme, 5Infectious Diseases Unit, Hospital Puerto Real, 6Hepatology Unit, Hospital Clínico, 7Infectious Diseases Unit, Hospital Infantia Elena, 8Infectious Diseases Unit, Hospital Gregorio Marañón, 9Infectious Diseases Unit, Hospital Torrecardenas, 10Hepatology Unit, Hospital Virgen del Rocío, 11Infectious Diseases Unit, Hospital Ramón y Cajal

Background and Aims: In our study, we evaluated the prevalence of HCV resistance associated substitutions (RAS) in a large cohort (HCVREsp-GEHEP004) of patients failing IFN-free direct antiviral agents (DAAs) regimens in Spain, how patients are being retreated, the efficacy of retreatment regimens, and how retreatment has adhered to treatment guidelines recommendations and resistance findings.

Methods: HCVREsp is a prospective multicenter cohort enrolling HCV infected patients treated with IFN-free DAA regimens at discretion of the investigators. For most of the patients, retreatment regimen was chosen after receiving a comprehensive resistance interpretation report, based on population-based sequencing of HCV NS3, NS5A and NS5B genes. RAS were scored according to Lontok et al, 2015 (doi: 10.1002/hep.27934)

Results: HCVREsp includes 6743 patients treated with DAAs across Spain. Data of 289 failing patients [GT-1a (n=104), GT-1b (n=80), GT-3a (n=59), GT-4a (n=9), GT-4d (n=37)] are shown. Patients had failed SOF/SIM (19.3%),
SOF/DCV (19.0%), SOF/LDV (39.1%), Paritaprevir/ Ombitasvir±Dasabuvir (14.9%) or Other (7.6%). Patients failing SOF/SIM developed RASs in NS3 in 79.0% of the GT1a infected patients and 47.8% of the GT1b, being RASs in positions 155 & 168 the most prevalent. To date, 47/56 patients failing SOF/SIM developed RASs in NS3 in 79.0% of the GT1a infected patients and 47.8% of the GT1b, being RASs in positions 155 & 168 the most prevalent. To date, 47/56 patients failing SOF/SIM have been retreated, 45 with Harvoni and 40 have reached 12 weeks post end of treatment, with 34 reaching SVR12 (85.0%). Almost all the patients failing SOF/DCV showed NS5A RASs, being Y93H highly prevalent in GT-1b (75.0%) and GT-3a (75.0%); to date, 25/55 patients failing SOF/DCV have been retreated, 18 have reached 12 weeks post end of treatment, with 13 reaching SVR12 (72.2%). Patients treated with SOF/LDV also showed a high prevalence of Y93H at failure, especially GT-1b (80.0%), in contrast to GT-3a infected patients (only 6.3% prevalence); of note, three GT-4 patients failing SOF/LDV harbored S282T. To date, 54/113 patients failing SOF/LDV have been retreated, 44.4% with SOF/SIM, 32 have reached 12 weeks post end of treatment, with 28 reaching SVR12 (87.5%). Most patients treated with 2D/3D developed RASs, and 14.0% showed RASs against the three drugs; only 14/43 patients failing 3D/2D have been retreated.

**Conclusions:** Genotype 1a & 1b patients failing DAAs in Spain harbor a high prevalence of RASs, especially in NS5A. Genotype 3 patients failing SOF/LDV are less prone to develop NS5A RASs than SOF/DCV failures. Retreatment of SOF/DCV failing patients was more difficult than SOF/LDV or SOF/SIM failing patients, and resulted in lower rates of SVR12. Resistance testing may help to guide the retreatment option in those patients who have failed a first line, but guidance on treatment duration and the need to use ribavirine is also needed.

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**Abstract 19**

Clinical relevance of accurate HCV genotype and subtype assignment by HCV Sanger and next generation sequencing in the era of new direct acting antiviral agents

Aragri M1, Di Maio V.C1, Belloccchi M.C.1, Carioti L1, Teti E2, Rossi P3, Masetti C1, Giannerra L4, Pieri A5, Palitti V6, Cento V7, Bertoli A8, Barbaliccia S1, Lenci P9, Francioso S10, Cacciatore P11, Polilli E12, Landonio S13, Melis M14, Paoloni M15, Micheli V16, Nicolini L17, Marendo S18, Lambiasi L19, Milana M1, Madia F1, Sarmati L20, Pellicelli A21, Nosotti L22, Bilotti E23, Marignani M24, Sozio F25, Minichini C26, Romagnoli D27, Bruzzone B28, Siciliano M29, D’Ettorre G20, Lichtner MP21, Sarreccia C22, Greco A23, Morisco F24, Mastroianni C25, Vecchiet J26, Puoti M27, Caporaso N28, Bruno S29, Craxi A30, Tarquini P31, Maggi C32, Babuiecri S33, Taliani G34, Vullo V35, Picciotto A12, Andreoni M13, Pasquazzi C14, Parruti G15, Angelico M16, Perno C.F.1, Ceccherini-Silberstein F1

1Department Of Experimental Medicine And Surgery, University Of Rome Tor Vergata, 2Infectious Disease, University Hospital of Rome “Tor Vergata, 3Hepatology Unit, University Hospital of Rome Tor Vergata, 4Infectious Diseases, Sant’Andrea Hospital – “La Sapienza” University, 5Infectious Disease Unit, Pescara General Hospital, 6Hepatology Unit, Pescara General Hospital, 7Division of Infectious Disease, Hospital Sacco of Milan, 8Infectious Diseases Unit, Department of Clinical and Experimental Medicine, University of Sassari, 9Infectious Disease Unit, Avezzano General Hospital, 10Unit of Microbiology, Hospital Sacco of Milan, 11Infectious Diseases, -AO IRCCS San Martino-IST, 12Hepatology Unit, -AO IRCCS San Martino IST, 13Hepatology Unit, San Camillo Forlanini Hospital, 14Hepatology Unit, National Institute of Health, Migration and Poverty, 15Infectious and Tropical Diseases Unit, Department of Clinical Medicine, “La Sapienza” University, 16Gastroenterology and Hepatology, AO S.Andrea “La Sapienza” University, 17Infectious Diseases and Viral Hepatitis Unit, Second University of Naples, 18Department of Biomedical, Metabolic and Neural Sciences, NOCSAE, 19Haemology Unit, -AO IRCCS San Martino-IST, 20Gastroenterology, Catholic University of Rome, 21Infectious Diseases, “La Sapienza” University, 22Infectious Diseases, “La Sapienza” University, 23Liver Transplant Unit, Catholic University of Rome, 24Department of Clinical Medicine and Surgery, University “Federico II” of Naples.
Background: The selection of the most effective Direct-Acting-Antiviral-Agents (DAAs) regimen to treat HCV infection requires definition of HCV genotype/subtype. Since commercial genotyping assays can be at times inaccurate, this study was aimed at evaluating the concordance between commercial-assays and HCV sequencing in the subtype/genotype assignment.

Methods: Genotypic-resistance-testing (GRT) for NS3-protease, NS5A, NS5B and/or core regions was performed by standard Sanger-sequencing or ultra-deep-pyrosequencing (UDPS), using home-made protocols. Phylogenetic analysis was performed to assess HCV subtype/genotype and concordance with previous classification by commercial-assays.

Results: A total of 1813 HCV-infected patients with GRT pre- or post-therapy performed between 2011 and 2017 were analyzed. According to the GRT requests, Sanger-sequencing was performed on the 3 targets of DAAs: NS3-protease (94.5% of samples), with/or to NS5A (89.8%), and/or NS5B (54.4%).

HCV Sanger-sequencing and commercial-assays were concordant in 89.8% of cases analysed. In the remaining 10.2%, GRT identified 35/1813 genotypes discordant with the assignment provided by commercial-assays (commercial/sequencing: 1a/3a[N=4]; 1b/2c[N=7]; 1b/3a[N=2]; 1b/4d[N=3]; 2a/1a[N=4]; 2/3a[N=2]; 3/1a[N=2] and N=1 for 1/2c; 1/3a; 1a/2c; 1a/4d; 2/1a; 3a/1b; 3/4d; 4/1a; 4/1b; 4/1g; 4/2c) and 54/1813 discordant subtype HCV-1 cases (commercial/sequencing: 1a/1g[N=3]; 1a/1b[N=20]; 1b/1a[N=31]). Of interest, 16 cases revealed a discordant genotype only at the time of GRT performed at DAA failure. Furthermore, 97/1813 patients with a previous result of mixed (N=42/44) or indeterminate (N=19/19) or HCV-1 without subtype information (N=36/36) by commercial-assays, were instead precisely resolved by GRT. Two HCV mixed genotypes (1a/3 and 1a/4) were confirmed by Sanger-sequencing in all 3 genes. As a whole, 186/1813 (10.2%) patients achieved the correct genotyping assignment thanks to HCV Sanger-sequencing.

Conclusions: When all 3 targets of DAAs per patient were analysed (N=911), phylogenetic results were 100% concordant in NS3+NS5A+NS5B genes, confirming the specific genotype/subtype assignment. In addition, for 19 patients with HCV mixed-genotype assignment by commercial-assays, further analyses were also performed by UDPS in NS5B-region and/or by Sanger-sequencing in the HCV core, to identify potential mixed or recombinant infections, respectively. Interestingly, the NS5B UDPS confirmed the Sanger mixed-genotype results, and showed additional occurrence of 3/19 mixed infections (commercial/Sanger/UDPS: 1a+1b/1a/1a[55.0%]+1b[45.0%]; 1a+4/1a/1a[14.4%]+4d[85.6%]; 1+3+4/4d/1a[12%]+4d[88%]).

The phylogenetic analysis of the HCV core sequences obtained by Sanger showed discordant results in 3/18 patients in comparison of NS3+NS5A+NS5B sequences: core/NS3+NS5A+NS5B: 4a/1a[N=2]; 2b/1b[N=1]. Of interest, the patient infected with presumed recombinant HCV-1b/2b is from Philippine, and the HCV core sequence clustered with a published inter-genotypic 2b/1b recombinant found in the Philippines. Interestingly, this patient treated with ledipasvir+sofosbuvir+ribavirin for 12 weeks showed a sustained-viral-response (SVR24). The sequencing of 5'-UTR region is currently ongoing to confirm the potential recombination event in these patients with presumed mixed infection.
Abstract 20

HIV-1 drug resistance testing using Nanopore technology – a long-range low error sequencing approach using rolling circle amplification

Thielen A1, Engel N1, Förster A1, Memmer A1, Welter B1, Thielen B1, Düämer M1

1Institute Of Immunology And Genetics

Background: HIV drug resistance testing by Next-Generation-Sequencing technologies has already been established occasionally, providing information on mutation frequencies with unprecedented sensitivity. Third-Generation-Sequencing by nanopore technology (e.g. MinION®) is additionally capable of generating reads, hundreds of kilobases (kb) in length, potentially enabling long-range covariation analysis. However, an extremely high error rate results in a highly overestimated sequence variability. The goal of this work was to establish a proof of concept, clonal concatemer sequencing approach using Oxford Nanopore’s MinION®, resulting in a significantly increased accuracy of the reads. With this, we elucidate the feasibility of using the MinION® for HIV-1 drug resistance testing.

Material and Methods: Barcoded 3.1kb amplicons comprising the pol-region encoding for Protease, Reverse Transcriptase and Integrase were generated from 16 patient samples. Amplicons were pooled equimolarly, self-circularized by T4 DNA ligase and isothermal amplified using phi29 polymerase and random hexamer primers. This resulted in approximately 100kb large products that were sheared with g-tubes® from Covaris. A size of 20kb with theoretical 7 concatemeric copies of the original amplicon was anticipated. Resulting products were sequenced on a MinION using the 1D protocol (RCA-1D approach). As controls non-concatemerized amplicons were run on the MinION (1D-only approach) and on a MiSeq after Nextera XT tagmentation (Illumina). Analysis was performed with in-house developed bioinformatics pipelines. Covariation was determined using the R-package covaRius. MiSeq data were used as the gold standard.

Results: The “1D-only run” generated approx. 240,000 reads which could be mapped to HIV-1 with median read lengths of about 1.3kb (range: 31-3098bp). The median error rate per position for 1D-only was 14.7% (6.4% substitution errors, 5.6% deletions, and 1.1% insertions). The “RCA-1D” run resulted in approx. 198,000 HIV-1 mappable reads with median read lengths of 1698 bps (3671bp on average, range: 173-82,556bp). Regarding the error rate, similar results were found for the “RCA-1D” approach if only a single copy of the amplified region was considered: 14.7% (6.0% mismatches, 6.3%, deletions 0.8% insertions). By considering 5 copies in one read, the error rate decreased to 6.1% (1.2%, 4%, 0%) and to 1.2% (1.2%, 0%, 0%) when having regard to 11 copies.

When using 5 copies, the frequency of miscalled mutations at a sensitivity cut-off of 10% (15%, 20%) was 1.8% (0.9%, 0.52%). Similar results were found with more replicates. All of these miscalled mutations were located within or adjacent to homopolymeric regions.

Conclusions: Here we show that the MinION nanopore technology is capable of sequencing amplicons containing the full protease, reverse transcriptase, and integrase genes with an acceptable accuracy, when combined with an concatemer library preparation. Although the raw error rates are quite high, we could show how they can be reduced significantly to levels below 2%. Following this protocol, reliable linkage information over long genomic distances can be inferred.
Abstract 21

Next Generation Sequencing in Reverse Transcriptase and Protease of HIV-1 helps to discriminate recent from chronically infected newly diagnosed patients

Fernandez-Caballero Rico J1, Chueca N1, Alvarez M1, Mérida M1, Sánchez A1, López J1, García F1

1Clinical Microbiology Service. Hospital Universitario San Cecilio. Instituto De Investigación Ibs.

Background and aim: In some cases it may be difficult to discriminate recent from chronic infection in newly diagnosed patients. Although some serological tools, such as STARHS have been proposed, there is no gold standard. Here we have used the results of Next Generation Sequencing (NGS) of RT & Protease used for routine baseline resistance testing to discriminate recent from chronic infection.

Patients & methods: Plasma samples from newly diagnosed patients sent to our reference laboratory for baseline resistance testing through October 2015 to January 2017 were studied with 454 GS Junior. Recent infection was defined as having at least one of the following criteria: positive p24Ag, negative Ab test in the prior 3 months, and/or clinical symptoms of acute HIV infection following high-risk exposure. Each of the 4 amplicons used for baseline resistance (RTA, RTB, RTC & RTD) were analysed with the GS Amplicon Variant Analyzer and filtered for quality values (QV) >30 and read length using USEARCH. Additionally, we used Mesquite software to build a consensus sequence using a 20% cut-off to search for ambiguous codons. MUSCLE was used for sequence alignment and MEGA 6.06 to build phylogenetic trees using Neighbor joining. To study viral variability we used the relative proportion of the major sequence of each of the four amplicons.

Results: We have studied RT & Pro sequences from 192 patients. Thirty-three patients met at least one of the criteria to be considered recently infected; this population had a median age of 37 (IQR 30.5-45), 90.9% were male, 87.7% Spanish and infected by B subtype. with a median viral load (log copies/ml) and CD4 count (cells/ul) of 6,30 (IQR 5,73-6,95) and 587 (462-751). Transmitted drug resistance mutations were found in 19 patients with chronic infection (11.9%) and 6 with recent infection (18.2%). We found a higher number of the median number of ambiguous bases in the chronically infected group than in the patient with recent infection [17 (IQR 13-23) vs 3 (IQR 1.25-4.5, respectively; p<0.001). In patients with recent infection, the major sequence represented a median of 97.2% (IQR 95.7-98.25) of all the viruses detected with NGS, while for those with chronic infection the median was 54,3% (28.6-71.8) (p<0.001). Phylogenetic analysis also displayed a higher abundance and diversification of viral quasispecies in the chronically infected group.

Conclusion: Viral quasiespecies diversification may be identified with appropriate analysis from baseline routine testing performed with Next Generation Sequencing tests, as well as by studying the number of ambiguous bases from Sanger based sequences. We propose a cutoff of 95% viral diversity and 10 ambiguous bases to discriminate chronic and recent infection in newly diagnosed patients.
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Treatment Strategies & Antiviral Drug Resistance

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Abstract 22

Sequence ambiguity determined from routine pol sequencing is a decent indicator of changing trends in HIV epidemic in real-time

Lunar M1, Poljak M1

1Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana

Background: Distinction between individuals infected with HIV recently (RI) and individuals with a long standing infection (LSI) is of utmost significance when trying to detect changes in HIV epidemic dynamics. This can be achieved by using different laboratory techniques, e.g. serologic assays. Low HIV sequence ambiguity (SA) has been described as another indicators of RI in previous studies and has been estimated from the proportion of ambiguous base calls determined in routinely genotyped sequences of pol region. The aim of this study was to explore the utility of SA of baseline pol sequences for the surveillance of changing trends in HIV epidemic on a national level.

Materials & methods: A total of 260 partial HIV-1 pol sequences of approximately 1,000 base pairs obtained from therapy naïve newly diagnosed HIV-infected persons between the years 2000-2012 in Slovenia were studied. SA was measured by counting ambiguous base calls using the Bioedit package and Python script and reported as a percentage of ambiguous nucleotides. Patients were characterized either as RI or LSI using Aware BED EIA HIV-1 Incidence Test (Calypte Biomedical Corporation, Portland, Oregon) (BED) to the same baseline sample. A determined RI corresponded to a mean duration of recent infection of 155 days. Additional 131 patients were tested with BED, however baseline sequence was not obtained, therefore this results could subsequently be included only in the time trend analysis. T-test was performed using JASP version 0.8.0.0 and ROC curve analysis using MedCalc Statistical Software version 16.8.4. P-value of <0.05 was considered significant.

Results: Firstly, we verified whether SA is discriminatory enough to differentiate RI from LSI. In the group of RI, a mean SA of 0.29% was observed when BED results were used as gold standard. Welch’s t-test determined that sequence ambiguity of RI vs. LSI differ significantly (P<0.001).

In the second part of the study we tested how SA method performs at various thresholds. For this reason, receiver operating characteristic (ROC) curve was obtained with the area under the curve (AUC) of 0.76 (±0.03). According to ROC, optimal SA cut-off was set at 0.30%, exhibiting best sensitivity and specificity of 69%.

In the third part of the study we examined how SA performs as a discriminator of RI from LSI in a nation-wide setting. BED data were available for 391/428 (91.4%) of patients diagnosed with HIV in 2000-2012 and were therefore representative of Slovenian HIV epidemic. SA method results were analysed with the following cut-offs: 0.15%, 0.2%, 0.3% and 0.45%. A good indication of the trends of RI vs. LSI could be inferred from observing SA whatever the cut-off used, however for Slovenian situation it seemed that lower cut-offs were more appropriate.

Conclusions: Our data suggests that sequence ambiguity method could be used as real-time surveillance tool for measuring changing trends in HIV incidence, especially in countries where baseline HIV resistance genotyping is performed routinely for each diagnosed patient, rendering this approach cost-effective.
Abstract 23

Molecular Epidemiology of Newly Diagnosed HIV-1 Infected Patients in Cyprus (2010-2012) Reveals Active Transmission Networks Among Young Men Having Sex with Men and Low Transmitted Drug Resistance

Hezka J1, Stylianou D1, Kostaki E2, Andreou M1, Kousiappa I1, Demetriades I1, Paraskevis D2, Kostrikis L1

1University Of Cyprus, 2National and Kapodistrian University of Athens, 3AIDS Clinic, Larnaca General Hospital

A molecular epidemiology study of HIV-1 infection was conducted in one hundred HIV-1 diagnosed and untreated patients in Cyprus representing 65.4 percent of all the reported HIV-1 infections in Cyprus between 2010 and 2012. Eighty-two patients were newly diagnosed (genotypic drug resistance testing within six months from diagnosis), and eighteen patients were HIV-1 diagnosed for a longer period or the diagnosis date was unknown. Phylogenetic trees of the pol sequences obtained in this study with reference sequences indicated that subtypes B and A1 were the most common subtypes present and accounted for 41.0 and 19.0% respectively, followed by subtype C (7.0%), F1 (8.0%), CRF02_AG (4.0%), A2 (2.0%), other CRFs (7.0%) and unknown recombinant forms, URFs (12%). Most of newly-diagnosed study subjects were Cypriots (63%), males (78%) with median age 39 (Interquartile Range, IQR 33-48) reporting having sex with other men, MSM (51%). A high rate of clustered transmission of subtype B drug-sensitive strains to reverse transcriptase and protease inhibitors was observed among MSM. Twenty-eight out of forty-one MSM study subjects (68.0%) infected were implicated in five transmission clusters, two of which are subtype A1 and three subtype B strains. The two largest MSM subtype B clusters included nine and eight Cypriot men, respectively, living in all major cities in Cyprus. There were only three newly diagnosed patients with transmitted drug resistant HIV-1 strains, one study subject from the United Kingdom infected with subtype B strain and one from Romania with subtype A2 strain, both with the PI drug resistance mutation M46L and one patient from Greece with subtype A1 strain with the NNRTI drug resistance mutation K103N.

Abstract 24

Genetic analysis of HIV-1 variants circulating in Sakhalin island (Russia)

Gromov K1, Tumanov A1, Lomakina E2, Kazennova E1, Bobkova M1

1FSBI "N.F. Gamaleya FRCEM" of the Ministry of Health of Russia, 2Sakhalin regional AIDS Centre

Background: During the last two decades, HIV-1 has been spreading rapidly in the Russian Federation with subtype A1 variant A -FSU dominating in almost all territories. The studies in the Russian Far East (Blagoveshchensk, Vladivostok, Khabarovsk) in 2012-2013 showed very unusual for Russia molecular landscape of HIV infection epidemic. In addition to subtype A1 variants, there were also subtypes B, C, CRF063_02A1, and CRF02_AG identified. Further studies have been planned to analyze the distribution of various HIV-1 subtypes in other cities and regions of Russian Far East. The subject of this study was to characterize HIV-1 genetic strains currently circulating in Sakhalin region. The Sakhalin region consists of the largest island of Russia - Sakhalin and the Kuril Islands (low populated area). Sakhalin Island is an isolated territory separated from the mainland by the Tatar Strait and from the Japanese island of Hokkaido by the Strait of La Perouse, with rare air communication with Russia and China, Japan and the regular ferry service to the Khabarovsk Territory.

Materials & methods: Blood samples were collected, with informed consent, in 2014 from 53 HIV infected persons. Sequences of pol gene
Abstracts

were obtained by in house system. Genotyping, phylogenetic and recombinant analyses were carried out by COMET HIV-1 (https://comet.lih.lu/), REGA HIV-1 Subtyping Tool - Version 3.0 (http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/), RIP (https://www.hiv.lanl.gov/content/sequence/RIP/RIP.html), MEGA 6.06 and jpHMM (http://jphmm.gobics.de/), correspondingly.

Results: The results of genotype analysis revealed that 81% (43/53) of the samples studied belonged to subtype A1, 7.54% (4/53) - subtype B, 3.77% (2/53) - subtype G, 1.89%(1/53) - CRF63_02A1, 1.89%(1/53) CRF 11_cpx and two were recombinant forms of A1 and G. Phylogenetic analysis showed that all A1 variants from Sakhalin region clustered with A -FSU from other regions of Russia. Two B-variants clustered with B -FSU strains. All other (G, CRF63_02A1, CRF 11_cpx) viral sequences formed the clusters with reference subtypes. Two recombinants formed a sub-cluster between the A1 and G/AG clusters. Further jpHMM analysis showed that this recombination form had significant differences from CRF02_AG and was treated as URF_02AG.

Conclusions: Our study supports the view that the subtype A1 variant A-FSU dominates in the Sakhalin region as well as in Russian Federation as a whole. As to other subtypes the situation differs from the main territory because there were no CRF_02 AG viruses quite typical for Russia found, but only its derivatives as double recombinants. It indirectly points to the fact that the epidemic in Sakhalin is relatively young, and the random events such as labor forces migration played a large in its development.

Abstract 25

Phylogenetic analysis of subtypes A among HIV-1 infected individuals diagnosed in Warsaw center in 2015-2016

Dyda T1, Zabek P1, Cielniak I1, Siwak E2, Stanczak J1

1Hospital for Infectious Diseases, 2HIV Out-Patient Clinic

Background: In recent years we observe constant increasing acceleration in HIV-1 genetic diversity among Polish patients population. The prevalence of non-B subtypes continue to rise disproportionately in comparison to dominating subtype B. The most of the introduced subtypes are typical for neighbor East European countries. Evolving from a African ancestor Subtype A1 is responsible of most infection in post Former Soviet Union (FSU) countries. This variant initially spread through heterosexual contact in Ukraine, then begun to explosively propagate among injective drug users - IDU (A Fsu) through territory of Russia. The prevalence of subtype A1 in Western and Central Europe is approximately on level of 2%. Extensive epidemics of this variant is observed in some Mediterranean countries. Circulation of non-B clades in Poland has been significantly increased in association with socio-economical migration. Shifting HIV-1 subtype pattern is important because of epidemiological reasons and could be relevant in clinical settings. The aim of the study was to determine genetic diversity of HIV-1 A1 subtypes among Polish infected individuals diagnosed in 2015-2016.

Methods: Plasma samples from 102 HIV-1 A1 infected patients were obtained during the 2015 to 2016 years. Generation of the protease and reverse transcriptase sequences of the HIV-1 pol gene was performed using the Viroseq HIV-1 Genotyping Test (Abbott). The subtyping and DR mutation detection approaches were improved by using online tools provided by Stanford University HIV DR Database and REGA HIV-1&2 Subtyping Tool. One hundred seventy A1 and 3 A2 subtype sequences from the HIV Los Alamos database were downloaded for further analysis. Nucleotide alignments were prepared using MEGA6 software followed by manual editing. The best fitting
distance model of nucleotide substitution for the phylogenetic alignments was inferred to be the Hasegawa-Kishino-Yano with discretized gamma distribution (HKY+G) with bootstrap 100 replicates. We analyzed subsets of Polish and reference sequences by using the maximum likelihood method by online tool for phylogenetic reconstruction PhyML.

Results: We observe two groups of HIV-1 A1 infected patients which reveal significant genetic distance. We identified a closely related and separated cluster consisting of 56 taxons among sequences from Russia, Ukraine, Belarus. Remaining 46 strains were equally spread among subtype A_FSU sequences. None of analyzed sequences from Polish patients placed between African origin strains.

Conclusions: Genetic diversity of HIV-1 in Poland is become more complex. Our analysis revealed high genetic variety among A1 isolates. We identified a cluster of highly associated sequences of subtype A1 which are probably an effect of previous singular introduction. This group form a compact, homological rooted clade separated from rest of the A_FSU. A feature of this group is a high homogeneity. Second group of cases with A_FSU subtypes exhibit high genetic heterogeneity and not grouped closely each other. They form weakly related branches. A monophyletic lineage in most cases not formed clusters. The majority of the new subtype A_FSU was detected among MSMs.

These observations documented numerous single introductions of A_FSU into Polish population.

*This work was in part sponosred by Foundation for Research Development in Hospital for Infectious Diseases, Warsaw

Abstract 26

Immune dysfunction in Romanian Injecting Drug Users infected with different HIV-1 strains

Vlaicu O1, Banica L1, Jipa R1, Abagiu A1, Otelea D1, Paraschiv S1

1"Prof. Dr. Matei Bals" National Institute for Infectious Diseases

Background: In the last few years Romania has faced an HIV outbreak among injecting drug users (IDUs). This outbreak is characterized by circulation of two different HIV strains, the F1 subtype strain, typically observed in sexually and nosocomially infected patients and CRF14_BG strain, a recombinant form previously reported in Spain and Portugal within the same risk group. Most of IDUs infected with CRF14_BG were diagnosed in clinically advanced stages of disease, presenting lower CD4 counts and X4 tropic virus at baseline as compared with the IDUs infected with subtype F1. In the present study we aimed to define the immune dysfunction in IDUs infected with CRF14_BG in order to better understand the rapid CD4 depletion.

Materials and methods: Twenty nine IDUs infected with HIV, 19 infected with subtype F1 and 10 with CRF14_BG, with similar CD4 counts, were evaluated for CD4 and CD8 T cell subsets based on the expression of CD27, CD28, CCR7 and CD45RA by flow cytometry. Activated CD4 and CD8 T cells, defined as HLADR+CD38+, were measured using FACS Canto II system.

Results: IDUs infected with CRF14_BG had significantly lower activated CD8 T cells than the patients with subtype F1 (p=0.001). Moreover, CD8 T cells pool was less differentiated in the CRF14_BG patients group, the naive population (CD28+CD27+CD45RA+CCR7+) being significantly higher as compared to the F1 subtype group. This less differentiated CD8 T cell phenotype was accompanied by a reduced percentage of effector memory CD4 T cells (CD4+CD45RA-CCR7-CD28+CD27-).
Conclusion: Our findings reveal that, at the time of diagnostic, the IDUs infected with CRF14_BG strains present a specific immune pattern with both impaired CD4 and CD8 T cell profiles suggesting the strain specific factors are involved in the rapid disease progression observed in these patients.

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Abstract 27

High clustering of acute HCV infections among HIV positive men having sex with men

Todesco E1,2, Day N3, Amiel C4, Schneider V4, Elaerts S5, Kreplak G6, Roudière L3, Hué S6, Liotier J7, Gosset D8, Pialoux G8, Katlama C1,9, Marcelin A1,2, Valantin M1,9

1Sorbonne Universités, UPMC Univ Paris 06, INSERM, Institut Pierre Louis d’épidémiologie et de Santé Publique (IPLESP UMRS 1136), 2Department of Virology, Hôpital Pitié-Salpêtrière, AP-HP, 3Cerballiance Laboratory, 4UPMC Univ Paris 06, Centre d’Immunologie et de Maladies Infectieuses (CIMI) UMRS CR7, Persistent Viral Infection (PVI) Team, Inserm U1135, APHP, Groupe Hospitalier Paris Est, Virology Laboratory, Tenon Hospital, 5Department of Internal Medicine, Hôpital Pitié-Salpêtrière, AP-HP, 6Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, 7Department of Infectious Diseases, Hôpital Bicêtre, APHP, 8Department of Infectious Diseases, Hôpital Tenon, APHP, UPMC, 9Department of Infectious Diseases, Hôpital Pitié-Salpêtrière, AP-HP

Background: Emerging acute hepatitis C virus (HCV) infections in HIV positive Men having Sex with Men (MSM) has been described in recent years. Moreover, a high rate of reinfection was recently emphasized among these patients, whereas the mode of contamination (sexual or by injecting drug) is still controversial. To study transmissions and better understand the epidemic, we explored virological relationships of acute HCV infections from a restricted geographical area of Paris.

Methods: Polymerase sequences (NS5b) were obtained by Sanger Sequencing (SS) from acute HCV infections, defined as a positive serology and/or a positive viral load < 12 months. Maximum likelihood phylogenies were estimated using FastTree 2.1 under a GTR+CAT model of nucleotide substitution with SH-like tests. More than 400 sequences from Pitié-Salpêtrière hospital HCV patients were used as reference for comparison. Clades with a branch support ≥80% and intra-clade genetic distances <0.03 nucleotide substitution per sites were considered as transmission chains. Informations were obtained from the existing electronic database or medical record.

Results: Sequencing of 80 acute HCV infections diagnosed during the period 2014-2016 from a neighborhood of center of Paris (« le Marais ») was performed.

Patients were infected with HCV genotype 1a (50%), 4d (40%), 3a (7.5%) and 2k (2.5%) and 92.5% (n=74) of them were coinfected with HIV (9 detectable HIV viral load: 20 to 13251 copies/mL (IQR: 29-364)). At least 81% (n=65) were MSM (15 with unknown sexual orientation). This was a HCV recontamination for 20 patients. Sexually Transmitted Infections (STI) were concomitantly detected in 20 patients (17 Treponema pallidum, 4 Neisseria gonorrhoeae, 4 Chlamydia trachomatis, 1 Giardia intestinalis). Twenty-two transmission chains were identified, including 53 acute Hepatitis C (of which 14 recontaminations) divided in 6 pairs of 2 patients and 14 clusters from 3 to 8 patients. Seven transmission chains were composed by only acute HCV whereas thirteen were mixed acute/chronic.

Conclusion: A high incidence of acute HCV infection has been found in HIV MSM patients in Paris and many of these HCV patients were part of a transmission chain. Many STI have also been found in these patients and 11,2% of them had a detectable HIV viral load. These results highlight the necessity of frequent STI and HCV testing even after a successful treatment. In this context of high clustering, treatment strategies must be rethought and HCV transmitted drug resistance mutations should be monitored in the future.
Abstract 28

Tracing HIV-1 sub-subtype F1 epidemic dispersal in Spain: evidence for spill-overs from Galicia to other areas

Kostaki E1, Koskeridis F1, Chueca N2, Pernas B3, Alvarez M2, Fernández-Caballero J2, Monje A4, Cañizares A5, Aguilera A6, Rodriguez J6, Grandal M2, Castro-Iglesias A6, Mena A3, Garcia F2, Paraskevis D1, Poveda E3, and CoRIS

1Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, 2Department of Clinical Microbiology, Hospital Universitario San Cecilio, Instituto de Investigación IBS, 3Division of Clinical Virology, INIBIC-Complejo Hospitalario Universitario de A Coruña, Universidad da Coruña, 4Centro Nacional de Epidemiología, Universidad de Alcalá de Henares, 5Service of Microbiology, INIBIC-Complejo Hospitalario Universitario de A Coruña, 6Service of Microbiology, Hospital Conxo-CHUS, and Department of Microbiology, Universidad de Santiago de Compostela

Background: A sub-epidemic of HIV-1 sub-subtype F1 with specific characteristics has been previously identified in Galicia. Our previous analysis on 88 F1 sequences sampled during 2009–2013 in Northwest Spain (A Coruña & Santiago de Compostela), revealed that the exponential growth of the sub-epidemic dated back in 2008. Our aim was to investigate the patterns of sub-subtype F1 dispersal across Spain, to identify potential transmissions among seropositives living in Galicia and other states of Spain, and also to estimate the transmission dynamics of the HIV-1 sub-epidemic in Galicia using molecular methods.

Materials & methods: We studied 325 F1 sequences isolated from HIV-1 diagnosed patients during 2000-2016 from 10 different states of Spain. Patients' samples were merged from four datasets: a) CoRIS (2004-2014, N=66); b) Eastern Andalucia Resistance Cohort (2000-2015, N=33); c) Galician Cohort (2009-2015, N=119), and d) the public Los Alamos HIV sequence database (2002-2016, N=107). HIV-1 subtyping was carried out using automated subtyping tools (COMET, REGA), and further verified by phylogenetic analysis. We analyzed phylogenetically 325 F1 sequences sampled in Spain along with all globally sampled F1 sequences available on Los Alamos HIV-1 database (N=1,025) as references. Phylogenetic trees were estimated by maximum likelihood (ML) method as implemented in RAxML v8.0.20 program. Phylodynamic analysis was performed using a Bayesian method as implemented in BEAST v1.8.0.

Results: Galicia (N=199, 61.2%), Madrid (N=59, 18.2%) and Andalusia (N=37, 11.4%) were the states where F1 was the most prevalent. Phylogenetic analysis revealed that the 29.8% (N=97) of the F1 sequences sampled in Spain clustered at different points in the ML tree. The majority of the unclustered sequences were originated from Spain (N=27, 27.8%), Romania (N=23, 23.7%) and L. America (N=18, 18.6%). The remaining 228 (70.2%) F1 sequences sampled in Spain formed a single monophyletic cluster (local transmission network). The 83.8% (N=191) of the clustered sequences were sampled from Galicia. The majority of the sequences (N=178, 78.1%) found within the network had been isolated from men having sex with men (MSM). Within the network, were also found 37 (16.2%) sequences sampled from five different states of Spain [Madrid (N=24, 64.9%), Andalucia (N=4, 10.8%), Castile & Leon (N=2, 5.4%), La Rioja (N=1, 2.7%), Basque Country (N=1, 2.7%), and unknown (N=5, 13.5%)] (spill-overs). Regarding the origin of spill-over transmissions, the 43.2% (N=16) had been isolated from seropositives with non-Spanish nationality. Additionally, we found a distinct subclade (sub-epidemic) consisting of lineages (N=7) from individuals living in Madrid (N=4, 57.2%) and Castile and Leon (N=2, 28.6%), within the network. The Bayesian skyline plot showed that the epidemic growth occurred during 2008-2009.

Conclusions: Our analysis showed that F1 has been introduced in Spain as a result of multiple introductions from different continents, probably due to labor migration from Romania and human mobility from L. America. The hot spot for the largest F1 regional epidemic in Spain is in Galicia associated with MSM risk group. The existence of sub-epidemics within the Galician network, suggest that several spillovers occurred from Galicia to other areas, including also individuals with a non-Spanish nationality.
Abstract 29

The trends of HIV-1 subtypes diversity in European part of Russia in 2008-2015

Laga V1, Lebedev A1, Kazennova E1, Gromov K1, Bobkova M1

1Iiv Fsbi «n.f. Gamaleya Frcem» Of The Ministry Of Health Of Russia

Background: Since 1996, Russia has been experiencing an explosive HIV-1 epidemic among IDUs with predominance of subtype A1 variant IDU-A. Over the years IDU-A viruses moved outside IDUs group and started spreading through the sexual contacts. As to subtype B viruses they circulated among different risk groups in Russia with most of them found in MSM group. In recent years, other subtypes and recombinant viruses were reported as single cases of infection. The aim of this study was to analyze the trends of HIV-1 subtypes diversity in European part of Russia in 2008-2015.

Methods: In this work we analyzed 670 HIV DNA samples that were collected with informed consent in 2008-2015 in 16 regions of European part of Russia (Murmansk, Arkhangelsk, Lipetsk, Samara, Saratov, Izhevsk, Moscow, Bryansk, Kaluga, Kirov, Nizhniy Novgorod, Voronezh, Yaroslavl, Perm, Krasnodar and Republic of Tatarstan). The collection included 36.1% IDUs, 46.1% heterosexuals, 13.3% MSMs, 2.7% mother-to-child transmission cases, 0.6% nosocomial infections, and 1.2% cases with unknown route of transmission. The sequences of pol gene were obtained either by ViroSeq HIV-1 genotyping System v. 2.0 or by in-house system. Genotyping and phylogenetic analyses were carried out by COMET HIV-1/2v.0.5 (http://comet.retrovirology.lu/), MEGA 6.06 and PhyML (http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html) programs.

Results: In 2008-2009 (n=175) the distribution of subtypes was as follows: subtype A1 – 92.5%, subtype B – 2.8%, CRF02_AG – 1.7%, subtype G – 1.1%, CRF03_AB, and CRF06_cpx – 0.95%, respectively. The distribution by two main risk groups was as follows: IDUs (n=71) - subtype A1 – 88.7%, subtype B – 3.8%, CRF02_AG, CRF03_AB and CRF06_cpx – 2.5% respectively; heterosexuals (n=101) - subtype A1 – 94.1%, subtype B – 3.9%, CRF02_AG - 2%.

By 2015 (n=670) the distribution of subtypes was as follows: subtype A1 – 92.4%, subtype B – 4.2%, CRF02_AG – 1.3%, subtype G and CRF03_AB – 0.67%, respectively, and C, D, CRF63_02A1 and CRF06_cpx – 0.19%, respectively.

The distribution by two main risk groups was as follows: IDUs (n=242) - subtype A1 – 96.7%, subtype B – 1.6%, CRF02_AG – 0.8%, CRF03_AB and CRF06_cpx – 0.45% respectively; heterosexuals (n=309) - subtype A1 – 91.9%, subtype B – 3.9%, CRF02_AG – 1.9%, CRF03_AB – 0.9%, subtypes C, D, G and CRF63_02A1- 0.35% respectively.

Conclusion: IDU-A variant of HIV-1 subtype A1 still dominates in European part of Russia. During the period of time studied the slight trend to subtype B proportion increase can be noted as well as a hint to expanding the genetic profile of HIV-infection with subtypes C, D and CRF63_02A1.

Abstract 30

Near full-length genomic sequencing and analysis of HIV infected individuals in a network-based intervention in Athens (TRIP): identification of novel recombinants

Frampton D1, Ferns R3, Grant P4, Kozlakidis Z1,5, Nikolopoulos G6, Kostaki E3, Hadjikou A7, Pavlitina E10, Hatzakis A2, Williams L8, Friedman S8, Nastouli E4,9, Paraskevis D2

1University College London, 2National and Kapodistrian University of Athens, 3UCLH/UCL, NIHR Biomedical

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The Transmission Reduction Intervention Project (TRIP) was a social network-based contact tracing study to detect individuals with recent HIV infection (within 6 months). Most of the participants were people who inject drugs (PWID). We herein report on their associated near full-length HIV-1 sequences, describing novel recombinants and highlighting the benefits of next-generation sequencing over traditional methods.

Paired end reads were generated for 120 samples using Illumina MiSeq next-generation sequencing. 107 HIV genomes were assembled de novo using methods developed under the Infection Response through Virus Genomics (ICONIC) project. Full-length genomic alignments were performed using MAFFT and the Los Alamos 2012 HIV Sequence Compendium alignment. Subsequent phylogenetic analysis was performed using FastTree, PhyML and ClusterPicker. Subtyping and recombinant analyses were performed using the REGA, RIP and COMET web-based tools and jpHMM. Analysis of pol sequences suggested most samples belonged to previously identified local transmission networks (LTNs) of PWID (hitherto classified as CRF14_BG, CRF35_AD, subtypes A1 and B) in the Athenian outbreak (n=76). However, fuller genomic analysis revealed a more complex picture, with 14/22 CRF35_AD and all 52 CRF14_BG sequences showing evidence of recombination, the majority of the latter displaying similar recombination breakpoints across p15, revealing a URF common to this cohort.

Analysis of near full-length HIV genomes has identified previously undetected recombinants in a recent PWID community outbreak. In doing so it has provided a more reliable description of CRFs (that would otherwise be misclassified) which will help better define transmission clusters in future work.

### Abstract 31

Gag-protease coevolution analyses define novel structural surfaces in the HIV-1 matrix and capsid involved in resistance to Protease Inhibitors

M Codoñer F2,3, Peña R1, Blanch-Lombarte O1, Jimenez-Moyano E4, Pino M5, Vollbrecht T6, Clotet B1,5, Martinez-Picado J1,5,6, Draenert R7, G Prado J1

1AIDS Research Institute Irsicaixa, Hospital Universitari Germans Trias i Pujol, 2Lifesequencing SL, 3Universidad Catolica de Valencia, 4Veterans Affairs San Diego Healthcare System, 5Universitat de Vic, 6Institució Catalana de Recerca i Estudis Avançats (ICREA), 7Medizinische Poliklinik, Klinikum der Ludwig-Maximilians-Universität München

**Background:** Despite the major role of Gag in establishing resistance of HIV-1 to protease inhibitors (PI), very limited data are available on the total contribution of Gag residues to resistance to PI. To identify in detail Gag residues and structural interfaces associated with the development of HIV-1 resistance to PI, we traced viral evolution under the pressure of PI using Gag-protease single genome sequencing and coevolution analysis of protein sequences (CAPS) in 4 patients treated with PI over a 9-year period.

**Methods:** We amplified the Gag-protease coding region of multiple longitudinal plasma samples by bulk and single-genome amplification (SGA). We performed CAPS in 171 HIV-1 Gag-protease sequences obtained by SGA. The sequences were equally distributed across patients and across the times from initiation of therapy with PI (PT1, n=44; PT2, n=38; PT3, n=41; and PT4, n=48). High-resolution HLA class I typing for alleles A, B and Cw was performed using sequence-based typing methods. HIV-1–specific immune responses of the Gag and protease regions were assessed over time in cryopreserved PBMCs by Elispot to exclude CD8+ T-cell immune pressure on Gag coevolving residues.
**Results:** Using CAPS we identified a total of 38 Gag residues correlated with the protease, 32 of which were outside Gag cleavage sites. These residues were distributed in 23 Gag-protease groups of coevolution, with the viral matrix and the capsid represented in 87% and 52% of the groups. In addition, spatial mapping of Gag coevolving sites uncovered the distribution of Gag correlated residues in specific protein surfaces of the inner face of the viral matrix and at the Cyclophilin A binding loop of the capsid.

**Conclusions:** Our findings suggest a tight interdependency between Gag structural proteins and the protease during the development of resistance of HIV-1 to PI. Coevolution analysis of HIV-1 proteins when combined with those of additional functional and structural studies, may serve as a roadmap to guide the design of small molecules to increase treatment efficacy against HIV-1.

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**Abstract 32**

**Long Terminal Repeat (LTR) regions of HIV-2 groups A and B display in vitro differences in LTR transactivation in response to cellular transactivation**

Le Hingrat Q1, Visseaux B1, Bertine M2, Chauveau L3, Schwartz O2, Collin F3, Damond F1, Matheron S4, Descamps D1, Charpentier C1

1IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France, 2Institut Pasteur, Equipe Virus et Immunité, 3Université Bordeaux, ISPED, Centre INSERM U897, Epidémiologie-Biostatistique, 4IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Service de Maladies Infectieuses et Tropicales, Hôpital Bichat, AP-HP, Paris, France

**Background:** The LTR region contains many transcription factors (TF) binding sites and its transactivation by cellular and viral proteins is crucial for HIV reactivation and production. We recently observed a higher genetic variability in the U3 region of the HIV-2 LTR, particularly in the sub-region which encompasses most of the known TF binding sites (nucleotides 374 to 508). Genetic diversity was higher in group B viruses. Interestingly, patients infected by group B viruses had less frequently quantifiable HIV-2 reservoirs in PBMC and those reservoirs were also smaller when quantifiable. The aim of this study was to assess the transcriptional activity of diverse HIV-2 LTR.

**Methods:** HEK293T and Jurkat cells were transfected by plasmids encoding the luciferase gene under the expression of different HIV-2 LTR: (i) ROD-LTR, (ii) EHO-LTR, reference strains for groups A and B respectively, (iii) ΔSp1-LTR which is the EHO LTR lacking the first binding site of Sp1, (iv) Rec-LTR which corresponds to a previously identified A/B recombinant LTR and (v) VAR-LTR, containing the ROD LTR with the PuB1-pets sub-region replaced by the corresponding sequence from EHO. Cells were co-transfected or not by HIV-2 Tat plasmid (pTat) and activated or not by phorbol myristate (PMA). Luciferase activity was measured 24 hours after transfection. In order to assess LTR transcriptional activity, data was normalized with MTT assay for cell viability and to ROD-LTR basal activity to obtain a normalized luciferase index.

**Results:** In HEK293T cells, EHO-LTR and ΔSp1-LTR had halved basal transcriptional activities compared to ROD-LTR (p<0.01 and p=0.01, respectively), whereas Rec-LTR activity was 2-fold higher than ROD-LTR (p<0.01). When co-transfected with pTat, transcriptional activity of ΔSp1-LTR remained 2-fold less than ROD-LTR (p<0.01) but also than EHO-LTR (p<0.01). Transcriptional activities of EHO-LTR and Rec-LTR transactivated by pTat were not significantly different from ROD-LTR. In Jurkat cells, ΔSp1-LTR basal activity was 2-fold less than ROD-LTR (p<1.10^-4), while EHO-LTR exhibited a basal activity similar to ROD-LTR (p=0.71). Rec-LTR had also a basal transcriptional activity similar to ROD-LTR (p=0.21) and VAR-LTR was slightly reduced compared to ROD-LTR (p=0.03). Co-transfection by pTat transactivated all LTRs by 3- to 5-fold but ΔSp1-LTR remained significantly less transactivated than ROD-LTR (p=0.01). After cellular activation by PMA, ROD-LTR exhibited a higher transcriptional activity than all other LTRs (p<1.10^-4): 10-fold more than EHO-LTR and
ΔSp1-LTR, and 2-to-3-fold more than Rec-LTR and VAR-LTR. In Jurkat cells co-transfected with pTat and activated by PMA, transcriptional activity of ROD-LTR was multiplied by a factor 100 and Rec-LTR by a factor 55 (p=0.08), while transcriptional activities of VAR-LTR, EHO-LTR and ΔSp1-LTR were only multiplied by a factor 20 to 30 (p<0.05).

Conclusion: In HIV-2 LTR, the loss of the first binding site of Sp1 is responsible for a 2-fold diminution of basal transcriptional activity. It also seems to impair transactivation by Tat. Differences in response to cellular activation between LTRs suggest a major role of PuB1-pets sub-region in the level of in vitro LTR transcriptional activity. This sub-region is absent from group B sequences which could contribute in the lower level of cellular reservoir.

Abstract 33

Cross-validation of HIV DNA assays to quantify different HIV-1 subtypes

Rutsaert S1, De Spiegelaere W2, Van Hecke C1, De Scheerder M1, Kiselinova M1, Vervisch K1, Trypsteen W1, Vandekerckhove L1

1HIV Cure Research Center (HCRC), Ghent University, 2Department of Morphology, Faculty of Veterinary Medicine, Ghent University

Background: Total HIV DNA is regularly used as an HIV reservoir biomarker in HIV-1 cure trials. The high genetic diversity represented by different HIV subtypes can pose inaccuracy in quantifying HIV DNA across people infected with different HIV-1 strains. Despite numerous assays that have been described in literature, a systematic cross-validation of existing HIV DNA assays is lacking. Moreover, most assays have been designed for HIV-1 subtype B assays, and the efficiency of these assays to quantify different HIV subtypes remains to be evaluated. We aimed to compare the most commonly used HIV DNA assays using an in silico and in vitro analysis.

Materials and methods: A systematic literature search was performed to identify PCR-based assays for HIV DNA quantification in patients. These assays were analyzed in silico using an in-house-developed bioinformatics pipeline. The best performing assays from the in silico analysis and the assays that are most frequently used in practice were selected to be analyzed on HIV-infected cells. PBMCs or MT4s were infected with an NIH-provided panel of patient-derived viruses from different HIV subtypes. Extracted DNA of five samples per subtype (A, B, C, D, CRF01_AE, CRF02_AG) was measured on the ddPCR platform using the selected assays. Subsequently, DNA from PBMCs of 93 HIV+ patients, categorized in thirteen different HIV-1 subtypes, were analyzed by ddPCR with the six superiorly performing assays selected from the first experiments.

Results and conclusion: Sixty-eight assays resulting from the systematic literature search were analyzed in silico and twenty assays were selected for further screening. Overall, all selected assays were able to capture subtype B samples, contrary to the circulating recombinant forms CRF01_AE and CRF02_AG which were more challenging to quantify. Two out of the twenty selected assays performed superiorly regarding all included HIV subtypes (in > 80% of the samples more than 90% of HIV DNA was captured). Four assays were able to capture more than 70% of the HIV DNA in >80% of the samples with varying HIV subtypes. Data generated on samples from HIV-1 positive patients confirmed the performance of these six assays. The six selected assays performed well on a broad range of HIV-1 subtypes.

Our analysis indicates that published assays do not always guarantee an optimal measurement of non-B subtypes and that careful selection of the assay for accurate HIV DNA quantification is crucial.
Abstract 34

Sensitivity of three automatic HIV-1 subtyping tools

Viciana I1, Gonzalez-Domenech C3, Sena G1, Palacios R1, Mora L1, Clavijo E1, Marquez M2, Santos J2

1Hospital Virgen de La Victoria, Microbiology Department, 2Hospital Virgen de la Victoria, Infectious Diseases Unit, 3Hospital Virgen de la Victoria, IBIMA

Introduction and objectives: The current HIV-1 epidemic is characterized by the expansion and emergence of new recombinants of the virus, which hampers correct subtyping, especially in non-B variants. The aim of this study is to compare the sensitivity of three automatic subtyping methods with phylogenetic analysis

Material and methods: HIV-1 subtype was determined in 757 naïve patients diagnosed at the University Hospital Virgen de la Victoria in Málaga (Andalucia-Spain) during the period 2004-2015. The partial sequence of the pol gene (protease+RT) and three automatic tools (Geno2pheno, REGA and COMET) were used. The results were compared with a phylogenetic reconstruction inferred by Maximum Likelihood using the FastTree program. The criterion for assignment to a subtype was a consistent clustering (bootstrap values ≥70%) with a reference sequence. Sequences that could not be associated with a known pure subtype or CRF were analyzed for recombination with Simplot program. The sensitivity values of the subtyping tools were obtained using the calculator available at http://www.hrc.es

Results: The sensitivity of the three automatic tools for subtype B classification was higher than 90% in all cases, although the percentage of sequences associated with it varied significantly according to the method (p <0.001); around 70% by means of phylogeny and REGA (73.8% and 75.6%, respectively) on the one hand, and over 80% with COMET (82.6%) and Geno2pheno (83.6%), on the other. Regarding non-B variants, we also found significant differences (p <0.003) depending on the subtyping option used. This discrepancy is maintained in both pure non-B subtypes and recombinant variants. Only REGA v3.0 approached a sensitivity of 50% (46.46%, CI 95: 39.52-53.41) to detect them. It also well identified complex recombinants as CRF19_cpx (21 sequences) or CRF12_BF (6 sequences). There were 31 (4.0%) sequences that were not associated to any reference and, according to Simplot, were new recombinants of known subtypes.

Conclusions: The automatic tools analyzed identified B subtype well, although both COMET and Geno2pheno overestimated the percentage. For non-B subtypes, both the pure subtypes and the CRFs, the automatic tool with the highest concordance to the phylogenetic analysis and, therefore, greater utility for subtyping, was REGA 3.0. 4% of the sequences were not associated with any reference either by the automatic tools or by phylogeny. REGA by its characteristics and its higher resolution would be the automatic tool of choice for the realization of HIV-1 subtyping in our cohort.

Abstract 35

Combining social and phylogenetic approach in exploration of ongoing and active human immunodeficiency virus transmission

Siljic M1, Cirkovic V1, Lukovic S1, Salemovic D2, Pesic Pavlovic I1, Jevtovic D2, Stanojevic M1

1Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, 2Infectious and Tropical Diseases University Hospital, Clinical Center Serbia, HIV/AIDS Unit, 3Virology Laboratory, Microbiology Department, Clinical Center Serbia

Background: Phylogenetic analyses have been used extensively in studies of HIV-1, and have proven to be an invaluable tool, revealing important aspects of disease progression, transmission of drug resistance mutations, evolution and outbreak investigation which are of great public health relevance. In this research we
combined phylogenetic and population based statistical approaches to assess HIV transmission dynamics and identify clusters that harbor strong potential for onward transmission of HIV.

**Materials and methods:** To identify putative HIV transmission clusters phylogenetic analyses of Serbian HIV sequences were performed using MEGA software. Phylogenetic tree was constructed with 1000 bootstrap resampling using both neighbor joining and maximum likelihood approach under GTR+G+I nucleotide substitution model. Identification of phylogenetic clusters was based on different sets of criteria involving bootstrap support, genetic distance, Bayesian posterior probability value and Phylopart analyses. The largest HIV-subtype B transmission clusters detected, were further subjected to logistic growth model analyses implemented in mathematical application Geogebra. The model assumed that in one year some subjects in an identified cluster were not yet diagnosed, and some had already been infected and diagnosed. Clusters were classified in three groups, based on a model as having low probability (<5%), moderate probability (5-15%) and high probability (>15%) of hidden and potential for future HIV transmissions, which was considered as ongoing and active one with high probability of hidden HIV infection and strong potential for onward transmission. Lastly, we examined the relationship between cluster size and social profiles of cluster members. For this investigation latent class analyses (LCA) was applied to different categorical covariates including: HIV risk factor, year of diagnosis, place of residence, education, age at diagnosis, another STI and clinical stage at the time of diagnosis.

**Results:** Phylogenetic analyses revealed the presence of 14 transmission clusters. The majority of clustering sequences, were from male patients living in Belgrade that predominantly reported MSM (men who have sex with men) as transmission category. Two largest transmission clusters detected, comprising 13 and 8 sequences were evaluated by logistic growth modeling. Of these, one cluster were identified as having very high probability (58%) of harboring undiagnosed infection and one with high moderate probability (10%). LCA identified four classes based on lowest AIC that were associated with cluster size. Class 1 included predominantly young (20-30yrs), MSM patients (85%), living in Belgrade, presenting in CDC stage A (84%) with high potential to be a part of transmission cluster. Classes 2 and 3 included predominantly older patients (>40yrs), with heterosexual contact as transmission risk and presenting in CDC stage C. In addition, patients within classes 2 and 3 were mostly diagnosed in the earlier time period than patients from class1. Class 4 included a mix of MSM and heterosexual transmission category with high prevalence of patient presenting at CDC stage C.

**Conclusions:** This study clearly emphasizes the need of targeted public health action, utilizing novel interventions, to prevent future HIV transmission events, which would be critical in resolving one of the most prominent viral pandemics in human history.

**Abstract 36**

**Analysis of transmission clusters in a cohort of HIV-1 patients**

*Viciana I, Gonzalez-Domenech C, Sena G, Palacios R, Mora L, Clavijo E, Marquez M, Santos J*

1Hospital Virgen de La Victoria, Microbiology Department,       
2Hospital Virgen de la Victoria, Infectious Diseases Unit,       
3Hospital Virgen de la Victoria, IBIMA

**Introduction:** To date, temporal trends in HIV epidemic characteristics have been inferred by correlating transmission cluster composition with imputed infection times. The objective of our study was to analyze the epidemiological, clinical and immunological characteristics of the different transmission clusters (TCs) for a cohort of patients with HIV-1 infection.

**Material and methods:** Phylogenetic analysis of 757 sequences from patients with a new diagnosis of HIV-1 at the University Hospital Virgen de la Victoria in Málaga (Andalucia-Spain) during the period 2004-2015. We performed a preliminary phylogeny using the partial pol gene sequence and MEGA v6.06 program with the
Neighbor Joining method. We eliminated all those sequences that were part of branches with bootstrap values <80%. Finally, we constructed a new phylogenetic tree by the method of Maximum likelihood with FastTree through CIPRES. We selected the clusters with a bootstrap value ≥90% and performed a study of the TCs in general as well as of those TCs represented by five or more sequences in detail. Epidemiological, clinical and analytical parameters of the patients were collected and resistance mutations in Reverse Transcriptase and Protease were analyzed. Statistical analysis was performed by SPSSS v 16.0.

Results: 451 out of 757 patients (59.6%) were grouped into 53 TCs, 17 of them with five or more individuals. The largest number of patients associated within a TC was 90. Patients in TCs were mostly MSM (79.1%), from Spain (77.2%) and recent seroconverters (30.1%). Patients in TCs had fewer AIDS events [72 (16%) vs. 78 (25.5%), p = 0.001] and lower percentage of late diagnosis [199 (44.1%) vs. 173 (56.5%), p = 0.001]. The percentage of subjects associated with TCs was higher in the non-B subtypes than in the B subtype (73.7% vs. 54.5%, p <0.001). 118 patients (15%) had transmission resistance mutations, 94 of them (79.6%) were included in some TCs. The most frequent mutations associated with clusters were T69D/N, L210W and K219E/Q, for NRTIs, K103N and G190A/S for NNRTI, and the I54L/M and L90M mutations for PIs. The prevalence for resistance to NNRTI was higher in TCs (13.7%). There were two TCs of peculiar non-B subtypes: CRF19_cpx, with 21 individuals, 16 of them (76.2%) with mutation G190A; and CRF51_01B with 39 patients, 20 of them with the K103N mutation.

Conclusions: In our cohort, more than 50% of patients with a new diagnosis of HIV-1 infection were included in one of the 53 TCs detected. Belonging to a TC was associated with MSM, Spanish origin, recent seroconverter, increased presence of resistance mutations and non-B subtype. Fifteen percent of the patients had transmission of drug resistance mutations. NNRTI mutations were the most frequent among patients included in TCs. Some TCs were associated with infrequent non-B subtypes, like CRF19_cpx and CRF51_01B, in both cases with primary resistance mutations.

Abstract 37

Analysis of non B subtypes detected in a cohort of patients with HIV infection in Southern Spain

Viciana I1, Gonzalez-Domenech C3, Sena G1, Palacios R2, Mora L1, Clavijo E1, Marquez M2, Santos J2

1Hospital Virgen de La Victoria, Microbiology Department, 2Hospital Virgen de la Victoria, Infectious Diseases Unit, 3Hospital Virgen de la Victoria, IBIMA

Background: The prevalence of non B subtypes is increasing in our country as result of population movements. The objective of our study was to know the prevalence of different non B subtypes in our cohort of patients and its relation with epidemiological, clinical and immunovirological parameters.

Material and methods: HIV-1 subtype was determined in 757 sequences corresponding to naive patients diagnosed in our center from 2004 to 2015, by phylogenetic analysis of the partial sequence of the pol gene using the Max Likelihood method and the FastTree program. The criterion of assignment to a subtype was a consistent clustering (bootstrap ≥70%) with a reference sequence. The sequences that could not be associated were analyzed with the Simplot program. Epidemiological, clinical and immunological data of these patients were also analyzed

Results: The prevalence of non-B subtype was 26.2% distributed as follows: 5(2,5%)A1, 6(3%)C, 3(1.5%)F1, 2(1%)F2 3(1.5%)G. The rest of the non-B subtype were recombinant forms: CRF51_01B 44(22,2%), CRF19_cpx 21(10,6%), CRF02_AG 14 (7%), CRF30_0206 14 (7%), CRF01_AE 9 (4.54%) and CRF42_BF 10(7%). There were 31 sequences (17.3% of the recombinants) that were not pooled with any known subtype or CRF. Of the 198 patients with non B subtype, 80,8% were male, with median age of 40 years, median baseline CD4 count of 311 cells/mL and median baseline viral load of 5 log10. 59,1% of the patients were born in Spain and 40,9% were immigrants. 59% were MSM and
37.4% reported heterosexual transmission route. Rate of transmitted drug resistance was 32.8%. 23.2% were recent seroconverters and 54% had a late diagnosis of HIV-1 infection.

**Conclusions:** the prevalence of non-B subtypes has stabilized in our country. There is a predominance of CRFs, being CRF51_01B and CRF19_cpx the most prevalent. The prevalence of primary resistance was high in patients infected with non-B subtypes. The 17.3% of the recombinant sequences could not be assigned to any known subtype or CRF.

**Abstract 38**

**Significant Primary Resistance Mutations and High Genetic Diversity in Newly Diagnosed HIV-1 Patients in Bulgaria (2012-2014) (Preliminary Analysis)**


1National Center of Infectious and Parasitic Diseases, 2Specialized Hospital for Infectious and Parasitic Diseases, 3Department of Infectious Diseases, Medical University, 4University hospital St Marina, 5Department of Infectious Diseases, Medical University, 6Clinic of Infectious diseases, University Hospital, 7Department of Infection and immunity, Luxembourg Institute of Health

**Introduction:** In Bulgaria 570 cases with HIV/AIDS were diagnosed from 2012 to 2014. Epidemiological data indicated great heterogeneity of HIV-1 positive population, including 238 (41.8%) heterosexual (HET), 227 (39.8%) men who have sex with men (MSM), 88 (15.4%) people who inject drugs (PWIDs), 10 (1.8%) were MSM who inject drugs and 7 (1.2%) infants infected by vertical transmission. The aim of the present study was to analyze the prevalence of primary resistance mutations (PRM) and HIV-1 diversity among newly diagnosed HIV-1 infected individuals in Bulgaria 2012-2014.

**Materials & methods:** PRM and HIV-1 subtype diversity was analyzed in 254 (44.6%) randomly selected naïve to antiretroviral therapy individuals. HIV-1 pol sequences were generated with TruGene and/or ViroSeq Genotyping Systems. PRM were determined using the Stanford HIVdb version 8.3. HIV-1 subtypes were defined using COMET v2.2. The sequence alignment contained Bulgarian sequences and reference sequences from Los Alamos database. Phylogenetic relationships and possible clusters with PRM were inferred by ML analysis with FastTree program.

**Results:** The overall PRM prevalence was 15.4% (39/254), comprising 8 (3.1%) persons with resistance to nucleoside reverse transcriptase inhibitors (NRTI), 32 (12.6%) to non-nucleoside reverse transcriptase inhibitors (NNRTI), 2 (0.8%) individuals to major protease inhibitors (PI) and 2 (0.8%) to accessory PI. Dual class PRM was identified in 3 (1.2%) patients with both NRTI and NNRTI. 41 (16.1%) persons reported infection abroad of which PRM was identified in 5 foreign individuals diagnosed in Bulgaria and in 1 Bulgarian citizen acquired infection outside country. Regarding transmission groups, 2 major PI, 3 NRTI and 18 NNRTI were found in MSM, 1 accessory PI, 5 NRTI and 14 NNRTI were found in HET and 1 accessory PI were found in PWIDs. The main HIV-1 clade was subtype B 162 (63%) followed by 11 different subtypes including: 31 (12%) CRF01_AE, 14 (5.5%) A1, 14 (5.5%) unclassified and 7 other subtypes and CRFs representing 33 (13%) of all individuals in the study. The highest prevalence of PRM was found in subtype A1. Phylogenetic analysis identified 4 clusters and one group of 6 closely related sequences, all with PRM to NNRTI. One cluster of subtype A1 sequences contained K103E and K103N, two clusters each of 2 pairs of subtype B sequences with E138A, one cluster of 2 subtype B sequences with V179D and a group of 6 subtype B sequences with different PRM including: E138G, K101E and V179E.

**Conclusions:** Our analysis identified significant number of diagnosed MSM with HIV-1 in recent years (2012-2014). The prevalence of different PRM in newly diagnosed HIV-1 individuals in Bulgaria varies and is highest to the accessory...
NNRTI and low to NRTI and PI. We found unequal distribution of PRM among different subtypes and phylogenetic clusters revealing possible local infections with resistance viral clades particularly to NNRTI. Our findings indicated that providing of detailed molecular biological surveillance of PRM in newly diagnosed individuals with HIV-1 in Bulgaria is of great importance to better manage first line antiretroviral therapy and understand development of the epidemic in the country.

Abstract 39

The RESINA data support the individualized therapy based on primary resistance testing

Böhm M1, Jensen B10, Müller C1, Schütler E1, Heger E1, Neumann-Fraune M1, Büch J11, Sierra S1, Oette M2, Lübke N10, Fätkenheuer G2, Hower M4, Knechten H6, Naeth G5, Schübel N1, Esser S7, Scholten S6, Qurishi N9, Römer K9, Häussinger D10, Kaiser R1, Knops E1

1University of Cologne, Institute for Virology, 2Krankenhaus der Augustinerinnen, 3Department for Internal Medicine, University Hospital Cologne, 4City Hospital Dortmund, 5Private practice, 6Clinical Center Osnabrück, 7University of Essen, 8Private practice, 9Private Practice Göttingen, 10Department for Gastroenterology, University Hospital Düsseldorf, 11Max-Planck-Institute for Informatics

Background: The RESINA study started in 2001 and was originally focused on the evaluation of primary resistance in patients at the time point of first therapy. Additionally, we could follow up these patients (RESINA cohort) since cART start by collecting the clinical, virological and immunological data.

Methods: The clinical, virological and immunological data were collected from 30 centers since 2001. Genotypic analysis of resistance-associated mutations (RAMs) was performed from viral RNA exclusively until 2012, since then additionally from proviral DNA and/or total NA. Resistance-associated mutations were detected by Sanger sequencing and since 2015 by next-generation sequencing (Illumina MiSeq technology). Additionally, we collect similar data from any therapy-experienced patient within the AREVIR project (about 11000 samples).

Results: Meanwhile the RESINA cohort consists of more than 4000 patients. Furthermore, we performed a total number of more than 15000 resistance tests from therapy-naïve and -experienced patients (RESINA and AREVIR data). During this time we could observe a decline in prevalence of resistance-associated mutations in treatment-experienced patients from 80 percent to about 40 percent over the last 16 years. In contrast to this decline of RAMs in therapy-experienced patients the frequency of primary resistance-associated mutations at the beginning of cART remains relatively stable at 10 percent. The majority of the primary RAMs were NRTI resistance mutations in which the mutation T215 shows the highest frequency throughout the whole time of observation. NNRTI resistance-associated mutations were on a low level and did not increase over time although the use of NNRTI increased in our cohort since 2001. We did not observe an increase in primary PI resistance-associated mutations and almost no primary INI-resistance mutations.

Conclusions: Despite the declining frequency of resistance-associated mutations in therapy-experienced patients the frequency of primary resistance mutations remains stable at approximately 10% and justifies routine primary resistance testing. We can further conclude from our data that the individualized therapies according to the DAIG therapy guidelines for therapy-naïve patients translate in a low number of NNRTI- and PI-resistance-associated mutations in therapy-naïve and -experienced patients and prevent the transmission of INI mutations.
Abstract 40

Unravelling the epidemic history of HBV genotype A using a full-genome phylogenetic and phylogeographic approach

Kostaki E1, Karamitros T2, Stefanou G1, Kramvis A3, Mamais I1, Angelis K1, Hatzakis A1, Paraskevis D1

1Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, 2Department of Zoology, University of Oxford, 3Hepatitis Virus Diversity Research Unit, Department of Internal Medicine, University of the Witwatersrand, 4Department of Health Sciences, School of Sciences, European University of Cyprus

Background: Hepatitis B Virus (HBV) infection is a global public health problem. The dissemination of HBV genotypes and sub-genotypes follows complex geographical patterns, with variable levels of heterogeneity. Genotype A is a globally distributed HBV genotype with high predominance in N. W. Europe, N. America, S. Africa and Brazil. Our aim was to study the global dispersal of genotype A, by estimating levels of regional clustering, in order to shed light on how the virus has been disseminated within different geographic areas.

Materials & methods: We analyzed 731 full-length non-recombinant genotype A sequences available in public databases. HBV genotypes were confirmed by the Oxford HBV Automated Subtyping Tool and phylogenetic analysis using 110 reference sequences from all previously known HBV genotypes. The number of sequences available for different geographical regions (according to Global Burden of Disease-GBD classification system) was: N. America: 245, W. Europe: 112, sub-Saharan Africa: 112, L. America: 62, Asia/Pacific: 53, Caribbean: 52, C. Europe: 37, S. Asia: 26, E. Europe: 17, S. E. Asia: 8, E. Asia: 4, N. Africa/Middle East: 2, and C. Asia: 1. Phylogeny reconstruction with bootstrap evaluation was conducted by the maximum likelihood (ML) method as implemented in RAxML v8.0.20. We defined as monophyletic clusters those having bootstrap values higher than 70% within which 70% of genotype A strains share the same geographic area of sampling. Phylogeographic analysis was conducted by reconstruction of ancestral states using the criterion of parsimony on the ML estimated phylogeny using Mesquite v3.2.

Results: The phylogenetic analysis showed that genotype A strains form two major clusters; I including sequences mostly from sub-Saharan Africa, and II sequences mostly from W. Europe and N. America. Cluster I consists of two sub-clusters. The first includes sequences mostly from sub-Saharan Africa (sub-cluster Ia) and the second from Caribbean, L. America, and S. Asia (sub-cluster Ib). Within subcluster Ia, we found five regional epidemics (local transmission networks, LTNs) comprised of sequences sampled in Haiti (N=20; 1 LTN), Cameroon (N=8; 1 LTN), and S. Africa (N=43; 3 LTNs). Similarly sub-cluster Ib, sequences from Haiti (N=24; 2 LTNs), and Brazil (N=20; 2 LTNs) formed four regional epidemics. For cluster II, we found ten regional epidemics comprised of sequences from Japan (N=11; 2 LTNs), Argentina (N=6; 1 LTN), Belgium (N=33; 1 LTN), Germany (N=5; 1 LTN), Panama (N=13; 1 LTN), and the USA (N=240; 4 LTNs). No monophyly patterns were observed for strains sampled from C. Europe, E. Europe, S. E. Asia, E. Asia, and N. Africa/Middle East. In addition, we found that sequences sampled from sub-Saharan Africa were located close to the root of the tree.

Conclusions: Genotype A epidemic probably originated in sub-Saharan Africa and expanded at different time points to Europe, Asia and the Americas. Genotype A showed a strong pattern of regional dispersal suggesting that the population movements associated with cross-border transmissions were limited. Given the putative origin of genotype A in sub-Saharan Africa, our findings reflect the effect of ‘out of Africa’ population movements, which occurred at different time periods.
Abstract 41

The detection of specific vaccine-escape HBsAg mutations in HBV genotype D infected patients correlates with high viremia and hampers HBsAg detection and quantification

Salpini R1, Piermatteo L1, Di Carlo D1, Battisti A1, Colagrossi L1, Bertoli A1, Fabenri L1, Fini V1, Iuvara A2, Ricciardi A2, Cerva C1, Sarrecchia C1, Lichtner M1, Mastroianni C1, De Sanctis G1, Paoloni M1, Martignani M1, Pasquazzi C2, Iapadre N1, Mari T12, Parruti G11, Romano S12, Visca M12, Moretti A13, Vecchiet L14, Samatti L1, Andreoni M15, Cappiello G12, Spanò A12, Grelli S13, Angelico M15, Perno C1, Svicher V1

1Tor Vergata University, Department of Experimental Medicine and Surgery, 2National Institute for Infectious Diseases L. Spallanzani, IRCCS, 3Tor Vergata University Hospital, Microbiology and Virology Unit, 4Tor Vergata University Hospital, Infectious Diseases Unit, 5“Sapienza” University, Department of Public Health and Infectious Disease, 6“Umberto I” University Hospital, 7“S. Filippo e Nicola” Hospital, Infectious Disease Unit, 8“S. Andrea Hospital”, Department of Gastroenterology, 9“San Salvatore Hospital”, 10“Nuovo Regina Margherita” Hospital, 11Infectious Disease Unit, Pescara General Hospital, 12Microbiology and Virology Unit, “S. Pertini” Hospital, 13Gastroenterology Unit, “San Filippo Neri” Hospital, 14Clinic of Infectious Diseases, Department of Medicine and Science of Aging, University “G. d’Annunzio” Chieti-Pescara, 15Tor Vergata University Hospital, Hepatology Unit

Background: Vaccine-escape HBsAg mutants can challenge vaccine efficacy. Limited information is available on their circulation and correlation with virological and serological parameters in HBV-infected patients (pts).

Methods: This study includes HBsAg sequences obtained from 631 HBV genotype D infected pts from clinical practice. Ten vaccine-escape HBsAg positions (116-120-126-129-131-133-141-142-144-145) based on Lazarevic,2014, are analyzed. dN/dS analysis is estimated by HyPhy to define positions under evolutionary selective pressure. Association between vaccine-escape mutations and each mutation in major hydrophilic HBsAg region (MHR, target of neutralizing antibodies) is assessed by hierarchical clustering. WT and mutated HBsAg are expressed in HepG2 cells; after 72h quantitative-HBsAg is tested (in triplicate) in cell-supernatants by a commercial assay.

Results: >1 vaccine-escape mutation is detected in 17% of pts. By multivariable logistic regression model, higher serum HBV-DNA and age are independently correlated with the presence of >1 vaccine-escape mutation (OR [95%CI]:1.23[1.1-1.4], P=0.001; 1.05[1.03-1.07], P<0.001).

By dN/dS analysis, vaccine-escape positions 126, 133 and 142 are under strong positive selective pressure (P from 10-2 to 10-5). Mutations at those positions are observed in 2.4% (N=15), 4.8% (N=30) and 0.6% (N=4) of pts, respectively.

The presence of >1 mutations at positions 126, 133, and 142 correlates with lower HBsAg levels (median [IQR]:781 [250-2,489]IU/ml vs 2,416 [460-7,816]IU/ml, P=0.05), and with the detection of atypical serological profiles (HBsAg negativity despite detectable HBV-DNA and co-positivity to HBsAg and anti-HBs) (26% with vs 9% without mutations, P=0.01).

By covariation analysis, vaccine-escape mutations at positions 126 and 133 lie on divergent genetic pathways involving different MHR mutations: T126I with G145R (bootstrap=0.83), M133I with L109I/M (bootstrap=0.87), and M133T with both T131N and G130N (bootstrap=0.99).

Notably, G130N and T131N+M133T introduce 2 N-linked glycosylation sites, and in vitro determine a 70% and 40% reduction in HBsAg quantification compared to wt, respectively.

Conclusions: Vaccine-escape mutations circulate in a relevant fraction of HBV genotype D infected patients, and correlate with higher serum HBV-DNA, atypical serological profiles and lower HBsAg levels. This suggests that, by evading antibody recognition, these mutations can promote viral fitness, and affect full-reliability of assays for HBsAg detection/quantification.
Abstract 42

The positivity rates of hepatitis B virus s - antigen in the pediatric population, 20 years after the introduction of the systemic antihepatitic B vaccination in Romania

Vintila S1, Gheorghe E1, Osman E1, Merisescu M1, Juguete G1,2

1National Institute for Infectious Diseases "Prof. Dr. Matei Bals", 2University of Medicine and Pharmacy “Carol Davila"

Background: Hepatitis B virus (HBV) is an important human pathogen due to its worldwide distribution and severe consequences associated with the chronic infection. Systematic vaccination of romanian children was initiated in 1996.

Material and method: We studied the rate of the positive hepatitis B virus s antigen (HBsAg) by retrospectively questioning the patient’s data base of the National Institute for Infectious Diseases „Prof. Dr. Matei Bals” – Bucharest. Data from January 2003 to December 2016 were collected. For the pediatric patients (age < 14) of whom the serologic status of the HbsAg was annalised, required by the patient’s phisician, using an ELISA testing method, we annalissed the prevelence rate of the positive test in correlation with the patients sex, age and vaccinal status.

Results: A total of 21.797 ELISA test were required, out of which 2147 (9.87%) associated positive result, with an obvious and sustained decrease of the positivity rate – a 17.5% positivity rate was registered in the first five years of the evaluated period (2003-2007) in contrast with a 6.99% positivity rate associated with the last 5 year of the monitored period (2012-2017). A gender difference was registered, the M:F ratio being of 1280:867 and a higher positivity rate was observed in the 0-3 year age group.

Discussions and conclusions: The constant decrees of the HBsAg positivity rate highlights the importance and the success of the national vaccination campaign but the obtained data, which present a 9.87% HBsAg positivity rate, most probably overestimate the real national prevalence of the positive HBsAg in the pediatric population, mainly based on the fact that the cohort does not completely superpose the general population pattern, the patients addressing our infectious diseases specialized institute associating a higher risk for VHB infection. The real burden of chronic VHB pediatric infection in Romania requires further structured populational studies since only regional or monocentric analysis are available.

Abstract 43

The prevalence and distribution of HCV genotypes and sub genotypes among chronically infected Serbian patients

Pesic I1, Jevtovic D2, Salesmovic D2, Jovanovic S1, Stanojevic M3

1CCS, Department of Microbiology, 2Belgrade University Medical School, Infectious Diseases Hospital, Clinical Centre of Serbia, 3Belgrade University Medical School, Institute for Microbiology

Background: While the diversity in HCV prevalence in Europe has been ranging from 0.4% to 22%, it was the estimated to be around 1.3% in Serbia, which is higher than in developed European countries and still lower than in Central Europe. However, insight into HCV genotypes (Gs) and sub genotypes (sG) allows us to analyze and understand its prevalence, essential for effective targeted treatment and prevention approaches. Thus a retrospective study was conducted to analyse HCV Gs and sG prevalence among chronically infected patients evaluated for anti-HCV therapy.

Patients and methods: Over a rather long period from 2005 to now-a-days, a total of 2977 HCV mono-infected patients were included into the study, of which 164, 2578 and 235 belonged to three study periods, from 2005-2006, 2010-2016,
and 2016-2017, respectively. They were analysed using virology techniques, for HCV viral load measuring (Amplicor Roche Monitor, COBAS TaqMan HCV Test 2.0, Roche). For further HCV genotyping and subtyping, gene sequencing of 5' NTR type specific PCR along with commercial kit (InnoLipa, Inn genetics, Genotyping Linear Array hepatitis C virus test, Roche Diagnostics) used for early patients, was followed by reverse hybridization, Linear Array Hepatitis C Virus Genotyping Test (Roche Molecular Systems, Branchburg, NJ, USA) in the second study period, and recently introduced Abbott Real Time HCV Genotype II and Cobas HCV GT Roche, performed in the third study period.

**Results:** The overall prevalence of G1, even though varies from 57.9%, over 59.1% to 67.6%, in three study periods, respectively, showed that this genotype was a more prevailed one. During the first study period the G 1 (mainly the subtype 1b) was the most prevalent one, while G3a and G1b and 3a, were far less common. Using reverse hybridization in the second study period, we registered the increasing prevalence of G1 while non-1 Gs (G2, G3 and G4) comprises with 1.9%, 28%, 8% and 10.1%, respectively. However, since new generation Abbott and Roche qualitative real time PCR techniques for HCV genotyping has become available, we found that out of 235 samples analyzed 67.6% remained within G1, while pure non 1G (2, 3 and 4) were registered in only 23.4%, whereas remaining were various recombinants and/or dual infections, including 1a/4, 1b/3, 2/4, 3/4/. Moreover, within G1 it turned to be 33.6%, 19.1% of 1a and 1b, sG, respectively. Various recombinants and/or dual infections including 1a/1b, 1b/2, 1a/4, 1b/3, were recorded in 5.9%, 0.4%, 1.2%, 0.4% and 0.8%, respectively.

**Discussion:** Taking together, this new PCR based molecular method allowed more precise and accurate insight into the epidemiology of this infection, sG prevalence variations, which could be essential for pre-treatment evaluation, in order to put more efforts in infection eradication using new treatment approaches.

**Abstract 44**

**Characterization of NS5A and NS5B regions in HCV genotype 3a patients treated with direct-acting antiviral agents**

Giombini E¹, Bartolini B¹, Taibi C¹, Lionetti R¹, Montalbano M¹, Visco-Comandini U¹, D’Offizi G¹, Capobianchi M¹, Garbuglia A¹

¹Inmi L Spallanzani Ircs

**Background:** Hepatitis C virus (HCV) genotype 3 (GT3) is the second most prevalent genotype worldwide and is associated with an increased risk of steatosis and development of cirrhosis and hepatocellular carcinoma. In this study, the genetic heterogeneity of the NS5A and NS5B regions in 45 GT3a patients who received SOF ± DCV treatment with or without pegIFN+RBV was evaluated in GT3 Italian patients. In patients who did not achieve SVR, ultra-deep pyrosequencing (UDPS) was performed on samples collected at baseline and after virological failure, to assess whether novel RAS emerged at virologic escape.

**Material and methods:** Plasma samples from 45 HCV GT3a-infected patients receiving DAA-based therapies were obtained from our clinical Center. Four patients did not achieve SVR12. The HCV NS5A and NS5B regions were amplified using specific NS5A primers spanning 6102–6738 nt in D17763 and NS5B pan-genotypic primers spanning 8037–8665nt in D17763, respectively. In patients who experienced virological failure, UDPS was performed.

**Results:** Sequences from 42 patients were available in the analysis; in NS5A, 42 amino acid positions had polymorphisms, with 13 positions harbouring multiple polymorphisms. Three of the polymorphisms were not detected in the Los Alamos or GenBank database GT3 NS5A sequences: T79S, T107K and T107S. M28L polymorphism was detected in one virologic failure. NS5A-A30K was observed in 9.5% (4/42) of baseline NS5A sequences, while only the reference amino acid was observed at position 31. NS5A-Y93H was detected at baseline in only one patient also harboured A30K. Amino acid
positions 24, 32, 38, and 93 were associated with a negative selective pressure while positions 28, 30, 31, 58, 62 and 92 appeared to be under neutral pressure. Only two positions (7 and 103) were shown to be associated with a positive selective pressure.

In NS5B, 32 amino acid positions had polymorphisms, with 8 positions harbouring more than one polymorphism. Four polymorphisms (G166R, Q180K, V235M and C274W) were not observed in GenBank; all 4 patients with these polymorphisms achieved SVR. Polymorphisms at amino acid positions 159, 282, and 321, associated with SOF resistance in GT3, were not detected. Polymorphisms at the other noted NS5B positions (298, 316 and 320) were not observed. In patients who failed treatment, additional novel baseline NS5B polymorphisms (298, 316 and 320) were not detected. Polymorphisms at the other noted NS5B positions (298, 316 and 320) were not observed. In patients who failed treatment, additional novel baseline NS5B polymorphisms (G188D, Q273P, L285F, A306V, N310S), that were enriched after virological failure.

Positions 159, 244, 282, 289, 316, 320, 321, involved in drug resistance, were under negative pressure while positions 309 and 310 appeared to be under neutral pressure in this analysis. The only amino acid position under positive pressure was 185; polymorphisms at this position was observed in 11.9% (5/42) of patients.

In patients who failed treatment, novel baseline NS5B polymorphisms were detected by UDPS (G188D, Q273P, L285F, A306V, N310S), that were enriched after virological failure. Three polymorphisms present at baseline at low-frequency (I184T, G188D, and N310S, each at 0.7%) were on the same haplotype and became the predominant viral species after virologic failure.

**Conclusions:** Analysis of substitutions by UDPS can help to identify substitution potentially involved in therapy DAA resistance.

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**Abstract 45**

**HCV genotype diversity among patients treated with antiviral medications in Georgia**

**Kamkamidze G**, Kajaia M, Abzianidze T, Gulbiani L, Gamezardashvili A, Bustashvili M

1Medical Clinic Neolab

**HCV genotype diversity among patients treated with antiviral medications in Georgia**

**Background:** Hepatitis C virus (HCV) infection is one of the major public health problems worldwide. The prevalence of viral hepatitis in the country of Georgia is high: the population serosurvey conducted by Georgian Ministry of Health with support of US CDC estimated HCV seroprevalence of 7% in general population of Georgia. Identification of HCV genotypes is important for outbreak investigations as well as for determination of antiviral treatment duration and prognosis of its outcome.

**Methods:** Consecutive patients with HCV infection treated during 2013-2016 in outpatient clinic NeoLab, which represents one of the main sites in Georgia responsible for HCV diagnostics and treatment, have been studied. 5 ml blood from each subject has been collected in EDTA containing tubes. HCV antibodies were defined by ELISA. Samples from antibody-positive subjects were investigated by HCV RNA real-time PCR (Sacace, Italy). Among subjects positive by HCV RNA PCR the HCV genotypes have been determined by HCV genotype real-time PCR assay (Sacace, Italy) or alternatively by Versant HCV Genotype v2 (Siemens, Ghent, Belgium).

**Results:** 3310 participants were involved in study. Among them 431 (13%) were females and 2862 (87%) were males. The mean age was 44.6. Out of 3310 patients enrolled 859 (30.1%) had genotype 1, 745 (26.1%) had genotype 2 and 1189 (41.6%) had genotype 3. Genotypes 4, 5 and 6 have not been detected in any of our patients. Significant difference has been observed in the distribution of genotypes between the patients with and without history of intravenous drug use (IDUs). Among 1101.
patients ever using injection drugs the genotype distribution was as follows: genotype 1 – 286 (26.5%), 2 – 299 (27.7%) and 3 – 477 (44.1%), while among 764 non-IDUs the genotype distribution was as follows: genotype 1 – 277 (37.2%), 2 – 203 (27.2%) and 3 – 251 (33.7%), The difference between these two subgroups was statistically significant (p<0.0001). The prevalence of mixed genotypes was significantly higher (p<0.01) among IDUs vs. non-IDUs.

Conclusion: Our study has shown the higher proportion of HCV genotype 3 and presence of mixture of two genotypes among IDUs in comparison with patients with no history of intravenous drug use.

Abstract 46

Genotypes of HCV in HIV/HCV co-infected patients from Grodno region of Belarus

Matsiyeuskaya N1

1Grodno State Medical University

Introduction: Today the number of HCV-infected people is 4 times higher than HIV-infected ones. Associated HIV/HCV-infection has different variants: simultaneous HIV and HCV infection; HIV superinfection; HCV superinfection.

Aim of the research: to detect prevalence of various HCV genotypes in patients with HIC/HCV co-infection living in Grodno Region of Belarus.

Material & methods: Study group on HCV genotypes detection included 145 co-infected HIV/HCV patients from Grodno region—the 1st group. The 2nd group contained 95 patients with HCV monoinfection living in the same region. There was no patients received antiviral HCV therapy.

HCV RNA extraction from plasma was performed by “RIBOsorb” (AmpliSens, Russia), HCV genotyping was performed by PCR method using a reagent kit “AmpliSens ® HCV-genotype” (Russia). Statistical analysis of the results was carried out using “Statistics” v.10.0. Probability of differences of 95% was considered to be statistically significant (p<0.05).

Results: Frequency of 3a genotype in the 1st group was 56 (38.6%), in the 2nd group - 44 (46.3%), p>0.05, test χ2. Genotype 1b frequency in the 1st group was 28 (19.3%), in the 2nd group - 43 (45.2%), p<0.05. Genotype 1a was detected in 31 (21.4%) patients of the 1st group and in 6 (6.1%) of the 2nd group, p<0.05. Patients with the HCV genotype 2 had insignificant frequency in both groups: 4 (2.8%) in the 1st group and 2 (2.1%) in the 2nd, p>0.05. Frequency of RNA HCV-negative patients was rather high (17,9%) in the group of HIV/HCV co-infection, besides antibodies to hepatitis C virus (anti-HCV) were detected in all patients.

Number of HIV/HCV co-infected patients infected by intravenous drug using (IDU) was 98 (67.6%), by sexual way of transmission – 47 (32.4%), p<0.05. Among IDU genotype 3a was detected in 40 (40,8%), genotype 1a - in 19 (19,4%), genotype 1b - in 21 (21,4%). Among HIV/HCV co-infected patients with sexual way of HIV transmission genotype 3a was detected in 16 (34,0%), genotype 1a - in 12 (25,5%), genotype 1b - in 7 (14,9%). The distribution of HCV genotypes in patients with HIV/HCV co-infection accordance to way of HIV transmission did not differ significantly (p>0.05).

The most common variant of co-infection in HIV/HCV co-infected patients was HIV super-infection together with earlier detected HCV-infection - 89 (61,4%) of cases. Variant of simultaneous co-infection by two viruses was established in 53 (36,5%) of patients. HCV super-infection with earlier diagnosed HIV-infection was detected in 3 (2,1%) patients.

In all HCV genotypes the basic variants of co-infection were HIV super-infection and simultaneous infection by both viruses. Among patients with negative results of HCV RNA test (anti-HCV positive) HIV super-infection was detected in 18 (69,2%), simultaneous co-infection – in 7 (26,9%), HCV super-infection – in 1 (3,9%).

Conclusion: In Grodno region among HIV/HCV co-infected patients 3a and 1a HCV genotypes prevail. Among HIV/HCV co-infected patients there is quite large RNA-negative group (17,8%), that is why further detailed analysis of undetected viraemia is necessary for solving of the question on indications for anti HCV therapy in these patients.
Abstract 47

Comparison of genetic variability and resistance profile among DAA-naïve and DAA-failed HCV 3 infected patients in Italy


1Department of Experimental Medicine And Surgery, University of Rome Tor Vergata, 2 Infectious Diseases, University Hospital of Rome Tor Vergata, 3 Infectious Disease Unit, University Hospital of Rome Tor Vergata, 4 Infectious Disease Unit, Pescara General Hospital, 5 Infectious Diseases, Sant’Andrea Hospital – “La Sapienza” University, 6 Hepatology Unit, Pescara General Hospital, 7 Hygiene Unit, IRCCS AOU San Martino-IST, 8 Molecular Virology, IRCCS Policlinico Foundation San Matteo, 9 Infectious Diseases and Viral Hepatitis Unit, Second University of Naples, 10 Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, ASL City of Turin, 11 Department of Internal Medicine, University Hospital of Messina, 12 Division of Infectious Disease, ASST Fatebenefratelli Sacco, 13 Clinical Microbiology, Virology and Bioenergencies, ASST Fatebenefratelli Sacco, 14 Infectious Disease, IRCCS AOU San Martino - IST Genova, 15 Infectious Diseases Unit, University of Sassari, 16 Gastroenterology, “P. Giaccone” University Hospital, 17 Department of Clinical Medicine and Surgery, University “Federico II” of Naples, 18 Division of Hepatology, IRCCS San Martino, IST Genova, 19 Internal Medicine, Humanitas University, Rozzano, 20 Unit of Gastroenterology, University of Turin, Department of Medical Sciences, City of Health and Science of Molinette Turin Hospital, 21 Division of Infectious and Tropical Diseases, IRCCS Policlinico Foundation San Matteo, 22 Institute of Infectious Diseases, University of Pavia, 23 Department of Infectious Diseases, Hospital Niguarda Ca’ Granda, 24 Department of Medical Sciences Experimental and Clinical.

Background: Hepatitis C virus (HCV) genotype 3, even today with the current direct antiviral agents (DAA) based regimens, remains a difficult to treat genotype. Little information is known about the HCV variability and resistance in HCV-3. Therefore, the aim of this study was to investigate the prevalence and characteristics of resistance-associated-substitutions (RASs) in HCV-3 infected patients, naïve to direct-acting antivirals (DAA) and/or failing an interferon-free regimen in Italy.

Materials and methods: A total of 257 HCV-3 infected patients (183 DAA-naïve and 98 DAA-failures, of them, 24 at both baseline and DAA-failure) were analyzed. era sequencing of NS3-protease and/or NS5A and/or NS5B was performed by homemade protocols, specific for genotype 3. Phylogenetic analysis was performed to evaluate appropriate genotype allocation and concordance with previous genotype/subtype assignment.

Results: The majority of patients were male (86.0%) and cirrhotic (59.4%). Twenty-three patients (9.7%) were HIV-coinfected, and 41.6% of patients, with available risk-factor information, were injection-drug-users. Phylogenetic analysis classified viral sequences as HCV-3a (99.6%) and HCV-3h (0.4%). Notably, 16/257 (6.2%) patients were previously misclassified as infected with indeterminate-genotype (N=6), non-3 genotype (N=9), or as mixed-infection (N=1).

Overall, 98 patients experienced a virologic failure to an interferon-free regimen: 47/98 (47.9%) to sofosbuvir+ribavirin and 44/98 (44.9%) to daclatasvir+sofosbuvir+/-ribavirin. Moreover, 6/98 (6.1%) were treated with 3D+/-ribavirin (paritaprevir/r+ombitasvir+dasabuvir), due to a wrong-genotype 1 assignment. At virological failure, 59/98 (60.2%) patients showed at least one RAS related to the DAA-regimen, of whom 3/7 (42.8%) in NS3, 46/50 (92.0%) in NS5A, 8/92
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(8.7%) in NS5B sofosbuvir-failures. Among 46 NS5A failing patients with NS5A-RAS, 15/46 (32.6%) failed the optimal sofosbuvir+daclatasvir+ribavirin 24-weeks regimen. However, among the total 16 patients who failed sofosbuvir+daclatasvir+ribavirin 24-weeks regimen, the NS5A-RAS prevalence was 93.7%. In DAA-naïve patients, overall RAS prevalence was lower (32/183, 17.5%) than in DAA-failing patients, as expected. Indeed, the NS3-Q80K was mainly detected in 3D-failures (33.3% vs 1.1% DAA-naïve, p=0.006), while the NS5A-Y93H RAS was found in 5/6 (83.3%) 3D-failures, in 38/44 (86.4%) daclatasvir+sofosbuvir-failures, vs 6/163 (3.7%) DAA-naïve patients (p<0.001). Interestingly, 3/6 DAA-naïve patients with natural Y93H were treated with daclatasvir+sofosbuvir+ribavirin for 24 weeks and 1/3 (33.3%) reached SVR. Natural NS5A RASs were detected also in 8/47 (17.0%) sofosbuvir+ribavirin failing patients (2=Y93H, 1=A30K+L31F and 5=A62L). In NS5B, sofosbuvir RASs L159F and S282T were both detected only at virologic failure of a sofosbuvir-containing regimen (5.3% and 2.1% prevalence, respectively).

Conclusions: Performance of HCV sequencing before first-line or second-line DAA regimens allows a personalization of DAA regimen by both definition of viral resistance profile, and assessment of “correct” HCV genotype, avoiding suboptimal and inappropriate treatments that may lead to multiple RASs development. Failures in HCV-3 are frequent associated with resistance, particularly with Y93H NS5A-RASs, whose role in reducing treatment efficacy deserves further investigation. This high prevalence of NS5A-RASs also in patients, who already failed an optimal NS5A-based regimen with long duration, makes retreatment of such patients a challenging scenario.

Abstract 48

Hepatitis Delta Antigen is characterized by an extensive degree of genetic variability that correlates with elevated levels of serum HDV-RNA

Colagrossi L1, Salpini R1, Scutari R1, Battisti A1, Piermatteo L1, Bertoli A1, Fabeni L1, Menichini C1, Trimoulet P3, Fleury H2, Nebuloso E1, De Cristofaro M1, Cappiello G1, Spanò A1, Malagnino V1, Mari T1, Barlattani A1, Iapadre N1, Lichtner M8, Mastroianni C1, Lenci I1, Pasquazzi C10, De Sanctis G1, Galeota Lanza A12, Stanzione M13, Stonaiuolo G13, Marignani M10, Sarmati L5, Andreoni M5, Angelico M5, Perno C1, Coppola N2, Svičer V1

1Tor Vergata University, Department of Experimental Medicine and Surgery, 2Second University of Naples, Department of Mental Health and Public Medicine, Section of Infectious Diseases, 3Hôpital Pellegrin tripode, Laboratoire de Virologie, 4Sandro Pertini Hospital, 5Tor Vergata University Hospital, Infectious Diseases Unit, 6Nuovo Regina Margherita Hospital, 7San Salvatore Hospital, 8Sapienza University, Department of Public Health and Infectious Diseases, 9Tor Vergata University Hospital, Hepatology Unit, 10Sanità Hospital, Department of Gastroenterology, 11Umberto I Hospital, 12AO Cardarelli, Gastroenterology Unit, 13Second University of Naples, Viral Unit, 14National Institute for Infectious Diseases L. Spallanzani-IRCCS

Background: HDV antigen (HDAg) is present in viral capsid and interacts with HBV surface protein (HBsAg). No information is available on the extent of genetic variability in HDAg and its impact on virological parameters.

Methods: Among 78 patients with chronic HBV+HDV infection, HDAg gen-1 sequences (aa:1-214) are obtained for 47 pts and HBsAg gen-D sequences (aa:1-226) for 31 pts. Shannon Entropy (SE) is used to measure the extent of amino acid variability at each HDAg and HBsAg position in overall population and by stratifying patients according to serum HDV-RNA: 18 pts with HDV-RNA<5logIU/ml defined as low-viremic and 29 with HDV-RNA>5logIU/ml defined as highly-viremic. Positions with SE=0 are defined conserved.
Results: Median (IQR) serum HDV-RNA and HBV-DNA are 4.7(2.5-6.1) and 2.0(1.3-3.1)logI.U/ml while median (IQR) ALT and AST are 68(43-127) and 66(34-101)U/L. By SE, a lower % of conserved residues is observed in HDAg than HBsAg (49.5% for HDAg vs 69.2% for HBsAg, P<0.001). The degree of HDAg genetic variability varies according to HDAg domains. In particular, HDAg domains with a lower % of conserved positions are multimerization domain (MD, aa:31-52) and RNA-binding domains (RDBs, aa:2-27; 97-107; 136-146), followed by nuclear localization signal (NLS, aa:68-88) and viral-assembly signal (VAS, aa:195-214) (% conserved residues: 27.3%, 31.3%, 52.4% and 70%, respectively). Notably, stratifying patients according to serum HDV-RNA, a higher degree of genetic variability is observed in highly-viremic than in low-viremic pts (% conserved residues: 55.6% vs 65.9%, P=0.037). In addition, HDAg mutations S6R, A22S and L90S significantly occur more frequently in highly-viremic than in low-viremic patients (S6R: 24.1% [7/29] vs 0% [0/18], P=0.034; A22S: 72.4% [21/29] vs 33.3% [6/18], P=0.015; L90S: 31% [9/29] vs 0% [0/18], P=0.008). In particular, median (IQR) serum HDV-RNA in presence of >1 of these mutations is 5.7 (5.1-6.3) vs 4.4 (3.8-5.6) in their absence (P=0.003). S6R and A22S reside in RDB-I, while L90S is close to NLS (domains crucial for HDV life cycle), suggesting that these mutations can confer a replicative advantage to HDV.

Conclusions: An extensive genetic variability in HDAg correlates with elevated serum HDV-RNA, suggesting a still ongoing evolutionary HDV adaptation to human host. Specific HDAg mutations correlate with higher HDV-RNA and might have an impact on long-term disease progression. This genetic variability should be taken into account for the design of novel pharmacological targets.

Abstract 49

Report of a novel C1483W mutation in the hepatitis E virus polymerase in patients with acute liver failure

Kar P1, Borkakoti J2, Ahmed G2

1Max Super Speciality Hospital Vaishali, 2Department of Medicine, Maulana Azad Medical College, University of Delhi

Background: Here, we report the molecular alterations in the HEV genome from patients with acute liver failure (ALF) and acute viral hepatitis (AVH) from North India, including pregnant women and its association with the poor outcome of the disease.

Materials and methods: Partial sequencing of the RNA Dependent RNA polymerase (RdRp) region of the ORF 1 protein in the HEV genome from representative samples from patients with ALF and AVH was done.

Results: Two novel mutations were identified- Cysteine 1483 Tryptophan and Asparagine 1530 Threonine in 100% (25/25) of the patients with ALF compared to none (0/30) in the patients with AVH (P < 0.0001). Disease severity parameters along with viral load corresponding to the samples with C1483W and N1530T mutations were significantly higher compared to those lacking the mutation showing significant association with the outcome in ALF patients.

Conclusion: The nucleotide substitutions in the RdRp region may play a crucial role in enhancing HEV replication thus leading to disease severity.
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M184V/I as unique NRTI resistance mutation does not impact the efficacy of an abacavir/lamivudine/dolutegravir use as switch in patients with a fully suppressed viral load

Marcelin A1,2, Charpentier C3, Wirden M1,2, Peytavin G3, Soulie C1,2, Simon A4, Yazdanpanah Y6, Katlama C2,6, Descamps D1, Calvez V1,2

1Hôpital Pitié-Salpêtrière, Virologie, 2Sorbonne Universités, UPMC Univ Paris 06-UMR_S 1136 Pierre Louis Institute of Epidemiology and Public Health, Inserm, 3IAME, UMR 1137-Université Paris Diderot, Sorbonne Paris Cité, Inserm, 4Hôpital Pitié-Salpêtrière, Médecine Interne, 5Hôpital Bichat-Claude Bernard, Maladies Infectieuses, 6Hôpital Pitié-Salpêtrière, Maladies Infectieuses

Background: M184V/I NRTI resistance mutations can be selected by lamivudine/emtricitabine and abacavir conferring a full lamivudine/emtricitabine resistance and an intermediate abacavir resistance. There are controversies about the use of abacavir/lamivudine/dolutegravir combination in HIV-1 treated patients with a fully suppressed viral load and harboring M184V/I as unique NRTI resistance without resistance to integrase inhibitors. The aim of this study was to assess the efficacy of ABC/3TC/DTG combination when used in pretreated patients with an undetectable viral load and harboring M184V/I as unique NRTI resistance and without any resistance to integrase inhibitors.

Materials & methods: 59 patients with a fully suppressed HIV-1 plasma viral load (< 50 copies/ml) treated by TDF/FTC+r/DRV (n=20) or TDF/FTC+r/ATV (n=28) or ABC/3TC+r/DRV (n=4) or ABC/3TC+r/ATV (n=7) and switched to a ABC/3TC/DTG regimen with M184V/I as unique NRTI resistance mutation in their therapeutic history were retrospectively analyzed at M3 and M6 after the switch to ABC/3TC/DTG.

Results: at Day 0, the median CD4 cell count was 603 cell/mm3 and all patients had confirmed viral load < 50 copies/ml for at least 6 months prior to switch. The history of their resistance testing showed for NRTI: M184V in 54 cases or M184I in 5 cases; for NNRTI at least one IAS NNRTI resistance mutation in 27 cases and for PI in 2 cases.

During the first 6 months of follow-up, two patients had a blip (106 copies/ml in one case and 53 copies/ml in the other) at month 3 followed by a subsequent viral load < 50 copies/ml.

Conclusion: M184V/I as a unique NRTI resistance mutation, regardless of selection by regimens containing 3TC or FTC or ABC, does not affect the response of virologically suppressed patients who switch to ABC/3TC/DTG for at least 6 months.

Abstract 51

Efficacy and durability of maintenance therapy with lamivudine and darunavir/ritonavir or atazanavir/ritonavir in virologically controlled patients from the clinical practice enrolled in ARCA

Di Carlo D1, Bezenchek A2, Battisti A1, Incardona F2,3, Bavaro D.F.4, Zuccalà P5, Bai P6, Salomoni E7, Giachè S7, Gagliardini, R.8, Di Giambenedetto S9, Pecorari M9, Zazzi M10, De Luca A10, Lo Caputo S10

1University of Rome "Tor Vergata", Experimental Medicine and Surgery, Rome, Italy, 2IPRO - InformaPRO S.r.l., Rome, Italy, 3EuResist GEIE, Rome, Italy, 4University of Bari "Aldo Moro", Clinic of Infectious Disease, Bari, Italy, 5University of Rome, "Sapienza", Department of Public Health and Infectious Diseases,Rome, Italy, 6Clinica di Malattie Infettive, Ospedale San Paolo, ASST Santi Paolo e Carlo, Dipartimento di Scienze della Salute, Università degli Studi di Milano, 7University of Florence, Tropical and Infectious Diseases, Florence, Italy, 8University of Rome, Laboratorio virologia Cattolica, Rome, Italy, 9Polyclinic of Modena, Virology, Modena, Italy, 10University of Siena, Department of Medical Biotechnologies, Siena, Italy
Background: To evaluate the durability and the maintenance of virological suppression (VS) in antiretroviral-experienced HIV-1 infected patients with viral load (VL) <50 copies/mL switching to lamivudine+atazanavir/ritonavir (3TC+ATV/r) or lamivudine+darunavir/ritonavir (3TC+DRV/r) in clinical practice.

Material & methods: Survival analysis was used to estimate the probability and predictors of virological rebound (VR, the first of two consecutive VL >50 copies/mL or one single VL >200 copies/mL after switching) and of treatment discontinuation (TD). Cumulative drug resistance (CDR) to nucleos(t)ide reverse transcriptase inhibitors (NRTIs), non-NRTIs (NNRTIs) and protease inhibitors (PIs) (IAS/Stanford HIVDb 2017 lists) was evaluated in all plasma genotypic resistance tests (GRTs) available before switching.

Results: We analyzed 314 patients, 134 under 3TC+ATV/r (group-A), 180 under 3TC+DRV/r (group-B). Main differences between groups were due to the prevalence of B-subtype (group-A: 50.7%; group-B: 65.6%; p=0.008), of patients under VS pre-switch >36 months (group-A: 46.3%; group-B: 61.1%; p=0.009), and of patients switching from tenofovir/emtricitabine+ATV/r (group-A: 67.2%; group-B: 8.9%; p<0.0001), and from tenofovir/emtricitabine+DRV/r (group-A: 0%; group-B: 31.7%; p<0.0001).

By 36 months after switching, the overall probability of VR was 9.7%, without significant differences between groups. By Cox multivariable model (adjusted for demographic/viro-immunologic/therapeutic confounders) male patients and those having a period of VS pre-switch >36 months showed a lower risk of VR compared to female patients (adjusted hazard ratio, aHR [95% confidence interval]: 0.29[0.10-0.86]; p=0.025), and to patients having a period of VS <12 months (aHR 0.10[0.02-0.59]; p=0.011), respectively. Patients showing at least one viral blip during VS pre-switch (aHR 5.85[1.71-19.99]; p=0.005) and those treated with multiple prior regimens (aHR per 1 regimen increase 1.2[1.04-1.40]; p=0.013) showed a higher risk of VR. Dual therapy type was not associated with VR.

Among patients having at least one previous GRT pre-switch (N=209), patients with concomitant CDR to 3 drug-classes (NRTI+NNRTI+PI) showed a higher probability of VR compared to those having CDR to 2, 1 and none drug-class (33.3%-11.8%-4.8%-6.1%; p=0.002).

For 2 patients experiencing VR (both treated with 3TC+ATV/r), a GRT at VR was available. One of them, without any CDR to PIs, showed DR to PIs at VR (V32I-M46L-I50L-V82A).

Overall, 148 patients had a TD. Toxicity was the main reason of TD (62-42%), followed by simplification (27-18%), drug-interactions (12-8%), and patient choice (8-6%). By 36 months after switching, the overall probability of TD was 44.4%, and was significantly higher in group-B compared to group-A (50.4%-36.4%; p=0.021). By Cox multivariable model, the calendar year of starting dual was the only variable associated with a higher risk of TD (aHR per 1 year increase 1.15[1.03-1.29]; p=0.016). There were no significant differences found in the aHR of TD according to treatment group. The overall probability of TD due to toxicity was 19% (group-A: 15%; group-B: 22%; p=0.162).

Conclusions: Our analysis confirms data from clinical trials on the high efficacy of maintenance therapy with lamivudine+darunavir/ritonavir or atazanavir/ritonavir in a more polymorphic population from the clinical practice, without differences between type of PI used. A heavier previous treatment experience and extensive drug resistance may influence the risk of VR with these strategies.
Abstract 52

PBMC resistance evaluation predicts virological response after therapy switching in treated patients with undetectable HIV-1 RNA

Armenia D1, Zaccarelli M2, Borghi V1, Gennari W1, Di Carlo D1, Giannetti A2, Fortici F2, Bertoli A1, Gori C2, Fabeni L2, Pinnetti C3, Marocci R1, Latini A2, Ceccherini-Silberstein F1, Mastroianni C4, Mussini C3, Antinori A2, Perno C.F.2, Santoro M.M1

1University of Rome "Tor Vergata", Rome, Italy, 2National Institute for Infectious Diseases L. Spallanzani, IRCCS, Rome, Italy, 3Polyclinic of Modena, Italy, 4La Sapienza University Polo Pontino, Latina, Italy, 5San Gallicano Dermatological Institute, IRCCS, Rome, Italy

Background: We evaluated the impact of baseline resistance detected in PBMC genotypic resistance test (GRT) on maintaining virological suppression after therapy switch in combined-antiretroviral-therapy (cART) treated patients with undetectable HIV-1 RNA.

Materials & methods: Patients switching therapy with a GRT from PBMCs available before therapy change were included. Baseline resistance and genotypic susceptibility score (GSS) from PBMC GRT and previous cumulative plasma GRT (when available) were evaluated. Survival analysis was used to assess probability and predictors of virological rebound (VR: two consecutive viremia >50 copies/mL or 1 viremia >1000 copies/mL after switch). The following variables were considered: GSS, age, gender, subtype, CD4 nadir, virological suppression duration before switching, number of previous viral blips, previous treatments, antiretrovirals (number and type) administered at switch.

Results: Overall, 227 cART-treated patients with virological suppression lasting from a median (IQR) of 3.7 (0.9-7.0) years were analyzed. Patients were on ART since a median (IQR) time of 9 (4-16) years, with a CD4 nadir of 184 (80-336) cells/mm³. Patients had a median (IQR) number of previous regimens of 4 (2-7); of them, 55.9% experienced ≥3 antiretroviral classes. 18.9% and 28.2% of patients switched to a PI-based dual/monotherapy and INSTI-based therapy, respectively. At baseline, 43.2% of patients showed at least one primary resistance mutation in PBMCs (PI: 10.1%; NRTI: 33.9%; NNRTI: 22.0%; INI: 0.4%); 87.3%, 11.8% and 0.9% of patients showed fully susceptible, intermediate resistant and fully resistant GSS, respectively. Twenty-four months after therapy switch, the overall probability of VR was 15.3%. Patients showing intermediate or full resistant GSS had a higher probability of experiencing VR compared to those carrying a fully susceptible virus (27.2% vs. 13.7%, p=0.001). By multivariable Cox-regression, a higher adjusted hazard (AHR [95% Confidence interval, CI]) of experiencing VR was found in patients with: i) intermediate/full resistant GSS compared to those with fully susceptible GSS (4.01 [1.17-13.77], p=0.027); ii) a nadir CD4 cell count <100 cell/mm³ (4.65 [1.49-14.49], p=0.008); iii) a shorter time of previous virological suppression (per 1 year lower, 1.32 [1.08-1.61], p=0.007).

Among 114 patients with previous plasma GRT available, 103 maintained virological success; of them, 70 (68%) showed a susceptible GSS in both previous plasma and PBMC GRTs. Only one patient showed intermediate/fully resistant GSS in PBMCs but not in plasma, while 32 (31%) patients showed resistance only in plasma GRT, suggesting an overestimation of the predictive role of plasma resistance. Among the 11/114 patients experiencing VR after therapy switch, one patient (9.1%) with virus fully susceptible in PBMCs but not in plasma showed a discordant prediction due to underestimated resistance in PBMCs.

Conclusions: Virologically suppressed patients with an intermediate/fully resistant GSS in PBMCs, a low CD4 cell nadir and with a shorter previous virological control have a higher risk of rebound after therapy switch. Despite resistance can be underestimated in PBMC, PBMC genotyping might be a useful tool for tailoring antiretroviral treatment switch in patients with unknown previous information, and might reinforce the clinical relevance of previous plasma resistance.
Abstract 53

HIV Genotypic Drug Resistance test (GRT) for Integrase Strand Transfer Inhibitors (INSTIs) at low level viremia: light and shadow in the clinical practice

Mileto D1, Cattaneo D2, Mancon A1, Tamoni A1, Fiori L1, Meraviglia P3, Rusconi S1, Gismondo M1, Micheli V1

1Clinical Microbiology, Virology and Bioemergencies, ASST FBF-Sacco, L. Sacco University Hospital, 2Unit of Clinical Pharmacology, ASST FBF-Sacco, L. Sacco University Hospital, 3Department Infectious Diseases, ASST FBF-Sacco, L. Sacco University Hospital, 43rd Division of Infectious Diseases, ASST FBF-Sacco, L. Sacco University Hospital

Background: Despite their good tolerability and efficacy, INSTI-based regimens could be affected by virological failure too. The current guidelines recommend GRT at viral load (VL) >200 cp/mL. The aim of this study was to depict the different pathways of resistance mutations at virological rebound according to VL in INSTI-experienced patients as well as to investigate their prevalence in HIV+ naïve subjects.

Materials & methods: A retrospective study was conducted from 2009 to August 2016 on new HIV-1+ diagnoses and on experienced patients with loss of virological suppression while receiving Raltegravir (n=143), Elvitegravir (n=7) or Dolutegravir (n=9), for whom current antiretroviral regimen information was available. GRTs on PR/RT and integrase regions were performed on a total of 92 naïve and 159 experienced patients administered with INSTI-based regimens. The pattern of INSTI resistance mutations (major+accessory) was evaluated according to the following VL ranks <200, 201-500, 501-1000, 1001-10000, 10001-100000, >100000 cp/mL and over calendar years. GRT results were interpreted by means of Stanford algorithm version 8.2. In addition, Therapeutic Drug Monitoring (TDM) of Raltegravir was conducted in a subgroup of patients (n=83) to better depict the virological rebound.

Results: GRTs were performed at median (IQR) VL 4.85 Log (4.20-5.38) and 3.41 Log (2.77-4.20) cp/mL, in naïve and experienced patients, respectively. New HIV-1 diagnoses harboured clade B in 56.5% versus 87.4% of experienced patients (p<.001). Among 92 naïve patients, no one carried INSTI major mutations, while 14 (15%) presented accessory mutations at codon 74, 157 and 163. Regarding INSTI recipients, 83 (52%) showed at least one major mutation, 16 (10%) at least one accessory mutation, while 60 (38%) harboured a wild-type virus. The highest probability to detect a virological rebound in presence of INSTI major mutations lies in the 500-1000 cp/mL rank versus <500 cp/mL (p=.002) and >1000 cp/mL (p=.02), being 32.4% of GRTs performed at VL <1.000 cp/mL. Among patients harbouring INSTI major mutations, the most commonly observed were N155H (48.8%), Q148H/K/R (33.7%), Y143A/C/H/R (10.8%), T66K/I (10.0%) and E92Q (1.2%); five patients (6.0%) showed a more complex pattern with the presence of two major mutations, regardless of VL. Moreover, only the mutation at 148 codon presented an increasing prevalence related to VL ranks (p=.01). As regards the years of INSTI administration, there was a decreasing prevalence of mutation at codon 143 over the time, it being present in the first years of INSTI administration, when these drugs were not so optimally flanked. The prevalence of mutation at codon 155 and 148 is quite similar if VL >1,000 cp/mL versus <1,000 cp/mL (p=.03). TDM for Raltegravir highlighted that 76% of patients with Raltegravir concentration <20 ng/mL presented none INSTI major mutation versus 38% with >20 ng/mL (p=.002).

Conclusions: GRT for INSTIs at low VL is useful in the clinical practice, even if a better understanding of the virological rebound could advantage from TDM. Considering both the GRT and TDM results jointly the virological failure in absence of drug resistance mutations for INSTI-based regimens seems to be an overestimated phenomenon.
Abstract 54

Typhoid fever presenting as acute psychosis in a patient with HIV infection

Ramos E1

1Philippine General Hospital

Background: Typhoid fever remains to be an important cause of life-threatening illness in the developing world. Neuropsychiatric manifestations of Salmonella infection have been described in case reports and case series. Infection with the Human Immunodeficiency Virus (HIV) is a rising epidemic worldwide. Its presence can lead to altered clinical presentation of disease among patients.

Case presentation: We report a case of a 32 year-old Filipino male who developed acute onset febrile illness, with temperatures from 38-39C associated with behavioral changes, blank stares and decreased verbal output. He was admitted in our hospital on the fourth day of illness. The patient was received awake, disoriented to person, place and time and does not follow commands. He had a BP of 110/70 mmHg, regular pulse rate of 112 and temperature of 38.6C. The physical examination only showed oral thrush, seborrheic dermatitis on the face and scalp. There were no abdominal tenderness, hepatomegaly generalized rash and focal neurologic deficit. The patient had supple neck.

The patient was managed as a case of meningitis and septic encephalopathy. He was gven ceftriaxone 2g IV 12 hourly and acyclovir 700 mg IV 8 hourly. Laboratory investigation showed a normal leukocyte count, unremarkable urinalysis and chest x-ray. HIV testing showed a viral load of 869,000 copies/mL and CD4 count of 7. Bacterial culture studies showed Salmonella typhi bacteremia sensitive to ceftriaxone. The cerebrospinal fluid had no bacterial growth on culture. On the sixth hospital day, the patient had improved sensorium and was discharged.

Discussion: We present a case of typhoid fever presenting as acute psychosis in a patient infected with HIV. Salmonella infection remains common in the developing world. The mechanism by which it causes neuropsychiatric disorder is not yet known. In patients with HIV, common bacterial infections may present atypically. A high index of suspicion is necessary to institute proper diagnosis and management.

Abstract 55

Therapeutic Challenges in a Heavily Treated Patient with Multiple Resistance Mutations in All Antiretroviral Classes

Raducanu I1, Tudor A1, Tomozei M1

1“Prof. Dr. Matei Bals” National Institute

Background: Romanian HIV epidemic is characterized by a large number of young adults diagnosed during childhood who experienced a very high number of antiretroviral regimens in the last 20 years. In patients who fail multiple regimens, it is very likely to find highly resistant viral making difficult to maintain the viral suppression for long time.

Case report: We report the case of a 28 years old young woman diagnosed with HIV infection at 2 years old. At the moment of HIV diagnosis she was severe immunosuppressed, CD4 count =25 cells/ mm3, cachectic, and she had recurrent pneumonia, purulent chronic otitis, HIV encephalopathy (memory and learning disorders). She started antiretroviral therapy in January 1996 with zidovudine monotherapy. After 10 month zalcitabine was added and after another 2 years she received triple therapy with protease inhibitor (ritonavir) and reverse transcriptase inhibitors as backbone (lamivudine plus stavudine). This was the start of the eighteen antiretroviral regimens containing combinations with protease inhibitors and nucleoside inhibitors. Later on she also received integrase inhibitor (raltegravir) and CCR5 antagonist (maraviroc).
She did not obtain viral suppression in this period despite of tailored therapy with genotypic resistance tests. In the same time she had developed allergic reactions to abacavir and efavirenz. The adherence to therapy was mostly low and fair, with variations over time, creating the conditions to add more and more resistance mutations for all the approved antiretroviral drugs. In April 2016 the CD4 count was 255 cells/mm3, viral load was 82886 copies/ml. We performed a phenotypic test and despite of the high level viremia we found a lot of resistance mutations: NRTI: K65R, T69T/I, K70K/R, M184V, K219K/E, NNRTI: V90I, PI: L10V, I13V, K20R, V32I, L33F, E35D, M36I, M46L, I47V, I50V, F53L, I54L, L63T, T74A, I85V, L89M, L90M. The interpretation demonstrated resistance to abacavir, didanosine, emtricitabine, lamivudine and tenofovir for reverse transcriptase inhibitors. We found susceptibility to stavudine, zidovudine, efavirenz, nevirapine, etravirine nad rilpivirine. There was no resistance to integrase inhibitors, but many resistance mutations to all boosted protease inhibitors in use today (atazanavir, darunavir, lopinavir). The tropism test showed dual and mixed viral population suggesting lack of activity for CCR5 antagonist. In June 2016 the patient started a new regimen containing boosted darunavir (1200/200 mg), etravirine (400 mg), fixed dose zidovudine- lamivudine (600mg +300 mg), dolutegravir 50 mg once daily. Follow up visits showed suppression of viral replication. At 4 weeks, the viral load was 182 copies/ml. The viral load was below detection limits (40 copies/ml) at 12 and 24 weeks. CD4 count increased to 353 cells mm3 after 3 months and to 473 cells/ mm3 after 6 month of treatment.

Discussion: Low adherence to cART and multiple resistance mutations to more than three antiretroviral classes create challenges in finding an active regimen. In our patient the fully active drugs were actually dolutegravir and etravirine, explaining the good outcome despite of highly resistant strains. In the same time the adherence was very good, because she was planning to have a baby.

Abstract 56

Comparison of genotypic and phenotypic drug susceptibility evaluation of a rare case of multidrug resistant HIV-1

Saladini F1, Giannini A1, Martini A1, Poggi A2, Vichi F2, Del Pin B2, Boccuto A1, Vicenti I1, Di Pietro M2, Zazzi M1

1Department of Medical Biotechnologies, University of Siena, 2Infectious Disease Unit, S. Maria Annunziata Hospital

Background: Current HIV-1 treatment options are very effective in the control of viral replication, resulting in a continuous decline of the frequencies of acquired drug resistance mutations among treated patients. However, rare cases of multiple resistance to HIV-1 inhibitors still occur and the clinical management of patients harbouring multidrug resistant strains remains a challenge. In such cases, the assessment of drug resistance through laboratory testing is essential for the selection of the best treatment option. Here we report the genotypic and phenotypic drug susceptibility evaluation of a rare six-class drug resistant HIV-1 strain.

Materials and methods: The patient was diagnosed HIV-1 positive in 1990 and started a dual NRTI therapy in 1993, followed by more than 20 treatment changes including all drug classes. The patient has been followed for almost ten years by intensive genotyping analysis due to multiple treatment failures, mainly due to poor adherence. Since 2012, viral load has been >100,000 copies/ml and CD4 count <100 cells/µl. REGA, HIVdb, GRADE, ANRS and AntiRetroScan prediction algorithms were queried to compare the predicted levels of resistance to PIs, NRTIs, NNRTIs, and INIs based on the last available as well as cumulative genotype. Phenotypic investigation was carried out at the last time point by recombinant virus assays specific for each of the viral drug target coding regions, under virological failure of a therapy including DTG, DRV, TDF and RPV. The drugs tested included ATV, DRV, LPV, ABC, TDF, AZT, RPV, ETR, RAL, DTG and ENF.
**Results:** Genotypic testing revealed a complex pattern of mutations conferring high levels of resistance to PIs (L10I, V11L, K20R, V32I, L33F, E35D, K43T, M46L, Q58E, A71I, V82F, I84V, L89M, L90M), NRTIs (M41L, E44D, D67G, V75M, M184V, L210W, T215Y, K219N), NNRTIs (K101H, V179F, Y181C, G190A) and INIs (L74M, T97A, E138K, G140A, Q148R), while Geno2Pheno Coreceptor algorithm identified the viral strain as X4 (FPR 1.7). Although prediction algorithms were mostly in agreement, some discrepancies were found in the predicted activity of DRV, LPV, TDF, RPV (intermediate vs. complete resistance). According to the Phensense assay cut-offs, phenotypic investigation confirmed high-level resistance to the PIs DRV (FC 578), ATV (FC 755) and LPV (FC 1270), the INIs RAL (>5000) and DTG (FC 606), the NRTI AZT (FC 20.7), and the NNRTI RPV (FC 25.6), while intermediate resistance was detected for TDF (FC 1.6, cut-off range 1.4-4) and for ETR (FC 7, cut-off 2.9-10). Interestingly, ABC retained full activity, despite close to the lower cut-off (FC 4.3, cut-off range 4.5-6.5). The uncommon substitution N43Q was found in gp41 coding region and the resulting FC value of 23.7 indicated resistance to ENF. Phenotypic tropism assay confirmed a dual tropic variant.

**Conclusions:** Although multidrug resistance is uncommonly selected under latest treatment strategies, this clinical case highlights that a minority of patients still exists who harbour challenging multidrug-resistant virus as a result of progressive accumulation of treatment failures. In such cases, phenotypic investigation could be considered by clinicians to identify potential residual drug susceptibility assisting treatment decisions.

**Abstract 58**

**HCV inter-subtype 2k/1b recombinant detected in a DAA treated patient in Italy**

Paolucci S1, Premoli M1, Mondelli M2, Ludovisi S2, Baldanti F1

1Molecular Virology Unit, Virology and Microbiology Department, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy; 2Department of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy

**Background:** DAA drug combinations are potent and effective antiviral drugs that now represent recommended treatment options for chronic HCV infection. However, outcomes might be impaired by different predictors of lack of treatment response; genotypes more complex to treat are one of them. Herein, we report a case of DAA treatment failed in a HCV infected patient carrying a recombinant genotype 2k/1b.

**Materials and methods:** In this study we reported a 55-year-old lady of Russian origin acquired HCV infection after receiving blood transfusion for miscarriage in Russia. She was treated with sofosbuvir in combination with ribavirin for 20 months as EASL clinical practice guidelines. HCV RNA level (Abbott HCV-RNA assay, Abbott Park, Illinois, U.S.A.) and genotype analysis by different commercial assay (Versant HCV Genotype 2.0 Assay LiPA, Siemens Healthcare Diagnostic Inc., Tarrytown, NY USA; Abbott RealTime HCV Genotype II, Abbott Park, Illinois, U.S.A.) were performed at the baseline and after failure respectively. In addition, HCV 5’UTR, NS3, NS5A and NS5B genes nucleotide sequences was performed by direct sequencing. Evaluation of nucleotide sequences were conducted by comparing viral strain obtained from the patient with the sequences available in GenBank. Phylogenetic analysis was performed and genetic distance between strains was evaluated with pairwise analysis using the best substitution evolutionary model selected by MEGA software (version 5.0).

**Results:** Genotype performed at the baseline revealed that she was infected with HCV genotype 2a. While after treatment failure the genotyping showed the presence of two viruses 1b and 2. HCV 5’UTR, NS3, NS5A and NS5B direct sequencing showed that patient was infected by a recombinant form 2k/1b of HCV RNA. Phylogenetic analyses showed that all the strains sequences isolated from the patient belonged to HCV subtype 2k/1b. Genetic distances in the three genes NS3, NS5A and
NS5B among patient HCV sequences and the 2k/1b reference strains were lower (4-7% of genetic distance) as compared to others 1a, 2b, 2k subtypes (20-76% of genetic distance), while sequencing analysis in the 5'UTR with 98% homology for genotype 2k confirmed the presence of HCV subtype 2k/1b. Phylogenetic tree analysis showed that the 2k/1b recombinant virus strain carrying from the patient formed a monophyletic cluster with the previously described recombinant form 2k/1b sequences in all three genes. **Discussion:** The present case, to our knowledge, is the first report of a recombinant form 2k/1b in Italy. Although the possibility of a reinfection with a recombinant strain in Italy cannot be ruled out, it appears more likely that the patient was infected in Russia. Even though, the presence of recombinant forms of HCV might begin to circulate or might be already present by now even in Italy. The commercial tests in use are not able to accurately identify their presence. In this patient, given that no RAVs was detected, it is likely that the therapeutic failure of sofosbuvir plus ribavirin was due to inadequate treatment for the genotype 2k/1b recombinant. However, further study of HCV recombination need to be conducted to improve the clinical impact of the use of DAAs.

**Abstract 59**

**Prevalence of NS5A resistance associated variants in patients experienced a virological failure**


1Department of Health Sciences (DiSSal), University of Genoa, 2Department of Internal Medicine, Gastroenterology Unit, University of Genoa, 3Infectious Diseases, IRCCS AOU San Martino-IST, 4Hygiene Unit, IRCCS AOU San Martino-IST, 5Department of Infectious Diseases, E.O. Galliera Hospital

**Background:** Direct-acting antiviral agents (DAAs) represent the new standard against hepatitis C virus (HCV), showing an excellent sustained virological response (SVR) rate. However, not all patients achieve SVR with this novel anti-HCV compounds, as their viruses often develop resistance-associated variants (RAVs) to one or to all classes of the drugs used in the therapeutic regimen. This study aimed to evaluate the prevalence of NS5A RAVs in patients who experienced a virological failure (VF).

**Materials & methods:** HCV-RNA was extracted using NucliSENS easyMAG system (bioMérieux, Boxtel, The Netherlands) and genomic regions were amplified with/b specific HCV genotype/subtype primers, in two steps using reverse transcriptase PCR. Subsequently, cDNA of the NS5A genomic target (1-213 aa) was purified and sequenced by 3130-Avant Genetic Analyzer (Life Technologies, NY, USA). Sequences were aligned by SeqScape Ver. 3.3 Software (Life Technologies, NY, USA). Mutations and predictions of phenotypic resistance were obtained using Geno2pheno tool (latest version available at the time of our analysis) (http://www.geno2pheno.org/). We evaluated and compared RAVs at baseline (when available) and at failure in 58 patients. Thirty-two/58 patients were treated with an NS5A (Ledipasvir or Daclatasvir or Ombitasvir administered in 3D regimen) + NS5B (Sofosbuvir or Dasabuvir in 3D) ± Protease Inhibitor (Paritaprevir in 3D) ± Ribavirin (RBV) , and 26 received a Sofosbuvir + RBV regimen.

**Results:** Among 32 patients failing a DAA IFN-free regimen, 23 (71%) had at least one mutation in NS5A gene, with this distribution: 93H (60%), 28M and 30R (8.6%), 31M (17.4%), 58S, 28V, 30E/G and 92V/F/N (4.3%). High prevalence of NS5A RAV was detected in HCV-1b and 3a-infected patients compared to HCV -1a (26%), HCV-4d (8.7%) and HCV-4a (4.3%) -infected patients. We observed a different picture in the group of patient treated with NS5B alone ± RBV-containing regimen, only 5/26 patients developed RAVs with 93H, 28M, 30H and 30M (the more prevalent). Genotype distribution among patients who experienced a VF after Sofosbuvir regimen was: 1b (60%), 1a (20%) and 3a (20%).

**Conclusions:** Even though the high efficacy of DAA treatment reported in clinical trials, a 5-10%
VFIs occur, with the presence of clinically relevant RAVs. In particular NS5A RAVs demonstrated important implications in terms of treatment failure, due to its long persistence as far as two years and longer that impacts on the subsequent retreatment strategies.

The high prevalence of RAVs observed in our patients treated with NS5A Inhibitor ± RBV is sustained by their low genetic barrier to resistance despite their broad genotypic activity and high potency, with respect to Sofosbuvir + RBV regimen. Our data confirm the relevance of HCV resistance test at baseline to limit the risk of failing the selection of an optimal therapeutic regimen.

Abstract 60

Caution towards chronic hepatitis C in advanced HIV

Avihingsanon A1, Gatechompol S1, Tangkijvanich P2

1Hiv-nat, Thai Red Cross Aids Research Centre, 2Faculty of Medicine, Chulalongkorn University

This is an interesting case of someone we thought had acute HCV but in fact, had something totally different. This is a complicated case which we had not encountered before. This case is of a 41 year old Thai MSM. HIV and TB was diagnosed in May 2014. His CD4 was 11 cells/mm3. Anti HCV, and HBsAg were negative. He was treated with tenofovir+lamivudine + efavirenz in September 2014. In 29 Sep 2015, his HIV RNA was 383,151 copies/ml and CD4 was 20 (1%). Since his HIV RNA levels were high, we performed resistance testing which revealed that he had K65R, T69N, Y115F, M184V, K103 N, Y181C, and G190A mutations. We screened for HCV and HBV prior to new ART. Both results were negative. In 27 Oct 2015, his ALT was 30 U/L. We changed his HIV regimen to AZT/3TC + Dolutegravir. After 12 weeks on the new regimen, his CD4 was 63 cells/mm3, VL<50 copies/ml, and ALT was 39 U/L. His ALT remained normal until week 36 of ART. At week 36 (12 Jul 2016), his CD4 was 63 cells/mm3, VL<50 copies/ml, and ALT=282 U/L. Due to high ALT, we tested him for HCV. Finally, his anti HCV test became positive. So in 01 Aug 2016, ART was discontinued due to his ALT which was 275 U/L. When we followed him in 31 Aug 2016, his CD4 was 42 cells/mm3, VL=96699 copies/ml, and ALT was 67 U/L. We then decided to restart AZT/3TC+LPV/r. In 8 Sep 2016, his ALT/AST was 648/473 U/L, and total/direct bilirubin was 4.6/2.8 mg/dl. We immediately discontinued his ART. We performed liver biopsy and found out that he had chronic hepatitis of which the activity was moderate to severe. His HCV was genotype 1 and he had HCV RNA of 10,869,000 copies/ml. In 22 Sep 2016, his ALT/AST was 153/104 U/L, CD4 was 31 cells/mm3, and VL was 111522 copies/ml. Because of this, TDF/FTC+ATV/r was started. One week later, he developed symptomatic hepatitis. His ALT/AST was 382/351 and ART had to be stopped again. In October, he started HCV treatment with generic sofosbuvir/ledipasvir. In November, his ALT/AST returned to normal (19/12 U/L) so TDF/FTC+ATV/r was resumed.

For this case, his HCV was negative many times but he developed hepatitis after 36 weeks of ART. It was only until then that his HCV finally became positive. We thought he had acute HCV but in fact, he had chronic HCV. This was revealed to us only after having had a liver biopsy done. If we had not treated him with HCV therapy, then he would have had repeated ART-related hepatotoxicity, recurrent TB and/or would have died. His negative HCV results were misleading. Lesson learned from this case is that patients with very advanced HIV may yield false negative for HCV infection.
Abstract 61

Inadequate daclatasvir blood levels in a liver transplantation recipient, treated with sofosbuvir + daclatasvir in association with ursodeoxycholic acid: a case report

**Bussini L**, Guardigni V, Badia L, Rinaldi M, Conti M, Viale P, Verucchi G

1Infectious Disease Unit, Department of Medical and Surgical Science, University of Bologna, 2Research Centre for the Study of Hepatitis, Department of Medical and Surgical Sciences, University of Bologna, 3Central Laboratory, S. Orsola-Malpighi Hospital

**Background:** The safety and efficacy of new Direct-Acting Antivirals (DAAs) in eradicating HCV has become a key aspect in liver transplantation (LT), where a HCV reinfection could lead to severe complications, such as accelerated fibrosis and organ rejection. In patients with hepatocellular carcinoma (HCC), treatment initiation after LT is usually preferred, raising the issue of drug-drug interaction (DDI) among antiviral, anti-rejection and other drugs used for hepatic function restoration. Although the therapeutic drug monitoring (TDM) of DAAs has not a clear role in clinical practice yet, it could be useful in evaluating possible DDI and patient’s compliance to antiviral treatment.

**Methods:** We report a case of a HIV-positive patient treated for HCV after LT. Periodic tests with HCV-RNA and TDM of sofosbuvir (SOF), GS-331007 (sofosbuvir active metabolite) and daclatasvir (DCV) concentration at the end of dosing interval were performed at week 4, 6, 7, 8, 9, 12, 16 and 20 from the start of therapy.

**Case report:** An HIV/HCV (genotype 3a) coinfected male with cirrhosis and HCC received a cadaveric liver transplant. Two days after surgery he started therapy with SOF and DCV, planned for 24 weeks. The patient was naive for any anti-HCV treatment. He was taking antiretroviral treatment with rilpivirine and dolutegravir with good immunological response (CD4+ >200/mm3). In addition, he started anti-rejection drugs (tacrolimus and prednisone), ursodeoxycholic acid (UDCA), nystatin, trimethoprim/sulfamethoxazole, pantoprazole and aspirin.

Baseline HCV-RNA was 130,617 IU/ml; treatment was well tolerated and the patient reported proper compliance. At week 4 HCV-RNA was 69,728 IU/ml, plasmatic drug levels of SOF and GS-331007 were within expected ranges, while DCV was undetectable (Figure 1). UDCA was then discontinued because of its potential interaction with DCV.

At week 6 HCV-RNA exceeded the baseline viraemia (271,031 IU/ml), so ribavirin was added. Resistance genotyping test was performed and no resistance mutations were detected. A progressive decay in HCV-RNA was then documented, and undetectability was reached at week 9 and maintained in following timepoints (treatment still ongoing).

**Conclusion:** In our case, the observed primary virological failure of the DAA-based regimen was likely due to DDI. The known interaction between DCV and UDCA seems to have led to a complete loss of therapeutic efficacy of DCV: the pattern of virological decline and rise in the first weeks was indeed consistent with a SOF monotherapy. Careful monitoring of co-medication is mandatory in patients treated with DAAs after LT; TDM can play a key role in this setting, allowing an early identification and correction of sub-optimal drug exposition.

Our findings highlight the need to completely avoid prescription of UDCA during DCV-based treatment.
Abstract 62

Acute Hepatitis B Virus Infection in children – clinical forms

Gheorghe E1, Osman E1, Vintila S1, Merisescu M1,2, Jugulete G1,2

1National Institute For Infectious Diseases “prof. Dr. Matei Bals”, 2University of Medicine and Pharmacy “Carol Davila”

Introduction: Acute hepatitis B virus infection is still a priority at European level by the extent of the impact on public health. Acute hepatitis B virus infection can preset under multiple clinical forms, from mild to fulminant disease courses, which in some cases can lead to death, despite appropriate treatment.

Material and method: We conducted a retrospective study on cases of acute hepatitis B virus infection in children who were hospitalized in Pediatric Department of the National Institute of Infectious Diseases “Prof. Dr. Matei Bals” during 1996 – 2017. For the these cases we have managed the following parameters were annalised: age, sex, clinical evolutive forms. Positive diagnosis was established on clinical, epidemiological and laboratory data.

Results: During the studied period we registered 390 cases of acute hepatitis B virus infection in children who were hospitalized in our Pediatric Clinic. The most affected age group gathered children younger than 12 months (57%), with a male predominance (72%). For 57% of the cases we registered medin severity forms of diseases, 29% of the patients presented severe forms and 14% of the managed patients associated mild form of the acute disease. We registered 4 cases (1.02%) in children diagnosed with acute hepatitis b virus infection who died.

Conclusion: Based on the study we performed, we noticed a downward trend to the number of cases of acute hepatitis B virus infection, the decrease in the incidence rate is secondary to the implementation of the nationwide free immunization program of children against hepatitis B virus, national program initiated in 1996. Another obvious success of the immunization program reflects into the fact that there were no new acute pediatric VHB infection registered, in our institute, in the first trimester of 2017.

Abstract 63

Prevalence of integrase inhibitors drug resistance mutations among patients treated in Warsaw Center in 2013-2016

Zabek P1, Dyda T1, Cielniak P, Kowalska J, Pulik P2, Stanczak J1

1Molecular Diagnostic Laboratory, Hospital for Infectious Diseases, 2Outpatient Clinic, Hospital for Infectious Diseases, 3Hospital for Infectious Diseases

Background: Raltegravir (RAL) is the first drug of a new class of HIV drugs, integrase inhibitors (also known as integrase strand transfer inhibitors - INSTIs), which recived FDA approval. Ten years after introduction of INSTIs, there are three drugs in this class, elvitegravir (EVG) and dolutegravir (DTG) beside RAL. The usage of INSTIs in current treatment schemes is increasing. Last HIV treatment guidelines, recommend drug resistance testing for INSTIs in two cases: I) patients who experience virologic failure while taking an INSTI-containing regimen, and II) if transmitted resistance is a concern. In Hospital for Infectious Diseases in Warsaw, drug resistance testing in this class of drugs is available since 2013 until now.

Materials and methods: Since 2013 until 2016, we have been tested 79 plasma samples obtained from 49 (62%) INSTIs naïve and 30 (38%) experienced patients. HIV viral load testing was performed using m2000sp/rt system (Abbott), mean VL – 127373 copies/mL, ranging from 166 to 3294791 copies/mL; 12 (15%) samples had VL <1000 copies/mL. Sixty three of patients (80%) were men. The RNA isolation, amplification and sequencing were performed using ViroSeq HIV-1 Integrase Genotyping Kit (Celera) and 3130-Avant Genetic Analyzer (Life Technologies); for interpretation of the data ViroSeq HIV-1 Integrase Software 1.0 and
Stanford HIV DR Database were used. Subtype determination was done basing on integrase sequence.

Results: Among naïve patients no INSTIs resistance was detected. However, 9 (18%) out of 49 samples carried E157Q mutation, which has low, if any effect on integrase inhibitors susceptibility. In the group of patients experiencing virologic failure, 10 (33%) out of 30 cases harbored drug resistance mutations. Following mutations in different combinations were detected: once E138K, G140S, S147G; twice N155H; triple T66A/K and Q148R/H; four times E92Q/G. Recognized substitutions had mostly great impact on RAL and EVG susceptibility, with low or no effect on DTG. However, two mutation patterns (G140S, Q148H and T66A, E138K, S147G, Q148R) increased resistance to DTG to intermediate level, apart from high level resistance to RAL and EVG. Subtype distribution, in tested group of patients was: 85.9% subtype B, 10.2% subtype A_FSU, 1.3% CRF02_AG, 1.3% CRF06_cpx and 1.3% subtype D.

Conclusions: Presented data showed that no major drug resistance mutations occurred in INSTIs naïve group of patients. This observation is consistent with other studies published. In the tested cohort, one third of patients experiencing virologic failure on INSTI regimen carried HIV strains with high level resistance mutations to RAL and EVG with very low effect on DTG. However, in two strains the presence of complex mutation patterns (G140S, Q148H and T66A, E138K, S147G, Q148R) caused intermediate resistance to DTG. It is worth noting that appearance of N155H mutation in this two cases will result in high resistant variants to DTG. Introduction of new more potent integrase inhibitors with different genetic profile and higher genetic barrier, like BIC, will help clinicians to cope with the problem of very resistant INSTIs variants.

Abstract 64

Baseline HIV-1 resistance testing for integrase strand transfer inhibitors not yet warranted in Belgium and Luxembourg

Vancutsem E1, Ruelle J2, Mortier V3, Debaissieux L4, Delforge M4, Depypere M4, Devaux C6, Fransen K6, Garcia Ribas S6, Piérard D7, Stoffels K7, Vaira D7, Van den Wijngaert S7, Van Laethem K8,9, Verhofstede C3

1Universitair Ziekenhuis Brussel, Department of Microbiology and Infection Control, Aids Reference Laboratory Vrije Universiteit Brussel, 2Université Catholique de Louvain, Aids Reference Laboratory, 3Ghent University, Department of Clinical Chemistry, Microbiology and Immunology, AIDS Reference Laboratory, 4Université Libre de Bruxelles, Hôpital Erasme, Aids Reference Laboratory, 5Luxembourg Institute of Health, Department of Infection and Immunity, Esch sur Alzette, 6Institute of Tropical Medicine, Department of Clinical Sciences, Aids Reference laboratory, 7UMC Sint-Pieter, AIDS Reference Laboratory Vrije Universiteit Brussel site Sint-Pieter, 8Université de Liège, Aids Reference Laboratory, 9KU Leuven – University of Leuven, Department Microbiology and Immunology, Rega Institute for Medical Research, 10University Hospitals Leuven, AIDS Reference Laboratory

Background: Drug combinations containing Integrase strand transfer inhibitors (INSTI) are recommended as first-line treatment regimens in HIV-1 infections. The “first generation” INSTIs, raltegravir and elvitegravir, have a low genetic barrier to resistance while the “next generation” dolutegravir, has a more robust resistance profile. Baseline genotypic drug resistance testing is recommended for protease and reverse transcriptase inhibitors but international guidelines do not yet recommend baseline analysis of INSTI resistance as in general the prevalence of transmitted INSTI resistance remains extremely low. In this study, we defined the occurrence of INSTI resistance and its impact on the success of first-line regimens for patients treated in Belgium and Luxembourg.

Material and methods: The Belgian and Luxembourgish Aids Reference Laboratories retrospectively performed baseline INSTI drug
Results: Of the 244 patients, 30 were on a raltegravir, 96 on an elvitegravir and 118 on a dolutegravir containing regimen. VL at diagnosis and before treatment was available for 243 and 242 patients respectively. CD4 values before treatment and after six months of treatment were available for 242 and 230 patients, respectively. Treatment success was achieved in 219 patients (89.8%). Twenty-five patients had a detectable VL at six months, ranging between 54 and 977 copies/mL. Overall, population-based Sanger sequencing revealed RAMs against one or more INSTI in 18 samples (7.4%). Mutations observed were mostly polymorphic or accessory mutations: 157Q (N=7), 95K (N=1), 97A (N=5), 97A+163K (N=1), 138K (N=2), 230R (N=1), 147G (N=1). The positive predictive value of the GSS scores was low for both algorithms but especially for ANRS. Overall, good virological response against the INSTIs containing regimens was obtained, independent of the GSS scores. Treatment failure was significantly associated with higher baseline and pre-treatment VL (p<0.0001) but not with lower pre-treatment CD4 counts or presence of INSTI RAMs.

Conclusion: The results of this study confirmed findings of others that the prevalence of pre-existing INSTI resistance is low. No association was found between virological failure on INSTI-based first-line regimens and presence of INSTI RAMs at treatment initiation. The findings indicate that baseline INSTI resistance testing in Belgium and Luxembourg is currently not warranted. These data will serve as a benchmark for follow-up studies on INSTI resistance within our local epidemics.

Abstract 65
Management of a Human Immunodeficiency virus type 1 infected adult with discordant antiviral drug resistance profiles in cerebrospinal fluid compared to plasma

Bang D¹, Johansen I², Fonager J³

¹Virus & Microbiological Special Diagnostics, Statens Serum Institut, ²Department of infectious diseases, Odense University Hospital

Background: The management of HIV drug resistance is challenging. Few studies describe differences in genotypic resistance profiles in plasma compared to other organ compartments such as cerebrospinal fluid (CSF). Poor compliance and co-infections with other pathogens may complicate treatment management. The aim of this study was to identify and describe the management and treatment of a case of HIV-1 infection with discrepant drug resistance profiles in plasma and CSF.

Materials & methods: A case with discordant HIV-1 genotypic drug resistance profiles in plasma and CSF was identified through genotypic resistance analysis of the HIV-1 pol gene. The case record was identified and searched for basic demographic information, clinical data, antiretroviral therapy (ART), CD4 counts, HIV RNA viral load compared to genotypic drug resistance data, found in both plasma and CSF over time.

Results: A male of West African origin co-infected with HIV-1 and Hepatitis B virus treated for cerebral toxoplasmosis, despite receiving ART, was described with regards to clinical, various ART strategies, CD4 counts and viral load monitoring over time and HIV genotypic drug
HIV-1 genotypic resistance analysis of the pol gene showed the development of plasma nucleoside reverse transcriptase inhibitor (NRTI) mutation M184V, and non-nucleoside reverse transcriptase inhibitor (NNRTI) mutations K103N and E138EK during the first years of ART with poor compliance. During subsequent years, the plasma drug resistance pattern reverted to susceptible for the NRTI/NNRTIs, however the protease inhibitor (PI) mutation L89V appeared, only later to revert to fully susceptible again. After ten years on ART the patient developed neurological symptoms. A cerebral MR-imaging showed toxoplasmosis sequelae and unspecific changes in the white matter. A brain biopsy performed suggested a differential pathological diagnosis of astrocytoma. The Epstein-Barr virus level in the CSF was increased. The astrocytoma diagnosis was unconfirmed and the diagnosis of HIV encephalopathy with sequelae after cerebral toxoplasmosis was concluded. Both plasma and CSF had the same NRTI mutation M184V, however discordantly the PI T74TP mutation was only found in the plasma but not CSF.

Conclusions: The management of an adult HIV-1 infection with AIDS due to cerebral toxoplasmosis and chronic hepatitis B co-infection with discordant HIV-1 genotypic drug resistance profile in plasma compared to CSF was described. Cerebral MR-imaging results indicated HIV encephalopathy. The discordant findings of PI mutations in plasma but not in CSF indicate poor penetration of PI’s into the CNS due to the selective pressure of PI drug treatment in plasma but not CSF. An intensified ART regimen including drugs with good CNS penetration improved conditions and cerebral MR-imaging. This case shows the importance of measuring HIV drug resistance in CSF fluid, which might differ from resistance detected in plasma samples.

Abstract 66

Prevalence of Transmitted Drug Resistance Mutations among Naïve HIV-infected patients (2014-2016) in Northwest Spain

Pernas B1, Tabernilla A1, Grandal M1, Cañizares A2, Castro-Iglesias Á1, Mena Á1, Borrajo A1, Poveda E1

1Grupo de Virología Clínica. Instituto de Investigación Biomédica de A Coruña (INIBIC)-Complejo Hospitalario Universitario de A Coruña (CHUC), SERGAS, Universidade de A Coruña (UDC), 2Servicio de Microbiología. Instituto de Investigación Biomédica de A Coruña (INIBIC)-Complejo Hospitalario Universitario de A Coruña

Background: Transmitted drug resistance (TDR) in naïve HIV-infected patients has been associated with suboptimal response to antiretroviral drugs. Nowadays, integrase inhibitors (INI)-based regimens are recommended as first line therapies. However, testing integrase genotypic resistance is only recommended if a high risk of TDR is suspected. The goal of the study was to evaluate the prevalence of TDR to reverse transcriptase, protease and integrase inhibitors (NRTI & NNRTI, PI, & INI) among naïve HIV-infected patients in a medical area of the Northwest of Spain.

Materials and methods: All naïve HIV-infected patients in clinical follow-up during 2014-2016 were identified. Epidemiological and immunovirological characteristics were recorded. Reverse transcriptase, protease and integrase regions were amplified and sequenced from plasma samples and FASTA format sequences were obtained. The presence of TDR was evaluated following the last HIV drug resistance update of WHO and International AIDS Society (2017). HIV-1 subtypes were assigned using the Stanford University Drug Resistance Database algorithm.

Results: Overall, 92 naïve HIV-infected patients were included, of whom 64 had been diagnosed of HIV infection during 2014-2016 and the remainder before 2014. Mean age was 37±11 years, 91.3% were male and 86.4% were Spanish. Routes of HIV transmission were: men
who have sex with men (66.3%), heterosexual (27.9%) and intravenous drug users (5.8%). At diagnosis time, CD4+ cell count and HIV-RNA were 503±287 cells/mm3 and 4.75±0.74 log copies/mL, respectively. Late diagnosis was observed in 27.9% of patients. Regarding viral hepatitis co-infection, 2.9% had positive HBsAg and 8.7% positive HCV antibodies. The distribution of HIV genotypic subtypes was: B (64.1%), F (23.9%) and other subtypes (12%). Overall, TDR mutations were identified in 7.6% of naïve HIV-infected patients. Prevalence of TDR mutations for NRTI, NNRTI and PI were 3.3%, 5.4% and 1.1%, respectively. All of them were identified in patients who had been diagnosed during 2014-2016. TDR mutations were identified in patients who had been diagnosed during 2014-2016. TDR mutations were: M41L (2.2%), T69D (1.1%) and T219R (1.1%) for NRTI; K103N (2.2%), E138A (1.1%), Y181V (1.1%) and G190A (1.1%) for NNRTI; and D30N (1.1%) and N88D (1.1%) for PI. Protease polymorphisms were common (57.6%). Although no significant differences in TDR prevalence were observed between subtypes B and F, the protease polymorphisms L10V (86.4%) and M36i (90.9%) were more common in subtype F compared to subtype B (1.7% and 18.6%, respectively) (p < 0.001). None major TDR mutations for INI were identified. However, the accessory T97A mutation was recognized in 2.2% of naïve HIV-infected patients, all of them diagnosed before 2014.

Conclusions: TDR prevalence in naïve HIV-infected patients was 3.3%, 5.4% and 1.1% for NRTI, NNRTI and PI, respectively, similar to those previously reported. Although no major TDR for INI were identified, mutation T97A was identified in 2.2% of patients. These results suggest that testing resistance to INI is not necessary yet before initiating treatment among HIV-infected naïve patients.

### Abstract 67

**High virological suppression rates regardless to the genotypic susceptibility score after switching to a dolutegravir-based regimen: W48 results in a prospective cohort**


1IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France, 2IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Laboratoire de Pharmacologie, Hôpital Bichat, AP-HP, Paris, France, 3IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Service de Maladies Infectieuses et Tropicales, Hôpital Bichat, AP-HP, Paris, France

**Background:** To assess, in virologically-suppressed patients, based on the genotypic susceptibility score (GSS), the virological response after switching ARV-treatment to a dolutegravir (DTG)-based regimen. Methods: A prospective observational single-center cohort enrolling all patients with a plasma viral load (pVL)<50 c/mL initiating a DTG-based regimen between September 2014 and March 2016. pVL were performed using CobasTaqman HIV-1 V2.0 assay. GSS of the ARV regimen was calculated using the ANRS algorithm including DTG, translating the interpretations "susceptible", "possible resistance" and "resistence" into scores of 1, 0.5 and 0, respectively. A virological failure (VF) was defined as two consecutive pVL >50 c/mL.

**Results:** Among 297 patients who switched to a DTG-based regimen, 239 (80%) had historical available genotypes. Taking into account all historical genotypes, 28 (12%), 70 (29%) and 141 (59%) had a total GSS equal to 1 or 1.5 (group 1), 2 or 2.5 (group 2) and 3 (group 3), respectively. Median time since the last genotype was 9 (IQR=4-12), 10 (IQR=3-13) and 5 (IQR=2-8) years in groups 1, 2 and 3, respectively. History
of infection differed between groups regarding duration of prior cART or CD4 cell count nadir. Twenty-seven patients (11.3%) discontinued DTG-based regimen in relation with neuro-psychological adverse events (n=9), pregnancy (n=3), renal toxicity (n=2), headaches (n=1), cutaneous adverse events (n=2), virological failure (n=3), patients' decision (n=3) and others (n=4). During the first year following the switch to the DTG-based regimen, 4 patients (1.7%) experienced a VF (median pVL = 90 c/mL, IQR=81-94), 2 patients were in group 2 and 2 were in group 3. Plasma drug concentrations were available in 3 patients, all showing adequate or above the respective cut-offs DTG C24h (2522 ng/mL, 1444 ng/mL and 3177 ng/mL). The drugs associated with DTG were as follows: (i) TDF/FTC; (ii) DRV/r, (iii) ABC/3TC, and (iv) ABC/3TC + DRV/r. Integrase sanger sequencing was successful in 2 patients showing no selection of integrase resistance-associated mutations. Overall, 7.1% (n=17) of the patients experienced a viral blip with no significant difference between the three GSS groups. Among the 12 available plasma drug concentrations DTG C24h was >1000 ng/mL in 11 (92%).

Conclusions: In this observational cohort, we showed a high level of virological suppression maintenance in the first year following the switch to DTG-based regimen, including in patients with baseline GSS <2. These data suggesting that DTG remains potentially able to maintain viral suppression when combined with fully or incompletely active drugs in these long-term virologically-suppressed patients need to be confirmed in clinical trials.

Abstract 68

Primary Resistance to Integrase Strand-Transfer Inhibitors in Spain, 2015-2016


Background: Transmitted HIV resistance reduces the effectiveness of first-line antiretroviral therapy (ART). Epidemiological surveys have not detected significant transmission of INSTI-resistant HIV to date, even with deep sequencing. However, continued HIV resistance surveillance is warranted because transmitted INSTI resistance might increase over time.

Methodology: This was a cross-sectional study of ART-naïve HIV-1 infected subjects starting first-line INSTI-based ART in Spain between April 2015 and December 2016. Pre-ART plasma samples were tested for the presence of integrase (IN), protease and reverse transcriptase (RT) resistance using MiSeqTM (Illumina Inc.). Results were analysed with an in-house automated pipeline (https://paseq.org). The WHO 2009 list was used to determine the prevalence of primary PR and/or RTI resistance. Given the lack of such resource for IN, we performed separate assessments including non-polymorphic IAS-USA 2017 mutations and Stanford HIVdb (v8.3) mutations with scores >15 to at least one INSTI. Phylogenetics were used to explore the existence of HIV resistance transmission clusters. HIV subtypes were determined with the Rega HIV-1 Subtyping tool.

Results: 124/134 (92.5%) evaluable samples were successfully sequenced and analysed. HIV-1 subtypes were: B in 101 (81.4%) subjects, F in
6 (4.8%) subjects, recombinant of B and D subtypes in 5 (4%) subjects and other subtypes in 12 (9.6%) subjects. Using a 20% MiSeq sensitivity cut-off, we found INSTI resistance mutations in 2 subjects (prevalence: 1.6%), with either IAS-USA or HIVdb approach. Mutations detected were R263K and E138K (both at 99.9% virus frequency). Using a 1% cut-off, the prevalence of INSTI resistance increased to 6 (4.8%) with the IAS-USA list and 12 subjects (9.6%) with the HIVdb approach. Low-frequency IAS-USA mutants were Q148H (n=1; at 2.5% frequency in the virus population) and E138K (n=3; at 2.8%; 1.8%; 1.4%, respectively). Additional low frequency mutants detected with the HIVdb approach were H51Y (n=1), V151L (n=1), V151A (n=1), G163R (n=3), and S230R (n=1), all at frequencies between 1.1% and 3.5% in the virus population. Using the WHO mutations list (2009), we found NRTI resistance mutations in 9 subjects (7.2%), NNRTI resistance mutations in 11 subjects (8.8%) and PI resistance mutations in 7 subjects (5.6%). Twenty-nine subjects (23.3%) had resistance mutations to one treatment family, whereas 2 subjects (1.6%) resistance mutations to two different treatment families.

Conclusions: Primary INSTI resistance is beginning to arise in ART-naïve subjects in Spain. Europe-wide surveillance of transmitted HIV resistance should be reinforced to validate these findings in other European countries. If confirmed, these results would support systematic INSTI resistance testing before ART in clinical routine.

Abstract 69

Is the management of HIV-1 drug-resistance still a clinical concern? An update in the modern antiretroviral era

Armenia D 1 , Di Carlo D 1 , Borghi V 2 , Forbici F 3 , Bertoli A 1 , Gori C 3 , Alteni C 1 , Fabeni L 3 , Gennari W 1 , Continenza F 3 , Pinnetti C 3 , Zaccarelli M 3 , Mondi A 3 , Cicalini S 3 , Maffongelli G 4 , Montella F 5 , Calafieli M 4 , Marocco R 7 , Mastroianni CM 7 , Ceccherini-Silberstein F 1 , Andreoni M 4 , Mussini C 2 , Antinori A 3 , Perno CF 3 , Santoro MM 1

Background: Even though dynamics of HIV-resistance has profoundly changed over the years, it is still a concern for achieving and maintaining virological suppression under antiretroviral treatment (ART). Thus, resistance monitoring is crucial to preserve future therapeutic options and avoid the emergence of new resistance.

Methods: We included HIV-1 infected ART-experienced patients followed among several clinical centers from Central and North Italy from 1999 to 2016. All genotypic resistance tests (GRT) performed at virological failure (viremia >50 copies/mL under ART-pressure) from protease (PR), reverse-transcriptase (RT) and integrase (IN, if available) were analyzed. We studied cumulative drug-resistance, defined as the presence of ≥ 1 major mutation from IAS-Stanford lists 2017. Three-class resistance was defined as the occurrence of ≥ 1 major mutation against three of the following four drug classes: nucleoside/nucleotide RT inhibitors (NRTIs), non-NRTIs (NNRTIs), PR inhibitors (PIs), or IN inhibitors (INIs). The remaining treatment options were evaluated according to the genotypic susceptibility score (GSS) (Stanford HIVdb algorithm version 8.3).

Results: A total of 14189 GRTs (PR/RT: 12660; IN: 1529) from 6051 ART-experienced patients were analyzed. Overall, 40% of GRTs showed no resistance, while the prevalence of resistance to 1, 2 and ≥3 classes was 21%, 25% and 14%, respectively. Prevalence of resistance to NRTIs, NNRTIs, PIs and INIs was 47%, 38%, 26% and 10%, respectively. Resistance at failure significantly decreased from 1999 to 2010 (two class-resistance: from 42% to 14%; ≥3 class-resistance: from 30% to 6%, p<0.0001), in conjunction with a remarkable increase of failures without resistance (from 13% to 58%, p<0.0001). An increase of one class-resistance was also found (from 14% to 22%, p<0.0001). Beyond 2010, prevalence of resistance remained stable from 2011 to 2016 (no resistance: from 59% to 55%, p=0.713; one class:
from 24% to 28%, p=0.416; two classes: from 13% to 12%, p=0.562; ≥3 classes: from 4% to 5%, p=0.891).

New detections of ≥3 class-resistance dramatically decreased from 23% in 1999 to 2.3% in 2016 (p<0.001). Resistance to ≥3 classes was found more than once in 361/801 (45%) patients, in whom re-occurred from previous GRTs recorded in a median time of 3.2 (1.2-5.9) years before.

Regarding genotypic susceptibility, the proportion of GRTs with a fully susceptible virus increased over time settling in 2016 at >80% for NRTIs, >74% for NNRTIs, >90% for PIs (p<0.0001). Regarding INIs, the proportion of GRTs with a fully susceptible virus was stable from 2008, settling in 2016 at >88% and 95% for raltegravir/elvitegravir and dolutegravir, respectively. After 2008, INI-resistance contributed to cumulative resistance mostly in those GRTs with ≥3 class-resistance (1 class: 9.2%; 2 classes: 16.7%; ≥3 classes 41.6%, p<0.0001).

Conclusions: A dramatic drop of drug-resistance has been achieved, confirming a good clinical practice and ensuring a high number of treatment options for failing patients. However, drug-resistance is stable in the last 5 years, where resistance to ≥3 classes remains a clinical relevant issue. Its management deserves an appropriate use of diagnostic tools and currently available drugs. These findings reinforce the need of developing new drugs and new drug classes.

Abstract 70

A QIAGEN-developed bioinformatics pipeline for prediction of HIV-1 resistance variants using Next Generation Sequencing (NGS) data

Einer-Jensen K1, Lübke N2, Thielen A3, Javed S4, Wall G1

1Qiagen Aarhus, 2Institute of Virology, Heinrich-Heine-University, 3Institute of Immunology and Genetics, 4QIAGEN Ltd, 5QIAGEN GmbH

Background: Human immunodeficiency virus type 1 (HIV-1) is a retrovirus, that over time leads to acquired immunodeficiency syndrome (AIDS) if not treated. Thus antiretroviral therapy (ART) is intended to slow the progression to AIDS but as HIV-1 can rapidly develop resistance, incomplete viral suppression can lead to treatment failure. The prediction of resistance variants using Sanger sequencing has been part of standard of care for many years. It is a vital tool in maximizing treatment options, controlling the prevalence of drug resistant viruses and improving patient management.

Targeted NGS offers a viable alternative to Sanger sequencing for routine clinical diagnostics that furthermore enables detection of mutations at a lower frequency than possible to obtain when using Sanger techniques. This study focuses on the development of a bioinformatics pipeline that automatically predicts drug resistance based on HIV-1 NGS data.

Materials & Methods: A panel of 21 HIV-1 patient samples representing diverse HIV-1 subtypes with representative resistance mutations in the protease and the reverse transtripase regions (PRRT) and the integrase (IN) gene region of HIV-1 were selected. The panel was divided into two subsets: a) RNA purified directly from clinical samples (10/21) was used for generation of two RT-PCR amplicons of the PRRT and IN gene regions, respectively; b) RNA purified from cell culture propagated samples (11/21) was used for generation of a single pol gene RT-PCR amplicon. The amplicons were prepared for Illumina MiSeq using the Nextera XT library kit and in parallel for generation of Sanger sequencing data.

Three bioinformatics pipelines were applied in parallel for the data analysis: 1) “deeptypeHIV” in-house solution of the Institute of Immunology and Genetics; 2) a customised and automated pipeline based on CLC Genomic Workbench (GWB), QIAGEN; 3) an automated proof of principal pipeline developed according to IVD software development standards, QIAGEN.

Results: Sanger consensus sequences were extracted using the CLC GWB and analysed using the online “HIValg Program” (https://hivdb.stanford.edu/hivalg/by-sequences/). The hereby identified “major resistance mutations” were considered as
the correct result. Results of the NGS pipelines 1, 2 and 3 all agreed with the Sanger-based predicted drug resistance.

**Conclusions:** This comparison study included three independently developed bioinformatics solutions, which generated equal results across the 21 diverse samples when using Sanger sequencing as the benchmark. The QIAGEN proof of principal solution is fully automated and delivers unified reporting and documentation aimed at users with little or no bioinformatics expertise. Once feature development is completed and the tool is fully verified it has the potential to provide valuable biological insights as part of QIAGEN’s future QIAact HIV-1 NGS Genotyping Kit using the GeneReader® NGS System.

**Disclaimer:**
The QIAact HIV-1 NGS Genotyping Kit using the GeneReader® NGS System and bioinformatics pipeline is currently under development.

**Abstract 70**

**Prevalence of resistance-associated mutations in HIV-1 treatment naïve patients in a tertiary hospital in Portugal**

**Costa O¹, Dias I¹, Corte-Real R¹, Flores C¹**

¹Molecular Biology Laboratory, Hospital Centre of Central Lisbon

**Background:** Since the introduction of HAART a drastic decrease in mortality, morbidity and transmission of HIV was observed. However, due to the arising of primary and acquired resistances, patient treatment and future therapeutic options are being compromised. The prevalence of primary resistance is variable throughout different geographic regions, depending on socioeconomic factors, mainly access to antiretroviral drugs. Transmitted drug resistance (TDR) analysis has an epidemiological relevance to the elaboration of guidelines to different settings, as well as to know the chain of disease transmission. Recent studies estimate a primary resistance prevalence of 6% to 15% in Europe. Our main objective was to find the prevalence of resistance-associated mutations to antiretroviral therapy (PI, NNRTI and NRTI) in treatment naïve patients followed on “Centro Hospitalar de Lisboa Centra” (CHLC).

**Materials and methods:** In CHLC, 5200 persons with HIV infection are followed in outpatient clinics. Resistance tests are performed since 1999 by the Molecular Biology Laboratory. We’ve done a retrospective analysis of 230 treatment naïve patients 175 (76%) male and 55 (24%) female, being the median age 38 years old, from 01/01/2016 to 31/12/2016. Resistance tests were made using a genotypic system based on genome amplification and sequencing of the HIV-1 protease and partial reverse transcriptase (Sanger method). Population sequencing was performed using an automated sequencer. Stanford University HIV Drug Resistance DataBase was used for interpretation of resistance data. TDR mutations were defined according to the WHO 2009 list of surveillance drug-resistance mutations.

**Results:** In our study, resistance-associated mutations to antiretroviral therapy showed a global prevalence of 10.4%, and 4.8%, 3.8% and 2.2% for NNRTI, NRTI and PI respectively. Resistance to 2 different classes of antiretroviral drugs were found in 1.3% of patients. None were resistant to three classes. Most frequent mutations reported for each class of antiretroviral drugs were as follows: NNRTI – K103N, V106A, Y181C; NRTI – D67N, M184V, M41L; and PI – M46L, L90M. There was a high prevalence (3.4%) of E138A polymorphism, associated with low-level resistance to Rilpivirine.

**Conclusions:** On our study population, there was an overall prevalence of primary antiretroviral resistance of 10.4%. This percentage is similar to other national and European studies. NNRTI class had the most resistance rate (4.8%), with an elevated percentage related to resistance to Efavirenz and Nevirapine.
Abstract 71

Proviral DNA as a Target for HIV-1 Resistance Analysis in patients with low viral load

Ferrer P1, Sobarzo M1, Afani A1

1Hospital Clinico Universidad De Chile

Background: Genotypic assays usually use viral RNA. However, this is sometimes not possible since the patient has low-level viremia. In these situations it is appropriate to consider HIV proviral DNA for resistance analysis. The objective of this study was to evaluate the utility of Proviral DNA as a genetic target for these cases.

Materials & methods: A total of 75 adult patients were studied of which 75% were men who were confirmed for HIV infection and who were on antiretroviral therapy. First 10 samples were analyzed in parallel by RNA and proviral DNA that had an average viral load of 20,000 copies/mL (CobasTaqman v2.0). Subsequently, 65 samples that had an average viral load of 258 copies/mL were analyzed only for proviral DNA. In these patients the physician suspected of virologic failure. RT-PCR amplified reverse transcriptase and protease comprising 240 and 99 amino acids respectively. The amplicons were automatically sequenced using 8 primers and the sequences obtained were analyzed and assembled by the ReCall® software. Only the sequences approved by this software were used to detect mutations associated with antiretroviral resistance in the Standford database.

Results: Of the 10 samples analyzed in parallel by RNA and proviral DNA were found that 9 were coincident of which 2 were susceptible and 7 were resistant to the same antiretrovirals. In the discrepant sample more mutations were found associated to resistance by RNA than by proviral DNA. The mutations K65R, L100I, Y115F, K219R, and F227L were only detected by RNA. While mutations V108I and K70G only by proviral DNA. The coincident mutations between the two compartments in this sample were, K103N, M184V, H221Y. This discrepancy in the detected mutations generated differences in the prediction of resistance to the following drugs ABC, DDI, AZT, D4T, TDF, ETR and RPV. RNA detected more resistance to drugs than proviral DNA. In both compartments no mutations associated with resistance to protease inhibitors were found. Of the 65 samples analyzed by proviral DNA, 14 were resistant (21.6%), 43 were susceptible (66.1%) and 8 were non-reportable (12.3%). The following mutations were detected in the resistant samples: M184V (13.8%), K103N (7.7%), M41L (4.6%), K70R (3.1%), V108I A62V, V90I, Y181C, M230I, T215Y, T215D, H221Y (1.5%).

Conclusions: Our results demonstrated that proviral DNA is a suitable target for antiretroviral drug resistance testing. However, because of the discrepancy found between both compartments, proviral DNA appears to be adequate only in cases of viremias of <1,000 copies/mL. Therefore, it is not advisable to replace RNA by proviral. We recommend use proviral DNA only in cases where RNA analysis is not possible. In the 65 samples analyzed with virema <1,000 copies/mL, interestingly the mutations found gave resistance to the 3TC, FTC, ABC, EFV, NVP and IDI drugs that coincided with the patients therapy. The importance of our result is firstly low level viremia corresponded to virologic failure since they were related to the detected mutations associated with resistance and secondly that patients were able to change their therapy earlier, which is fundamental for success of your antiretroviral treatment.

Abstract 72

Impact of the HIV-1 integrase natural polymorphism E157Q on susceptibility to integrase inhibitors

Saladini F1, Giannini A1, Boccuto A1, Tiezzi D1, Vicenti I1, Zazzi M1

1Department of Medical Biotechnologies, University of Siena

Background: The resistance profile of HIV-1 integrase inhibitors (INI) has been not fully elucidated, particularly for the latest licensed
compound dolutegravir (DTG). While multiple substitutions at codons 138, 140 and 148 clearly have an impact on DTG activity, anecdotal cases have suggested a possible implication for few integrase polymorphisms including E157Q. This has been found to confer DTG resistance in the absence of major integrase mutations in a case report and to be occasionally selected under DTG treatment. The aim of this study was to evaluate the prevalence and role of E157Q on INI susceptibility.

**Materials & methods:** The prevalence of E157Q was analysed by querying the Italian ARCA database. To analyse the role of E157Q, six plasma samples from patients infected with HIV-1 viruses harbouring the E157Q polymorphism and no other INI resistance associated mutations were used to create recombinant viruses carrying patient derived integrase sequences. One patient was treatment naïve and one patient was under virological failure on raltegravir (RAL) at sample collection, while previous exposure to INIs was unknown for the other patients. In addition, the E157Q polymorphism was also introduced in the HIV-1 wild-type reference NL4-3 strain through site directed mutagenesis. The phenotypic susceptibility to RAL (obtained through the AIDS Reagent Program) and DTG (kindly provided by ViiV Healthcare) was determined through a single cycle replication assay performed on the TZM-bl reporter cell line and previously shown to correlate very well with Monogram Biosciences Phenosense Integrase in terms of FC values.

**Results:** The natural polymorphism E157Q was found in 70/2,960 (2.7%) integrase sequences stored in the ARCA database, with an increased prevalence in CRF02_AG with respect to subtype B strains (7.5% vs. 2.3%, respectively, p < 0.0001, chi-square test). Phenotypic analysis yielded median IC50 fold change (FC) values of 0.9 (range 0.7-1.5) for RAL and 2.3 (range 0.9-3) for DTG (p = 0.027, Wilcoxon Rank Sum test). According to the Phenosense assay susceptibility cut-off values (1.5 biological cut-off for RAL, 4-13 clinical cut-offs for DTG), 1/7 recombinant viruses had a borderline resistance to raltegravir (FC 1.5), while all the viruses were considered as fully susceptible to DTG. Among them, NL4-3+157Q mutant strain showed FC values of 0.7 for RAL and 1.2 for DTG.

**Conclusions:** The E157Q integrase polymorphism is differently distributed across subtypes, particularly it is more frequent in CRF02_AG. The role of E157Q on INI susceptibility appears to be minimal in this limited case series, suggesting that INI resistance apparently driven by E157Q could depend on additional as yet unidentified and possibly rare polymorphism(s). The relative role for RAL vs. DTG also remains to be elucidated since the interpretation of the different FC values is currently dependent on two different types of cut-offs for these two drugs (biological vs. clinical).

**Abstract 73**

**Evolution of transmitted HIV-1 drug resistance in Italy from 2006 to 2016**

**Rossetti B**1,2, Di Giambenedetto S2, Torti C3, Postorino M3, Punzi G3, Saladini P3, Gennari W4, Borghi V5, Monno L4, Pignataro A6, Polillo E7, Colafigli M8, Poggi A9, Tini S10, Zazzi M6, De Luca A7,8

1Clinic of Infectious Diseases, AOU Senese, 2Clinic of Infectious Diseases, Catholic University of Sacred Heart, 3Infectious Diseases Unit, 4Virology, Bari Hospital, 5Biotechnologies Department, Siena University, 6Virology, Modena Hospital, 7Infectious Diseases Unit, Modena Hospital, 8Infectious Diseases Unit, Bari Hospital, 9San Raffaele IRCCS, 10Virology, Pescara Hospital, 11Infectious Diseases Unit, S Maria Annunziata Hospital, 12Medicine Department, Città di Castello

**Background:** Previous studies showed a trend of declining transmitted drug resistance (TDR) in newly infected HIV-1 patients in Italy, updated to 2010. The aims of this study were to evaluate TDR prevalence evolution and to investigate associated factors in newly diagnosed patients from 2006 to 2016.

**Materials and methods:** The earliest available HIV-1 sequence from treatment-naïve patients performed between 2006 and 2016 was retrieved from the ARCA database. TDR was defined as the detection of at least one mutation among those included in the WHO-
recommended SDRM list (Bennett 2009). HIV-1 subtyping was automatically performed upon sequence upload by BLAST. The changes in the prevalence of TDR over time were evaluated using the chi² test for trend, predictors of TDR by logistic regression.

**Results:** We included 3,671 treatment-naïve patients: all had sequences of protease and reverse transcriptase and 474 also integrase. 3,206 (76%) were males, 2,830 Italian (77%), 384 from Sub-Saharan Africa (10%), 179 from Latin America and Caribbean (5%), 135 from Eastern Europe (4%) and 139 (4%) from other countries; 1,829 (43%) were followed at sites in Northern Italy, 1,565 in Central Italy (37%) and 828 (20%) in Southern Italy/islands. Median age was 38 yrs (IQR 30-46), time from HIV diagnosis 0.06 yrs (0.02-0.45), CD4 348/μl (169-521), HIV-1 RNA 4.67 log₁₀ cps/mL (4.06-5.27). The overall prevalence of any drug resistance mutation was 10%; NRTI, NNRTI, major PI and InSTI resistance mutations were detected in 6.0%, 5.0%, 2.4% and 0.0% of patients, respectively. The prevalence of any drug resistance mutations was significantly reduced in 2016 compared with 2006 (5% vs. 14%, p <0.001). The changes over calendar year were significant in the trends for NRTI and NNRTI resistance (p=0.01 and p=0.04, respectively) but not for major PI resistance (p =0.62). The overall proportion of B strains was 67% but decreased from 73% in 2006 to 49% in 2016 (test for trend, p=0.001). The CRF02_AG subtype showed an increase from 9% to 15% (p =0.04). Among Italian natives, 579 (20%) carried non-B viral strains; the prevalence of these subtypes increased over calendar years (16% in 2006–2009, 23% in 2010–2013, 32% in 2014–2016; overall p <0.001). The prevalence of TDR was higher in B than in non-B subtype (13% vs. 9%, p=0.001) carriers. The prevalence of TDR in subtype B and non-B was 8% and 2% for NRTIs (p=0.001), 5% and 4% for NNRTIs (p=0.007), 3% and 1.4% for PIs (p=0.005). In subtype B, TDR declined from 17.2% to 5.7% (p=0.002). At multivariable logistic regression analysis, factors independently associated with TDR were viral subtype B (vs. non B, OR 2.9, CI 95% 2.09-4.10, p<0.001), site in Southern Italy/islands (vs. Northern Italy, OR 0.38, CI 95% 0.26-0.56, p<0.001), viral load at genotype (OR 0.80, 0.71-0.90, p=0.001) and calendar year (per more recent year, OR 0.94, 0.89-0.98, p=0.01).

**Conclusions:** The prevalence of HIV-1 TDR continues to decline in Italy in the last 10 years, while an increasing proportion of naïve patients, including natives, carries non-B subtype virus.

**Abstract 74**

**Prevalence of resistance mutations to Integrase Inhibitors in INSTI-experienced HIV-1 infected patients in a large Italian cohort**

*Modica S¹, Rossetti B², Lombardi P³, Lagi F⁴, Maffeo M⁵, D’autilia R², Pecorari M⁶, Vicenti I⁷, Bruzzone B⁸, Magnani G⁹, Paolucci S¹⁰, Francischi D¹¹, Penco G¹², Sacchini D¹³, Zazzi M¹, De Luca A¹, Di Biagio A¹⁴*

¹Infectious Diseases Clinic, University of Siena, ²Clinic of Infectious Diseases, Catholic University of the Sacred Heart, ³Institute of Clinical Infectious Diseases, Catholic University of the Sacred Heart, ⁴Department of Experimental and Clinical Medicine, University of Florence, ⁵Department of Mathematics, University of Roma Tre, ⁶Unit of Microbiology and Virology, Modena University Hospital, ⁷Department of Medical Biotechnologies, University of Siena, ⁸Hygiene Unit, IRCCS AOU San Martino-IST, ⁹Department of Infectious Diseases, S. Maria Nuova IRCCS Hospital, ¹⁰Molecular Virology Unit, Microbiology and Virology Department, Fondazione IRCCS Policlinico San Matteo, ¹¹Infectious Diseases Clinic, Perugia University Hospital, ¹²Department of Infectious Diseases, Galliera Hospital, ¹³Clinic of Infectious Diseases, “Guglielmo da Saliceto” Hospital, ¹⁴Infectious Diseases Clinic, IRCCS AOU San Martino-IST

**Background:** Integrase Inhibitors (INSTI) are effective and well tolerated in patients with HIV-1 infection. Variable prevalence of selection of resistance and cross-resistance has been selected at virological treatment failure of INSTI therapy. We aimed to analyze the prevalence of resistance mutations in INSTI-failed HIV-1 infected pts from the clinical practice and its associated factors.

**Materials and methods:** We analyzed integrase genotypes, selected from the ARCA database, in INSTI-experienced pts collected between 2008 and 2016 for the presence of at least a low level
resistance (Stanford 8.3 algorithm) to raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG). Differences in the prevalence of resistance were assessed by χ-square test.

**Results:** We included 2,120 genotypes, from 1,721 pts: 74.7% carried a B subtype, 65.6% were males; at time of genotyping median age was 47 years (IQR 40-52), CD4 count 328 cell/μl (175-572), nadir CD4 count 104 (29-228) cell/μl, calendar year 2015 (2013-2016). Contemporary HIV-RNA (VL) was available for 541 with a median of 870 copies/ml (89-22,908). Low-level or higher resistance to any INSTI was found in 402 (19%) and the mutational pattern Q148HKR plus at least one of G140S or E138K, expected to decrease susceptibility to DTG, was detected in 114 (5.4%). The most frequent INSTI-mutations were N155H in 141 cases (6.7%), Q148H in 92 (4.3%), G140S in 94 (4.4%), E138K in 34 (1.6%), Y143R in 30 (1.4%). Three hundred and twenty one out of 1,583 (20.3%) experienced pts carrying subtype B had at least low-level resistance to an INSTI vs. 97/537 (18.1%) with non-B subtype (p=ns) with higher prevalence of Y143R and E138A in viral subtype B vs. non-B (1.7% vs. 0.4%, p=0.009 and 1% vs. 0% p=0.01, respectively); 93/1,583 (5.9%) experienced pts with subtype B had decreased susceptibility to DTG vs. 21/537 (3.9%) with non-B (p=0.05). Low-level or higher resistance to any INSTI was detected in 30/192 (15.6%) pts with VL<200 cps/ml, 15/47 (31.9%) of those with VL 201 to 500 cps/ml, 16/39 (41%) of those with VL 501 to 1,000 cps/ml, 48/100 (48%) of those with VL 1,001 to 10,000 cps/ml, 31/94 (33%) of those with VL 10,001 to 100,000 cps/ml, and 23/69 (33%) with VL >100,000 cps/ml. Decreased susceptibility to DTG was found in 1/192 (0.5%) of pts with VL<200 cps/ml, 2/47 (4.3%) of those with VL 201 to 500 cps/ml, 3/39 (7.7%) of those with VL 1,001 to 1,000 cps/ml, 19/100 (19%) of those with VL 1,001 to 10,000 cps/ml, 17/94 (18.1%) of those with VL 10,001 to 100,000 cps/ml, and 12/69 (17.4%) with VL >100,000 cps/ml.

**Conclusions:** Accumulation of resistance and within class cross-resistance in INSTI failures was modest but related to VL at failure, prompting accurate virological monitoring in RAL treated individuals. Viral subtype influences the type of mutations selected and the entity of cross-resistance to DTG.

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**Abstract 75**

**Baseline Resistance Associated Substitutions in the NS5A region of HCV genotypes 1a and 3 in Spain**

Pérez A1, Chueca N1, Álvarez M1, Fernández-Caballero J1, Pineda J1, Rivero A1, Salmerón F1, Von-Wichmann M1, Santos J1, Collado A1, Téllez F1, Omar M1, Fernández E1, Bernal E11, García F1

1Hospital Universitario San Cecilio, 2Hospital Universitario Nuestra Señora de Valme, 3Hospital Universitario Reina Sofia, 4Hospital Universitario Donostia, 5Hospital Universitario Virgen de la Victoria, 6Complejo Hospitalario Torrecárdenas, 7Hospital Universitario Puerto Real, 8Complejo Hospitalario de Jaén, 9Hospital de Poniente, 10Hospital General Reina Sofia

**Background and aim:** Elbasvir in combination with Grazoprevir (Zepatier, MSD) has shown a lower efficacy when used for 12 weeks in patients infected by genotype (GT) 1a that harbour specific Elbasvir Resistance Associated Substitutions (RASs). European guidelines recommend using Zepatier subject to a viral load threshold of 800,000 IU/ml. Velpatasvir in combination with Sofosbuvir (Epclusa, Gilead) has a lower sustained virological response (SVR12) in cirrhotic patients infected by GT 3. Guidelines recommend to add ribavirine (RBV) to Epclusa when Y93H is present. Hence, to know the prevalence of Elbasvir and Velpatasvir RASs may aid to make clinical decisions for individualized treatment.

**Patients and Methods:** as a part of the GEHEP004 baseline RASs study, we have used Sanger sequencing of the NS5A region (codons 1-95) of all the patients with chronic infection by GT1a and GT3 that were to start HCV treatment. For GT1a we considered NS5A class RASs as any change at positions 28, 29, 30, 31, 32, 58, 62, 93, and Elbasvir specific RASs as changes 28A/G/T, 30D/E/H/G/K/L/R, 31F/M/V, 93 C/H/N/S. For GT3, changes at positions 30K, 31M/P/V, 92K, 93H/N/R were considered as Velpatasvir RASs. In addition, for this study, we have recorded baseline viral load, cirrhosis, and...
prior treatment experience with interferon based regimens.

Results: Globally, 318 HCV infected patients have been studied so far, 130 infected by HCV GT 3 and 188 by GT1a. For GT3 infected patients, Velpatasvir RASs were detected in 18 patients [13.8%; changes at A30K (n=9) & Y93H (n=9)]. Of note, Y93H was detected with a prevalence of 6.9%, and was more frequent in cirrhotic patients (14%). For GT1a the prevalence of NS5A RASs was 16.5% [31/188; M28V (n=5), M28V+Q30H (n=1), M28V+H58P (n=1), M28T (n=1), Q30D (n=1), Q30E (n=1), Q30H (n=2), Q30R (n=3), L31M (n=1), L31M+H58P (n=2), P32H (n=1), H58D (n=1), H58P (n=9), H58Y (n=1) and Y93L (n=1)]. The prevalence of RASs specific for Elbasvir was 6.4% [12/188; M28T (n=1), Q30D (n=1), Q30E (n=1), Q30H (n=3), Q30R (n=3) and L31M (n=3)]. Only 7/128 (5.5%) patients had Elbasvir RASs and more than 800K IU/ml, while 5/43 (11.6%) had Elbasvir RASs when VL was below 800K IU/ml.

Conclusions: We describe the prevalence of RASs in NS5A that associated to the use of Zepatier and Epclusa in HCV genotype 1a and genotype 3 infected patients, respectively. Treating all patients with more than 800.000 IU/ml with Zepatier for 16 weeks instead of basing decisions on Elbasvir RASs may result in overtreatment. The prevalence of Y93H in Spanish HCV cirrhotic patients may justify the investigation of RASs in NS5A prior to using Epclusa, in order to decide whether Ribavirine use is needed or not.

Abstract 76

Hepatitis C virus genotype 1 NS3/NS5a resistance in Polish HCV-monoinfected and HIV/HCV-coinfected patients

Parczewski M1, Kordek J1, Janczewska E1, Pisula A5, Łojewski W7, Socha L2, Bociać-Jasik M5, Szmyczak A1, Cielniak P5, Urbanska A1, Wawrzynowicz-Syczewska M5

1Department of Infectious, Tropical Diseases and Immune Deficiency, Pomeranian Medical University in Szczecin

Introduction: HCV antiviral resistance may adversely affect sustained virologic response rates in the era of directly acting antiviral regimens. Data on drug resistance associated substitutions (RAS) to the most commonly used anti-HCV drug classes - NS5A and NS3 still remain sparse. This study aimed to analyze differences in subgenotype distribution and presence of RAS among Polish genotype 1 HCV-monoinfected and HIV/HCV-coinfected patients.

Material and methods: NS3 and NS5A genotype 1 sequencing was performed with Sanger methodology. Genotype 1 subtype and RAS were identified using geno2pheno HCV according to the rules within the algorithm, for interpretation of resistance any mutation scored as "reduced susceptibility" or "resistant" was included. Final dataset included 300 sequences, with 222 paired NS3/NS5A sequences, 53 with NS3 only and 24 with only NS5A sequence; 189(63%) patients were HCV-monoinfected and 111(37%) HIV/HCV-coinfected - this subgroup also included 33(10.92%) HIV(+) patients with acute hepatitis C (AHC). 71(23.67%) patients were previously treated with pegylated interferon/ribavirin (PEGIFN+RBV), 23(7.67%) with PEGIFN+RBV +protease inhibitor (PI) and 206(68.67%) were treatment naive. For statistics Chi2 test, two-sided Fisher's exact test or U -Mann-Whitney test was used, as appropriate.

Results: Genotype 1a (G1a) was more common among HIV/HCV coinfected (n=45, 40.54%) than genotype 1b (G1b) (n=66, 59.46%) compared to HCV monoinfected cases (n=13, 6.88% vs. n=176, 93.12%, respectively),p<0.001.

Frequency of G1a was also significantly higher among AHC cases (n=19,57.58% vs. n=14 for G1b(42.42%) compared to chronic HVC [n=36,13.85% for G1a vs. n=224,86.15% for G1b,respectively],p<0.001. NS3 RAS were observed in 47/275(17.09%) sequences, with
Abstracts

Resistance-associated substitutions among HCV1b virus populations in patients who failed DAA based regimens

Pavia G1, Marascio N1, Dierckx T2, Cuypers L3, Vrancken B2, Pisani V4, Barreca G5, Mirante T4, Malanga D5, Viglietto G5, Vandamme A2,6, Torti C3, Liberto M5, Focà A1

1Department of Health Sciences, Institute of Microbiology, School of Medicine, University of “Magna Graecia”, Viale Europa, Germaneto, 88100 Catanzaro, Italy, 2KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, Leuven, Belgium, 3Department of Medical and Surgical Sciences, Unit of Infectious and Tropical Diseases, School of Medicine, University of “Magna Graecia”, Viale Europa, Germaneto, 88100 Catanzaro, Italy, 4Centro di Servizio Interdipartimentale (CIS) – Genomica funzionale e Patologia Molecolare, University of “Magna Graecia”, Viale Europa, Germaneto, 88100 Catanzaro, Italy, 5Department of Experimental and Clinical Medicine, University of “Magna Graecia”, Viale Europa, Germaneto, 88100 Catanzaro, Italy, 6Center for Global Health and Tropical Medicine, Institute for Hygiene and Tropical Medicine, University Nova de Lisboa, Rua da Junqueira 100, 1349-008 Lisbon, Portugal

Background: Resistance-associated substitutions (RAS) are usually determined after treatment failure of direct-acting antiviral (DAA) drugs, before a new DAA regimen is started. We investigated the presence of RAS in HCV quasispecies after therapy failure and compared them with baseline sequences to assess the accumulation of RAS and their role in treatment failure.

Materials and methods: Frequent longitudinal serum sampling from four HCV1b chronic infected patients was performed. Deep sequencing of NS3 and NS5B regions, by Ion Personal Genome Machine Sequencer, was applied. Sample-specific consensus sequences were generated from error corrected and adapter cleaned reads using reference guided de novo assembly with Vicuna and the Viral Finishing and Annotation Toolkit. HCV subtyping was performed by manual phylogenetic analysis using PhyML, and by typing

Conclusions: 1. NS5A RAS prevalence is higher among HCV monoinfected patients compared to HIV/HCV coinfection which may influence DAA treatment efficacy in the first group. 2. Despite lack of exposure to NS5A inhibitors, L31M/I and Y93H mutations were notably more common among patients previously treated with antiviral regimens containing PEGIFN+RBV±PI. 3. Acute hepatitis C in HIV patients is associated with higher likelihood of infection with g1a with signature Q80K simeprevir resistance, which may indicate the probability of spread of other primary resistant HCV clades in this group.
tools COMET and Oxford. RAS were analyzed aligning HCV1b reference sequences and subsequently by Geno2pheno tool.

**Results:** All patients were DAA-naïve, not HIV/HBV co-infected. First line therapy for patients 1 and 2 consisted of telaprevir containing regimen, while patient 3 was first treated with simeprevir + daclatasvir and patient 4 with boceprevir in combination with peg-interferon/ribavirin. After failure, all of them were treated with simeprevir + sofosbuvir. Patient 3 had further failure to the last therapy. At breakthrough in majority rule consensus sequence of patient 1, we found NS3 54A RAS, which reduces susceptibility to telaprevir and boceprevir. At baseline this patient’s virus population had NS3 substitution 122G as majority variant, which is not a RAS but located at a hot spot position related to telaprevir resistance (NS3 122R). Concerning patient 3, the breakthrough viral population harboured NS3 168V RAS (simeprevir, asunaprevir, paritaprevir and grazoprevir resistances) as majority variant. This RAS was not detected at baseline and could no longer be detected as majority variant nine months after breakthrough. Additionally, in patient 3, the NS5B 159F sofosbuvir RAS and the NS5B 316N fitness-associated substitution were detected in the majority rule consensus sequence at all time points, these pre-existing RAS may have contributed to treatment failure. For two of the patients, only a baseline sample was available. In patient 2, the majority rule consensus sequence of NS3 contained the 170I substitution on a hot spot position related to boceprevir, simeprevir and telaprevir resistances (NS3 170A/T). In patient 4, the majority population harboured NS3 174T substitution on a hot spot position related to boceprevir and telaprevir resistances (NS3 174F/S).

**Conclusions:** Our data illustrate the importance of resistance testing at baseline and before retreatment after DAA therapy failure. As recently reported in published data, the V amino acidic substitution on 168 position is generally less fit in the absence of selective Protease Inhibitors pressure. Indeed, reversal of treatment acquired NS3 168V RAS after treatment failure in one patient suggests a fitness cost for this mutation. This reversion to DAA-sensitive virus in just a few months raises concern about time of testing and retreatment.

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**Abstract 78**

**High and unpredictable prevalence of resistance in all HCV genotypes at DAA failure may affect the retreatment options and decrease cure rates**


1Department of Experimental Medicine and Surgery, University of Rome Tor Vergata, 2Hepatology Unit, University Hospital of Rome Tor Vergata, 3Molecular Virology, Policlinic Foundation San Matteo, Pavia, Italy, 4Department of Internal Medicine, University Hospital of Messina, 5Infectious Diseases and Viral Hepatitis Unit, Second University of Naples, 6Infectious Diseases Unit, University of Sassari, 7Division of Infectious Disease, ASST Fatebenefratelli Sacco, 8Clinical Microbiology, Virology and Bioemergencies, ASST Fatebenefratelli Sacco, 9Gastroenterology, “P. Giaccone” University Hospital, 10Hygiene Unit, IRCCS AOU San Martino-IST, 11Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, ASL Città di Torino, 12Hepatology Unit, San Camillo Forlanini Hospital, 13Department of Clinical Medicine and Surgery, University “Federico II” of Naples, 14Virology, Siena University Hospital, 15Infectious Diseases, Siena University Hospital, 16Infectious Diseases, Sant’Andrea Hospital – “La Sapienza” University, 17Division of Hepatology, University of Genova-AOU IRCCS San Martino-IST, 18Infectious Disease, IRCCS AOU San Martino - IST, 19Department of Gastroenterology, Scientific Institute for Digestive Disease “Saverio de Bellis” Hospital, 20Department of Emergency and Organ Transplantation, Section of Gastroenterology, University Hospital, 21Department of Medicine and Surgery, University of Milan-
Background: About 5% of patients fail an interferon-free Direct-Acting-Antiviral (DAA) regimen. At failure, resistance-associated-substitutions (RASs) are commonly detected, limiting retreatment options, particularly with NS5A inhibitor or multiclass-resistance. The aim of this study was to characterize the presence of RASs in a large cohort of HCV-infected patients who failed a recommended interferon-free DAA-regimen.

Materials and methods: Within the Italian network VIRONET-C, a total of 300 patients infected with different HCV genotype/subtype (GT1a/1b/2a-b-c/3a-h/4a-d-n-r-v=69/114/32/49/36) who experienced a virologic failure to a recommended regimen according to 2015-2016 guidelines, and with available resistance test at failure, were analyzed.

Results: The majority of patients were cirrhotic (79.3%), 11.3% had a history of hepatocellular carcinoma and 7.0% had a previous DAA-treatment experience. Overall, failures following six different DAA-based therapies were studied: simeprevir+sofosbuvir+ribavirin (N=81), daclatasvir/ledipasvir+sofosbuvir+ribavirin (N=54/87), 3D/2D (paritaprevir/ombitasvir +dasabuvir)+ribavirin (N=47/2), sofosbuvir+ribavirin (GT2, N=29). Interestingly, 5.0% of patients were found infected with a different genotype at failure. Among these, 6/10 patients previously classified as infected with GT1 were actually infected with GT3, and all failed a 3D+ribavirin regimen.

In agreement with clinical trials, 87.3% of patients experienced a relapse and 79.3% showed at least one RAS related to the failed treatment. RASs prevalence was higher in breakthrough/non-responders than in relapers (97.2% vs 76.7%, p=0.009) and varied according to the DAA-class used (91.6% NS5A-RASs [N=190], 65.4% NS3-RASs [N=130], 40.4% dasabuvir-RASs [N=47]), 23.9% sofosbuvir-RASs [N=251]). In GT1b and GT3a NS5A-failing patients, Y93H was the most frequent NS5A-RAS detected at failure (90.0% GT1b; 87.2% GT3a), independently of the NS5A-inhibitor used. Differently, in GT1a and GT4 the prevalence of Y93H varied according to the type of regimen failed: 25.0% (GT1a) and 15.8% (GT4) with ledipasvir+sofosbuvir+ribavirin vs. 0% in both GTs with daclatasvir+sofosbuvir+ribavirin. Overall, the most prevalent NS5A RASs in GT1a were Q30RKHE (51.1%), M28TV (20.0%) and L31MV (17.8%). Interestingly, all 5 GT2c NS5A-failing patients showed NS5A-RASs, mainly F28C (60.0%). Furthermore, 34.7% of patients had >2 NS5A-RASs, with multiple-RASs more frequently in GT1b-failures (64.3%), followed by GT1a (26.7%) and in GT4ad (17.4%).

Failures to sofosbuvir-based regimens showed L159F±C316N in 15.9% of cases, particularly in GT1b (38.1%), followed by S282T in 3.2%. A novel pattern involving three sofosbuvir resistance positions (S282R+C316T+L320C) was disclosed in one GT2a sofosbuvir+ribavirin failing-patient.

Notably, 40.2% of patients treated with >2 DAA-classes showed multiclass-resistance and 9.0% of patients showed RASs in all 3 targets. Among patients treated without NS5A-inhibitors, 29.1% (32/110) showed also extra-target NS5A-RASs, more frequently in GT1b (31.8%) and GT2c (55.5%). Finally, 13 cirrhotic patients already experienced 2 subsequent virologic failures to interferon-free DAA-regimens.

Conclusions: In this real life setting, RASs prevalence at failure was high in almost all HCV genotypes/subtypes and in all genes tested (NS5A very frequent, NS3 frequent, NS5B less common). Failure in GT1b is rare, yet when it occurs resistance is frequent and complex, complicating future therapeutic options. The frequent and complex multiclass-resistance

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advocates for HCV resistance testing at failure in all 3 genes and for all HCV-genotypes for the best second-line therapeutic tailoring.

Abstract 79

Frequent de novo generation of HCV3a resistance-associated substitutions in Spain

Vrancken B1, Cuypers L1, Pérez A2, Chueca N2, Alados J3, De la Iglesia A4, Martínez-Sapiña A5, Pineda J6, Tellez F7, García-Bujalance S8, Von Wichmann M9, Salmeron F10, Aldamiz-Echevarria T11, Vandamme A1,12, García F2

1KU Leuven – University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, 2Clinical Microbiology. HU San Cecilio. Instituto de Investigación Ibs., 3Clinical Microbiology. Hospital Universitario Jerez, 4Clinical Microbiology. Hospital Juan Ramón Jimenez., 5Clinical Microbiology. Hospital Miguel Servet., 6Infectious Diseases Unit. Hospital Universitario de Valme., 7Infectious Diseases Unit. Hospital Universitario de Puerto Real, 8Clinical Microbiology. Hospital Universitario La Paz, 9Infectious Diseases Unit. Hospital Universitario Donostia., 10Hepatology Unit. Hospital Universitario San Cecilio. Instituto de investigación Ibs. Granada, 11Infectious Diseases Unit. Hospital Gregorio Marañón., 12Center for Global Health and Tropical Medicine, Microbiology Unit, Institute for Hygiene and Tropical Medicine, University Nova de Lisboa

Background: HCV subtype 3a, responsible for approximately 17% of HCV infections in Spain, remains a difficult-to-treat genotype despite the availability of highly effective treatments based on direct-acting antivirals. Current treatment regimens often combine a NS5A inhibitor with NS5B inhibitor (sofosbuvir). Resistance-associated substitutions (RASs) can have a profound impact on treatment response, especially in cirrhotic patients, with NS5A variant Y93H of particular interest due to its substantial fold-decrease in susceptibility to all NS5A inhibitors. For this reason, it is of interest to evaluate the virus epidemic history for patterns that can be of public health relevance.

Methods: We combine publicly available with newly generated HCV3a NS5A and NS5B sequence data to elucidate the international HCV3a migration network with a focus on the role of Spain. Bayesian phylogenetic inference methods were used to estimate the epidemiological relations between the sampled virus lineages and to reconstruct the historical transmission patterns. Migration rates between locations were inferred using a discrete phylogeographic model in which rates from and to locations can differ.

Results: There were no clear associations between the sample’s origin and amino acid usage patterns for NS5B RASs S282T, C316N/Y and V321A and for NS5A RASs M28T/V and L31M/V, while Q30L and Y93H appear overrepresented in Pakistani (p=0.009) and Spanish strains respectively (p=0.052). Reconstruction of ancestral sequences shows that the Y93H RAS is usually de novo generated on external branches, dispersed over the whole phylogeny. Thus there is no founder effect for Y93H, as opposed to what is seen for HCV1a NS3 variant Q80K. The strengths and intensities of migration links between locations vary between the NS5A and NS5B datasets. Spain acts as a sink for HCV3a in both datasets but while most HCV3a import into Spain originates from Germany according to the NS5A data, the NS5B data points towards UK as the main source. Virus movements from Spain are usually towards other European countries (in particular to Portugal and Germany) and English-speaking countries (the so-called Anglophone, which encompasses the Australia, Canada, India, Pakistan, the UK and the USA). The inconsistencies in the dominant origin location of HCV3a migration into Spain across datasets point out that each genomic region represents a different sample from the epidemic, and its combined phylogeographical analyses create a complementary picture of relevant migration patterns. This illustrates the usefulness of incorporating data from multiple genomic regions, the added value of longer genomic regions, and the need for broader sampling strategies.

Conclusions: Spain can become an important ‘host-spot’ region of Y93H dissemination in the future, due to frequent de novo generation of this NS5A variant. Furthermore, while the inferred higher-level migration patterns are robust to the
available sampling for a genomic sub-region, the details of the migration links between Spain and other locations vary by dataset. Our results indicate a need for the analyses of larger genomic regions, and a worldwide sampling of the HCV3a epidemic to more reliably infer the most important sources of HCV3a in Spain.

Abstract 80

High incidence of lamivudine-resistant HBV mutants and presence of liver damages in HIV-positive eastern Indian patients harbouring HBV/D2 strains during long-term HAART and Poster presentation

Pal A1, Sarkar N1, Saha D2, Guha S2, Saha B2, Chakravarty R1

1Indian Council Of Medical Research (ICMR) Virus Unit, Kolkata, India, 2Department of Medicine, Calcutta School of Tropical Medicine

Introduction: Carrying the third largest population of HIV infection and the second largest population of chronic hepatitis B infection in the world, India is an important reservoir for HIV-hepatitis B virus (HBV) co-infection. Studies from the treatment-naïve HIV-HBV co-infected individuals reveal a high frequency (11.3%) of chronic HBV infection among HIV-positive individuals from eastern India (EI) and the predominance of HBV sub-genotype D2 (HBV/D2) among the HBV variants. Since 2004, HIV-HBV co-infected patients, in India, have received Lamivudine (3TC) as the only anti-HBV agent being a part of highly-active anti-retroviral therapy (HAART). Tenofovir (TDF, the recommended drug for the treatment of HIV-HBV co-infection) has been introduced in India from 2013. The present study was aimed to characterise the HBV infection and treatment response among chronic HBV infected HIV-positive patients from EI who did not show HBV DNA suppression during long-term HAART.

Materials and methods: Among patients receiving 3TC as the sole anti-HBV treatment as a part of HAART in the Calcutta School of Tropical Medicine, the main ART centre in EI, thirty-six patients receiving long-term 3TC mono-therapy (mean duration 31.28±22.42 months) who did not show HBV DNA suppression (<20 IU/ml) were investigated. A few patients having TDF add-on to their treatment regimen were followed up for treatment response. Different virological parameters were studied- plasma HBV load quantification by real-time PCR, hepatitis B e antigen (HBeAg) detection by commercial ELISA, 3TC-resistant mutation analysis by direct sequencing of overlapping surface/polymerase gene region of HBV genome and HBV genotype/sub-genotype determination by phylogenetetic analysis. Descriptive statistics for continuous and categorical variables were employed.

Results: During long-term HAART, 36 HIV-HBV co-infected patients (mean age 36.91±6.99 years and mean CD4+ T-cell count 347.56±200.15 cells/mm3) showed the presence of virological failure to anti-HBV treatment as indicated by the high percentage of HBV DNA load >2000 IU/ml (55.56%), HBeAg positivity (88.89%) and high mean HBV viraemia (4.31±1.51 logIU/ml). HBV/D2 (41.67%) strains predominated over the other HBV variants-HBV/A1 (38.88%), HBV/D3 (11.11%), HBV/D1(5.56%) and HBV/C1(2.78%). Remarkably, 50% of the HBV/D2 isolates showed the presence of 3TC-resistant mutations including the double (rtL180M+rtM204V) and triple (rtV173L+rtL180M+rtM204V) mutations in the HBV polymerase gene region. Moreover among these drug-resistant HBV/D2 strains, 87.5% had the 3TC-resistant triple mutation associated vaccine-escape mutations (sE164D+sI195M), which was significantly higher than that found in HBV/A1 strains (20%, P=0.023). The 3TC-resistant HBV/D2 strains demonstrated high mean HBV viraemia (5.49±0.03 logIU/ml) and the signs of liver damages (elevated serum alanine aminotransferase level and high fibrosis score). Upon TDF add-on, the 3TC-non-responder HBV/D2 strains (N=5) showed delayed HBV suppression as indicated by the persistence of 3TC-resistant mutations with an increase in mean HBV DNA load >2 logIU/ml with time (4.55±3.68 logIU/ml in 21.33±1.53 months vs. 2.34±2.33 logIU/ml in 10.33±5.03 months).
Conclusions: The high incidence of 3TC-resistant mutations and its associated potential vaccine-escape mutations in HBV/D2 strains, the major HBV variants among HAART-experienced HIV-HBV co-infected patients in EI, retaining the high infectivity and increased liver damage potency, underscore the urgent requirement for the proper management of these mutants from clinical and public health perspectives.

Abstract 81

Reasons for integrase strand transfer inhibitors discontinuation in Spain (Instinct study)


1Lluita Contra La Sida Foundation, Hospital Universitari Germans Trias i Pujol, 2University Hospital La Paz, 3Infectious Diseases Unit, Santiago de Compostela Clinical University Hospital, 4General University Hospital from Alicante, 5University Hospital de La Princesa, 6Infectious Diseases Unit, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, 7Infectious Diseases Unit, La Fe University and Polytechnic Hospital, 8Infectious Diseases Department, Hospital Universitari Vall d’Hebron, 9Infectious Diseases Unit, Elche University General Hospital, 10Infectious Diseases Unit, Hospital Arquitecto Marcide, 11HIV Unit, Hospital Xeral, 12Infectious Diseases Area, Hospital San Pedro-CIBIR, 13University Hospital Virgen de las Nieves, 14Infectious Diseases Unit, Ramón y Cajal Hospital, 15HIV Unit, Infectious Diseases Service, Bellvitge University Hospital, Bellvitge Biomedical Research Institute, 16Infectious Diseases Unit, Donostia University Hospital, 17University Hospital San Cecilio, 18Service of Infectious Diseases, Hospital del Mar, 19Infectious Diseases Unit, Mataró Hospital, 20IrsiCaixa AIDS Research Institute, Universitat Autònoma de Barcelona

Background: Integrase Strand Transfer Inhibitors (INSTIs) are recommended for 1st line ART. Raltegravir (RAL), elvitegravir (ELV) and dolutegravir (DTG) have different pharmacokinetic, resistance and safety profiles.

We evaluated the reasons for INSTI-based regimens discontinuation at 24 weeks in the routine clinical settings in Spain.

Methods: This was a prospective, 2-year, multicenter and on-going observational study including HIV-1 infected adults who initiated RAL, ELV or DTG in 19 tertiary care centers in Spain between 1st April 2015 and 31st December 2016, either as first-line (ART-naive), ART switching strategies or ART-salvage regimens. Virological failures, blips and low-level-viremia were considered as Virological Reasons (VR) for discontinuation. All changes of INSTI-based ART because of simplification strategies, patient’s preferences, drug-drug interactions, proactive changes to avoid future toxicities, and voluntary ART interruptions were considered as Non Virological Reasons (NVR). Virological failure (VF) was defined as 2 consecutive HIV-1 RNA measurements ≥200 c/mL or a single measurement >1000 c/mL while receiving RAL, ELV or DTG, and at least 3 months after INSTI initiation.

Results: From a total population of 995 subjects, 715 with at least one follow-up visit were included in this analysis. Out of them, 165 (23.1%) individuals were ART-naive, 499 (69.8%) had initiated INSTI as an ART switching strategy, and 51 (7.1%) as a salvage ART regimen. ART-naive group: In this group, there were 10 (6.1%) subjects on RAL. Out of them, 5 (50%) patients experienced NVR. There were no ART discontinuations due to VR or adverse events. For the 54 (32.7%) subjects on ELV, NVR were observed in 7 (13%) patients, 3 (5.6%) experienced adverse events, and 1 (1.9%) developed VF. Of 101 (61.2%) on DTG-based ART regimens, 5 (4.9%) subjects experienced NVR, 4 (3.9%) developed adverse events and 2 (1.9%) experienced VF. Switching group: In this group, there were 45 (9.0%) subjects on RAL. Out of them, 11 (24.4%) patients, 3 (6.6%) experienced adverse events, and 1 (1.9%) developed VF. Of 101 (61.2%) on DTG-based ART regimens, 5 (4.9%) subjects experienced NVR, 4 (3.9%) developed adverse events and 2 (1.9%) experienced VF. Switching group: In this group, there were 45 (9.0%) subjects on RAL. Out of them, NVR were observed in 11 (24.4%) patients and 7 (15.6%) experienced adverse events. For the 120 (24.0%) individuals on ELV, 4 (3.3%) experienced adverse events and 3 (2.5%) discontinued due to NVR. Of 334 (66.9%) subjects on DTG-based ART regimens, 19 (5.7%) experienced adverse events, 2 (0.6%) experienced NVR, and 2 (0.6%) were changed because of blips. There were no discontinuations due to VF. Salvage group: In this group, there were 7 (13.7%) subjects on RAL. Out of them, 2
(28.6%) patients experienced NVR, whilst there were no ART discontinuations due to VR or adverse events. For the 15 (29.4%) patients on ELV, 3 subjects discontinued due to VF, blips, and adverse events (6.7%, each). NVR were not observed. Of 29 (56.9%) subjects on DTG-based ART regimens, 2 (6.9%) individuals developed adverse events, 2 (6.9%) discontinued because of NVR, and one (3.4%) experienced blips.

**Conclusions:** At 24 weeks, NVR were the main causes of changes in INSTI-based regimens, while the VR and adverse events were less frequent.

**Abstract 82**

**Darunavir/cobicistat monotherapy in experienced HIV-1 positive subjects**

*Sterrantino G*, *Trotta M*, *Rogasi P*, *Bartoloni A*

1*Azienda Ospedaliero-Universitaria Careggi/Sod Malattie Infettive e Tropicali*

**Background:** Several studies have analyzed strategies of switch to monotherapies with PI/r in pre-treated aviremic patients. The rationale for such strategies lies in the attempt to reduce or prevent toxicities associated with NRTI and has also the advantage of reduce treatment costs. Currently there are no studies evaluating the efficacy and safety of monotherapy with darunavir/cobicistat (DRV/COBI). The objective of the study is to evaluate the efficacy and safety of the switch to DRV/COBI in subjects with baseline undetectable viremia.

**Materials and methods:** This retrospective study includes all experienced-patients who were switched to DRV/COBI monotherapy at the SOD of Tropical and Infectious Diseases, Careggi Hospital, between April and December 2016.

**Results:** Thirty-four subjects were evaluated, most of whom came from a monotherapy with DRV/r (76.5%). Median age was 53 (females 29.4%), most were heavily therapy-experienced, median therapeutical lines pre-switch 5 (IQR 3-6), median years of infection 20 (IQR 12-23), historical resistance showed major resistance to RT in 19 (59.4%), and major resistance to PR in 2 (12.5%). Nineteen (50%) had previous virologic failures. Median duration of undetectable viremia before switch was 10 years (IQR 7-13). Median follow-up was 9.5 months (IQR 7-11). Reasons to switch were simplification in 28 (82.4%), toxicity in 6 (17.6%). One subject discontinued therapy to his will, none failed or experienced adverse events. As for safety, there were no significant differences in lipid profile, liver function (transaminases) and renal function (e-GFR, creatinine) between DRV/COBI and previous therapy.

**Conclusions:** In the heavily multi-experienced cases we analyzed, with a long history of virological suppression, DRV/COBI, monotherapy shows a formidable genetic barrier and seems to be effective and safe. Larger case studies and longer follow-up are needed to confirm this results.

**Abstract 83**

**The Use of New Integrase Inhibitor Molecule in Heavy Treated HIV Patients**

*Tudor A*, *Raducanu I*, *Tomozei M*

1*National Institute For Infectious Disease "Matei Bals"*

2*University of Medicine and Pharmacy "Carol Davila"*

**Background:** The use of a new antiretroviral drug in heavy treated HIV patients creates an opportunity but also a threat. Highly experienced patients’ potential harbor resistance mutation for more than two antiretroviral classes. A new molecule from an already in use antiretroviral class can potentially add new resistance mutations in the absence of an optimal backbone. Objective: to evaluate the use of dolutegravir, the newest integrase inhibitor approved in Romania, in patients with 20 years history of HIV infection and antiretroviral therapy.
Material and methods: We retrospectively collected data on heavily treated patients receiving regimens containing dolutegravir 50 mg once daily. The patients were followed up in National Institute of Infectious Disease “Matei Bals”. We focused on efficacy (viral load levels and CD4 count) and tolerability (clinical data and laboratory tests) of cART containing dolutegravir.

Results: We identified 35 patients, 20 women with median age of 27 years old, diagnosed in their childhood. Median number of antiretroviral regimens was six before started dolutegravir containing cART. The reason for switching to dolutegravir containing regimen was in almost half of the cases (17/35 cases) intolerance to therapy, in 14/35 cases the lack of viral suppression and in 4/35 cases replacing an old drug (didanosine or fosamprenavir). All patients switched for intolerance or for replacing an old drug (Group A) had undetectable viral load at initiation and mean CD4 level was 598.1 cells/mm3. In patients with virologic failure (Group B), the mean CD4 was 244.3 cells/mm3. In patients with virologic failure the most used drugs associated with dolutegravir was boosted darunavir and tenofovir (10/14 cases) and in five cases we used at least five drugs (two reverse transcriptase inhibitors, etravirine, dolutegravir and boosted darunavir). At 12 weeks, CD4 levels increased in average with 75.3 cells/mm3 in group A and with 34.4 cells/mm3 in Group B. In group A, three patients experienced viral load blips and the rest remained undetectable. In group B, five patients reached viral suppression in 12 weeks and another four after 24 weeks, two were lost to follow up after first month and the rest encountered two logs decrease in viral load at 24 weeks. From 35 patients, two experienced neurologic adverse events (one headache and one nightmare) and replaced dolutegravir in group A. Two patients from group A and one in Group B had grade 1 increase in hepatic enzymes, with no clinical effect and they continued the dolutegravir-containing regimen with subsequent normalization of the hepatic lab test.

Discussions: In our multidrug experienced patients introduction in therapeutic use of a new integrase inhibitor had good outcome, due to a carefully selection of patients and antiretroviral combinations, avoiding low active drugs or using four or five partially active molecules.

Hepatotoxicity induced by antituberculous treatment in patients coinfected with HIV, tuberculosis and chronic hepatitis C

Sukach M1

1O.O.Bogomolets National Medical University

Background: Hepatotoxicity due to antituberculous drugs limits treatment in patients coinfected with HIV, tuberculosis and viral hepatitis. Its risk increases in case of advance stage of liver disease. Objective of our study was to determine risk factors for hepatotoxic reactions during treatment of TB in patients coinfected with HIV and HCV.

Materials and methods: The study included 86 patients coinfected with HIV, tuberculosis and chronic hepatitis C: 25 women (29.1%) and 61 men (70.9%), mean age was 36.3±3.8 years. All patients underwent diagnostic tests such as complete blood count, urinalysis, blood chemistry, ultrasound of the abdomen. HIV infection was diagnosed with the detection of HIV antibodies (ELISA and Western blot) and HIV viral load (PCR). CHC was confirmed by detection of HCV RNA in the blood (PCR) and antibody to HCV (ELISA). Diagnosis of pulmonary and extrapulmonary TB was confirmed according to medical history, clinical data, results of X-ray or CT scan, bacteriological tests (spu tum smear microscopy, sputum culture for M. tuberculosis), cerebrospinal fluid tests, histological examination of biopsy samples of lymph nodes. Statistical analysis was performed using the software package Statistica 6.0 and Microsoft Excel 2010. Descriptive statistics of frequency distributions, summary measurements and variability measurements were used. The association of each variable with the presence of hepatotoxicity was evaluated by means of the Mann-Whitney test.

Results: Clinical and laboratory signs of hepatotoxicity during antimycobacterial therapy
were observed in 47 patients (57.0%) coinfected with HIV/TB/CHC. In majority of cases (69.4%) it developed during the first two weeks of therapy. There was a significant (p<0.05) increase in liver enzymes: ALT (up to 154.9±11.9 IU/l), AST (up to 145.4±13.0 IU/l), GGT (up to 87.8±9.9 IU/l), alkaline phosphatase (up to 144.5±29.1 IU/l) and total bilirubin levels (up to 53.7±8.7 μmol/l). Conducting ultrasound examination of the abdomen in dynamics showed significantly higher (p<0.05) rate of hepatomegaly (75.6%), changes in acoustic density of the liver (79.1%), heterogeneity of hepatic parenchyma (74.4%) and expansion of intrahepatic bile ducts (40.7%). Factors that significantly increase the risk of hepatotoxic reactions in patients with coinfection of HIV/TB/CHC are the number of CD4+-cells<200 cells/mm³ (OR=3.922, 95% CI 1,586-9,698, p<0.01), advanced stages of liver fibrosis (OR=8.533, 95% CI 2,842-25,618, p<0.01), baseline increased ALT and AST (OR=4.362, 95% CI 1,478-12,873, p<0.01) and hyperbilirubinemia (OR=3.214, 95% CI 1,184-8,724, p<0.05), the administration of HAART during the intensive phase of antituberculous treatment (OR=4.800, 95% CI 1,647-13,991, p<0.01), pulmonary tuberculosis (OR=2.923, 95% CI 1,183-8,724, p<0.05), bacterioexcretion (OR=3.214, 95% CI 1,184-8,724, p<0.05) and chronic viral hepatitis B coinfection (p<0.05).

Conclusions: Hepatotoxic events occur during the treatment of TB in majority of patients coinfected with HIV/TB/HCV. Multivariate logistic regression showed that the following factors increased the risk of hepatotoxicity: CD4+ count of <200 cells/mm³, advanced stages of liver fibrosis, baseline increased ALT and AST and hyperbilirubinemia, the administration of HAART during the intensive phase of antituberculous treatment, pulmonary tuberculosis, bacterioexcretion and chronic viral hepatitis B coinfection.

Results of pegylated interferon alfa-2b treatment in children with chronic hepatitis C

Nezgoda I1, Singh S1, Singh O1

1National Pirogov Memorial Medical University, Vinnytsya

Background: Assessment of effectiveness, safety and tolerance of pegylated interferon (PEG-IFN) alfa-2b in children with chronic hepatitis C (CHC).

Methods: The study included the observation of 79 pediatric patients with CHC (aged 3 to 18 years). All children were treated in 11 different regional pediatric infectious disease hospitals of Ukraine for the time period of year 2012 to 2014. The treatment plan included PEG-IFN alfa-2b 60 μg/m2 subcutaneous once a week and ribavirin 15 mg/kg daily. CHC-patients with genotype 1 (G1) were treated for 48 weeks, genotype 2-3 (G2-3) for 24 weeks.

Results: Among children 59.6% were boys. By age: 14 to 18 years (50.6%), 3 to 10 years (34.2%). 36.7% patients with CHC had concomitant pathology (hematological diseases (40%), hemophilia (16.6%) and asthma (15.3%)). Most children were infected with horizontal route of transmission (70.8%), but 18.9% were infected vertically. By genotype: G1b (72.2%), G3a (24%), G2 (2.5%) and G1a subtypes (1.3%) respectively. At the beginning of treatment in 60.7% patients viral load was > 600000 IU / ml., 45.6% patients had 1.5 times elevated levels of liver enzymes (ALT, AST), in 25.3% - twice elevated levels, but 29.1% patients had normal levels. During treatment the side effects of antiviral therapy such as hyperthermia syndrome, anemia and leukopenia were observed in 25.5% children.

Conclusions: In completely treated children with CHC a sustained virologic response (SVR) was attained in 63.2% cases. By genotype, the SVR was achieved in 57.8% of G1 and 81.8% of G2-3 HCV patients respectively.
Abstract 86

A Grounded Theory of HCV Treatment Adherence among People who Inject Drugs

Butler M1,2, Gillanders D1, Gillings K2, Power K2

1University Of Edinburgh, 2NHS Tayside Psychological Therapies Service

Background: Chronic hepatitis C virus (HCV) is a global public health threat. It is a primary cause of liver disease worldwide, leading to significant morbidity and mortality (Leask & Dillon, 2016). Treatment innovation has inspired declarations that HCV could be eliminated in the next 15 years (Watts, 2014). Adherence is crucial to the success of such efforts. The primary aim of this study is to gain a qualitative, psychological understanding of positive treatment adherence among a sample of people who inject drugs (PWID), who have successfully completed the ERADICATE HCV treatment trial based in North-East Scotland. Given the challenges and barriers to engagement among this population, this is a quite novel phenomenon and warrants exploratory investigation.

Methods: A qualitative methodology was employed to explore treatment adherence. Semi-structured interviews were conducted with fifteen adult participants (aged 18-70) who had successfully achieved SVR within the ERADICATE HCV treatment trial, within the 3, 6, or 12 month treatment follow-up period. Treatment was offered to actively injecting drug users. Incentives were employed to promote adherence e.g. participants were given vouchers for a supermarket for attendance at clinic. The Interferon based regime involved weekly clinic appointments and self-administrated daily medication in order to achieve SVR by the end of the recommended treatment duration (as indicated by the strain of the virus acquired). Analysis of interview data followed a robust synthesis, following social constructionist grounded theory principles including open and focused coding, categorising and theoretical comparison.

Findings: The resulting grounded theory is a developmentally orientated, contextual behaviour model of HCV treatment adherence among PWID. The theory outlines intra-psychic, socio-cultural and interpersonal processes arising from the data. As people who inject drugs, this population embedded within personal and publicly orientated mistrust, shame and guilt. Core themes include: hope, agency and purpose as qualities emerging from the navigation of conflicting positions regarding behaviour change and the context in which this took place. This was facilitated by specific conditions and contingencies of the trial structure (e.g. clinic accessibility) and the nature of care provided by staff.

Discussion: Applied psychologists are well poised to provide enhance understanding HCV treatment adherence in the service of optimising care for socially excluded populations. This grounded theory is of particular relevance to all professionals working in substance misuse and services for blood borne viruses. Results support a holistic, psychological understanding of the facilitators adherence among this population. Community based, integrated harm reduction and social care services, alongside person-centred, continuous care are key to supporting sustained treatment engagement. It is hoped that further interdisciplinary liaison between psychological and medical professionals working in HCV may will lead to the enhancement of policies and procedures for future treatment provision.

Abstract 87

HCV treatment outcome among HCV/HBV co-infected patients in Georgia


1Health Research Union

Background: Georgia is the country with high HCV prevalence (estimated 5.4% of adults are HCV RNA positive). From 2015, in collaboration
with CDC and other partners, national HCV elimination program was initiated in Georgia providing free treatment with Direct Acting Antivirals (DAAs) for all HCV chronically infected persons. The aim of this study was to evaluate HCV treatment outcome among patients co-infected with hepatitis B virus (HBV) – HBsAg positive individuals.

**Methods:** Data from one of the major clinical sites in Tbilisi, capital of Georgia, providing HCV treatment within elimination program were analyzed. The database contains sociodemographic, clinical and laboratory data, treatment regimens, and outcomes of treatment. Different treatment regimens were used: sofosbuvir and ribavirin with or without pegilated interferon and sofosbuvir/ledipasvir combination with or without ribavirin depending on genotype and disease severity. Treatment outcome was estimated by sustained viral response (SVR) at 12-24 weeks after treatment. Chi-square test was used to determine the association between SVR and presence of HBsAg at baseline.

**Results:** The total number of patients during the study period was 1128. The majority (90.3%) was male and about half of patients were at the age group of 45-60 years. HBsAg prevalence was higher among males compared to females (2.2% vs 0.5%). The SVR rate was not significantly different among patients co-infected with active HBV from those having HCV mono-infection (93.1% and 90.7%, respectively, p=0.48). The prevalence of anti-HBs was 27.9% with only 9 patients (<1%) being vaccinated against HBV infection. The SVR rate was similar among anti-HBs positive and negative patients (90.4% vs 91.7%, respectively, p=0.31).

**Conclusion:** The treatment outcome was similar among patients co-infected with HCV/HBV and mono-infected with HCV treated with DAAs within HCV elimination program in Georgia.

### Abstract 88

**Results of hepatitis C treatment program among previously treated HIV/HCV patients**

**Filippovych S1, Burgay O1, Pavlenko O1, Tsenilova Z1**

1ICF “Alliance for Public Health”

**Background:** According to WHO estimates Hepatitis C (HCV) infection rate among general population in Ukraine is 3% (about 1.2 million persons). HCV/HIV co-infection is registered in 38.4% of all new registered HIV cases.

In June 2015 International Charitable Foundation “Alliance for Public Health” launched a Project on HCV treatment with direct-active antiviral (DAA) sofosbuvir (SOF) for HCV-infected key populations (PWID, OST patients, MSM, SW).

**Aim:** To study treatment success rate of community-based HCV treatment with DAA among previously treated HIV/HCV patients form key groups

Methods: HCV treatment with DAA (sofosbuvir) covered 16 regions of Ukraine. The patients were attended by multidisciplinary teams, which incorporated doctor, nurse and case manager to provide integrated services aimed at testing, diagnostic, HIV treatment, opioid substitution therapy (OST), counseling on HCV prevention and reinfection etc. 15 skilled NGOs were involved in community-based services of social support, rendered to patients on sofosbuvir-based treatment in 19 medical settings.

**Results:** As of March 1, 2017 totally 1209 patients were enrolled in HCV treatment. As few as 2% (N=26) were drop-off cases for different reasons, 4 patients started re-treatment. 1029 patients were tested at 12 weeks post-treatment point (SVR 12w) with 967 patients (94%) cured. 941 (78%) of the patients are PLWH, out of them 97% (N=909) patients receive antiretroviral therapy (ART). 816 HIV + patients (719 of them naive and 97 previously treated) were tested at 12 weeks post-treatment point (SVR 12w). 678 (94%) of HIV+ naive patients and 88 (91%) of previously treated HIV+ patients were cured.
patients achieved sustainable viral response at SVR 12w.

In analyzed cohort of 97 previously treated HIV + patients, that were tested at 12 weeks post-treatment point, gender proportion constitutes 22% (N=21) of female and 78% (N=76) of male patients.

Genotypes distribution in latter cohort showed G1- 74% (N=72); G2 - 5% (N=5); G3 - 21% (N=20) and fibrosis stage F1 in 6% (N=6); F2 in 43% (N=42); F3 in 23% (N=22); F4 in 28% (N=27). Age distribution: up to 25 years – 1% (N=1); 25-34 years – 8% (N=8); 35-44 – 69% (N=67); 45-64 – 20% (N=19); after 55 – 2% (N=2). 79% (N=77) of analyzed cohort were PWID, out of them 13% (N=10) OST patients and 5% of HIV/HCV experienced patients had HBV. 26 patients (27%) had TB in anamnesis.

In analyzed cohort of 97 previously treated HIV + patients with SVR 12w SOF-based 12 weeks regiments were administered in 92% (N=89) of patients with SVR 12w achieved in 90% (N=80). Among 8 patients who were prescribed 16w and 24 w regiments 100% achieved SVR 12w.

Conclusion: sofosbuvir-based HCV treatment regimens show high treatment success rate (91%) among experienced HIV/HCV patients form key groups, previously treated with Peg-IFN.

Abstract 89

Effectiveness and adherence of HCV therapy for people who inject drugs in frame of community initiated Hepatitis C treatment model. Example of Ukraine: Lviv, Poltava and Sumy regions

Vasylyev M1, Pavlyshyn O1, Ostapyuk L1, Koval T3, Piddubna A4, Sluzhynska M1, Chajka I1, Sluzhynska O2, Grushynska O2, Vorozhbyt O6, Ryabichenko V5

1Lviv Regional AIDS Center, 2Charitable Salus Foundation, 3Ukrainian medical stomatological academy, 4Sumy State University, 5Lviv National University named by Danylo Galatskyu

Background: Average prevalence of HCV-infection in Ukraine is 3 % which is approximately 1.17 million of people (WHO Report, 2016). According to the biobehavioural studies the estimated number of people who inject drugs (PWID) in the country is 310 000 with HCV prevalence of 55 %. Referring to the official data by 01.01.2016 there were 29 890 registered persons with HIV/HCV co-infection, including 2839 HIV/HCV/HBV co-infection cases.

HCV treatment is not widely available in Ukraine comparing to ART availability. HIV status may affect HCV treatment adherence in co-infected individuals. Adherence and treatment effectiveness of HCV monoinfected patients vs. HIV/HCV co-infected individuals has not being evaluated in Ukraine yet.

Methods: We aimed to compare adherence and treatment effectiveness to HCV treatment of HIV/HCV co-infected PWID vs. HIV monoinfected PWID.

123 patients were enrolled in the study, who received HCV treatment within community supported treatment model implemented by Charitable Salus Foundation (Lviv), Charitable association Light of Hope (Poltava), NGO Club “Shans” (Sumy) and financial support of Alliance of Public Health in 2016: 35 patients in Lviv Regional AIDS Center (26 HIV/HCV co-infected and 9 HCV monoinfected), 39 HCV monoinfected in Poltava infectious hospital, and 49 in Sumy Regional Clinical Infectious Hospital (9 HIV/HCV co-infected and 40 HCV monoinfected).

Treatment effectiveness was evaluated by sustained virologic response (SRV) after 12 weeks of treatment. Adherence was measured using pill count, number of appointments with physician and patient reports. High treatment adherence was defined as >85% of prescribed doses taken.

Results: All participants reported former injective drug use, were predominately men (75 %), mean age 39±1,28 years. All HIV/HCV co-infected patients were on TDF ART containing regime. Distribution by HCV genotypes: 60 (48,8 %) patients with genotype 1 (HCV/HIV co-infected n=16, HCV monoinfected n=44), 7 (5,7 %) – 2 genotype( HCV/HIV co-infected n=3, HCV monoinfected n=4), 54 (43,9 %) – genotype 3
Abstracts

(HCV/HIV co-infected n=13, HCV monoinfected n=41) and 2 (1.6 %) people with genotype 4 (HCV/HIV co-infected n=2 HCV monoinfected n=0).

In terms of fibrosis according METAVIR scale distributed as follows - F2 – 64 (52,0 %) patients, F3 – 31 (25,2 %), F4 – 28 (22,8 %) patients.

HCV treatment regimes: 1, 3 and 4 genotype - sofosbuvir + pegylated interferon + ribavirin for 12 weeks - 83 patients (HCV/HIV co-infected n=29, HCV monoinfected n=54), 3 genotype - sofosbuvir + ribavirin 24 weeks - 33 persons (HCV/HIV co-infected n=4, HCV monoinfected n=29), 2 genotype - sofosbuvir + ribavirin 16 weeks - 3 people (HCV/HIV co-infected n=2, HCV monoinfected n=1) and 4 HCV monoinfected patients - sofosbuvir + daklatasvir + ribavirin 12 weeks.

Participant adherence had not significant differences on early (baseline week 4) and late (week 8-12) treatment stages in both groups and was 93.5 %.

Conclusions: Community supported HCV treatment models are highly appreciated by PWID and need to be expanded and extended. Treatment adherence was high in mono and HIV co-infected patients regardless of aniretroviral use. Virological response at the end of treatment was observed in all enrolled patients.

Abstract 91

Disability and Low adherence to HAART. The role of depression

*Crux J¹, Vega H², Rodriguez V²*

¹Clinica Especializada Condesa

Background: HIV and depression share risk factors. Mental health and HIV are closely interrelated. The prevalence of psychiatric disorders in persons with HIV is high, ranging from 38 to 85%. Among people living with HIV, it is common to find high rates of depression. Untreated depressive disorders may decrease immune system function, and adherence to antiretroviral therapy. These outcomes impact clinical stability, and potentially increase the risk of drug resistance, disability in physical, social and mental health.

Methods: This is a descriptive, cross-sectional study. Data were collected from 2012 to 2013. Demographic and clinical data were obtained from files. Disability was measured with WHODAS 2.0, and Beck D-I for depression. Chi 2 and Fisher’s tests were used. It was made a bivariate analysis, and then a regression model

Results: Data were obtained from 223 subjects. The mean age was 31.3 (±7.5) years. Mean of education was 11.3 (± 3.3) years. Most of the participants were singles (79.8%), only 36.8% had an employment, and only 10.8% had disability. The 90.1% of the sample reported difficulties the last 30 days due to their health status with a mean of 9 days (± 9.9). The 26% of the sample were depressed. Differences were found between groups with good and poor adherence. According to the regression model: Depression increased the likelihood of having disability (OR= 31.18). Dropping HAART during the last month increases the likelihood of developing disability (OR= 13.8).

Conclusions: This is one of the first studies that associates depression and disability in Latin America among people with HIV. The relationship of depression to chronic diseases such as HIV is undeniable.

Abstract 92

Evolution under DDAs treatment of HIV/HCV genotype 1b infected patients in South-East Romania

*Suceveanu A¹, Arama V², Voinea F³, Catrinioiu D¹, Mazilu L¹, Suceveanu A¹*

¹Ovidius University, ²Carol Davila University

The progression rate of liver disease in HIV/HCV co-infected patients is related with host and viral features. The HCV/HIV-coinfection has a three-fold greater risk to progress to decompensated
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Liver disease, even greater in patients with low CD4 T lymphocyte (CD4) cells (≤350 cells/mm3). We aimed to study the rate of progression to liver decompensation in a group of 50 co-infected HIV/HCV genotype 1 patients treated with DDAs, knowing that HCV eradication is predicted to decrease the mortality associated with co-infection and reduce the toxicity of HIV treatment. The combination of paritaprevir/ritonavir/ombitasvir and dasabuvir was indicated in our coinfectd HCV/HIV patients for a period of 12 weeks, this being the only DDAs regimen reimbursed and approved for use in our country. The significant interactions between the 3D regimen with ART is known based on literature data. Still, the ART switch allowed us to use the 3D regimen in 34 patients (68%), the rest of 16 patients (32%) developing liver decompensation. We concluded that 1/3rd of HIV/HCV co-infected patients will be recommended to switch ART prior to use of 3D regimen in order to prevent liver decompensation. ART and DDAs interactions are still an unresolved issue and a challenge in HIV/HCV coinfection management, especially in resource-limited settings.

Abstract 93

Prevalence of resistance-associated variants (RAVs) to NS5A inhibitors in a cohort of direct acting antiviral (DAAs)-naïve Mexican patients infected with hepatitis C virus genotype 1b. Results of an initial exploratory study

Angulo-Medina L1, Rodriguez-Diaz R1, Orta-Resendiz A1, Sixtos-Alonso S2, Sanchez-Avila J2, Soto-Ramirez L1

1Department of Infectious Diseases, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, 2Department of Gastroenterology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán

Background: NS5A inhibitors targeting domain I of NS5A protein, have demonstrated high potency and pan-genotypic antiviral activity, however, it has been reported that several baseline polymorphisms of direct-acting antiviral agents (DAAs) resistance-associated variants (RAVs) would affect the treatment outcomes of patients infected with hepatitis C virus (HCV). There is a limited data in Latin America and none in Mexico on the RAVs frequency. The aim of this study is to explore the prevalence of RAVs in the HCV NS5A gene among Mexican DAAs-naive patients.

Methods: Direct Sanger sequencing of the NS5A gene (codon 1-288) was performed on plasma derived viral RNA from 9 HCV infected patients DAAs naïve. Amino acids changes in HCV NS5A domain I at codon positions 28, 30, 31, 32 and 93, reported to confer reduced susceptibility to certain NS5A inhibitors were examined (geno2pheno algorithm - last updated on Feb 24, 2017).

Results: Nine mono-infected patients with chronic hepatitis C genotype 1b were studied of which 66% were female with an average age of 55 years; all of them have failed to INF or PegIFN + Ribavirin previous treatments. Their mean viral load was 6.51 log UI/mL (range 4.72 – 7.09 log UI/mL), 9 had a class A Child-Pugh and 6/10 had F4 grade fibrosis. Amino acids substitution (Y93H) associated with high level resistance to NS5A inhibitors were detected in 1/9 (11.1%) HVC-1b samples who had not been treated with NS5A inhibitors. Secondary mutations were not found.

Conclusions: This is the first report of HCV NS5A RAVs in DAAs treatment naïve Mexican patients. Due to the prevalence of Y93H RAV found in our population, pretreatment sequencing of the HCV NS5A gene should be recommended when patients infected with GT1b HCV plan to receive DAAs-containing treatment. These results must to be confirmed in the ongoing prospective larger study.
Abstract 94

Possibilities and limitations in the use of NGS to detect HIV-1 minority variants

Fonager J1, Vorborg K1, Højlund Christensen L1, Kalsen Fischer T1

1Statens Serum Institut

Abstract: The use of deep sequencing to detect HIV-1 minority variants (MVs) involved in antiretroviral drug resistance can potentially allow for an earlier detection of drug resistance mutations (DRM), which could improve treatment strategies. Currently, it is not clear whether deep sequencing methods can reliably detect DRMs in samples from patients collected prior to emerging DRMs below the level detected by traditional Sanger sequencing. It is also currently not clear whether low frequency MVs represent replication competent HIV-1 viruses or if their detection and interpretation is more complicated in certain groups of HIV-1 patients. In order to investigate these issues, we performed next generation sequencing (NGS) on the HIV-1 POL gene amplified from baseline samples from patients, who later developed DRMs. We also investigated if the duration of an HIV-1 infection played a role in MV detection and interpretation.

Material and methods: Samples from 12 patients were used in this study. The patients were classified as: early presenters (EP; >350 CD4+ cells/mL), late presenters (LP; <350 CD4+ cells/mL) and long-time infected HIV-1 patients (LIHP). The POL gene was amplified in duplicate using in-house primers and a library for NGS was generated using the NexteraXT protocol with subsequent sequencing on the Illumina MiSeq benchtop sequencer. MiSeq reads were quality checked, mapped and further investigated using CLCbio. NGS consensus sequences were generated at 1%, 5%, 15% and 20% thresholds and each duplicated pair was evaluated for their identify and for common DRMs identified in a pair. Consistently identified DRMs were evaluated based on their impact on the identified drug resistance using the HIVDB algorithm at Stanford University and compared with DRMs identified by traditional Sanger sequencing.

Results and discussion: DRMs, missed by Sanger sequencing, were consistently detected in samples from 5/12 patients at a 1% cut-off. Two of these DRMs were identical with DRMs identified by Sanger sequencing of PCR products from later samples from the same patients. However, we also found that the sequence identity of 1% consensus sequences generated from the duplicated PCR products from LPs/LIHPS were significantly lower than the sequence identity for the EPs (91.6-91.7% vs. 97.3%). Furthermore, the 1% consensus sequences from LPs/LIHPS contained a higher proportion of INDELs, frame-shifts and Apobec mutation signals compared with the EPs (1.5/2.8 vs. 0.2 per paired consensus sequence). Although these differences diminished markedly at higher percentage cut-offs, this indicated that a diverse and low frequent population of potentially replication compromised HIV-1 strains might be present especially in the LP/LHIP group. This study showed that, although it is indeed possible to consistently identify DRMs early with NGS, not all DRMs detected at low levels of occurrence might have a clinical significance, since their low level of occurrence could also signify that HIV-1 viruses carrying them might have a compromised replicative capacity. Our findings have implications for the future use of NGS in the detection of HIV-1 minority variants.

Abstract 96

A novel primer design approach for the amplification of B-cell cDNA encoding broadly neutralizing antibodies against HIV-1

Döring M1, Kreer C2, Lehnen N2, Pfeifer N1,3, Klein F2

1Max Planck Institute for Informatics, Computational Biology & Applied Algorithmics, Saarland Informatics Campus, 2University Hospital of Cologne, Center for Molecular

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Background: Broadly neutralizing antibodies (bnAbs) are a promising treatment and prevention strategy for HIV-1 infection. The amplification of cDNA from B cells is challenging because multiplex polymerase chain reaction (mPCR) primers should simultaneously target several genes and bind to cDNA in the presence of somatic hypermutation. Thus the goal of our research was to develop a new approach for designing mPCR primers targeting IGH, IGK, and IGL, the three human immunoglobulin loci.

Materials & methods: We retrieved the variable regions of the functional genes from IGH (n = 147), IGK (n = 62), and IGL (n = 35) with complete leader sequences from the IMGT database. For designing primers targeting the less mutated leader region, we developed an algorithm consisting of three stages.

In the first stage, the algorithm constructs a degenerate set of primers by performing multiple sequence alignments of target region substrings, hierarchical clustering according to sequence similarities, and consensus formation on the resulting phylogenetic tree. Second, the initial primer set is filtered according to eleven physicochemical properties (e.g. GC clamp, secondary structures, and dimerization) giving rise to a reduced set of high-quality primer candidates. Third, to obtain a minimal set of primers maximizing the coverage of the template sequences, we solve an instance of the set cover problem using either a greedy algorithm or an integer linear programming formulation.

To evaluate the designed primers, we retrieved existing primer sets for IGH (n = 28), IGK (n = 28), and IGL (n = 14) from the IMGT database and the literature. We tallied the frequencies of fulfilled and failed constraints for all primer sets and then constructed contingency tables to compare the number of constraints that are fulfilled by our new primer sets and the existing primer sets. To test for significance, we performed a right-tailed Fisher's exact test on contingency tables and corrected the p-values using Bonferroni correction.

Results: We designed novel sense primer sets that bind with at most one mismatch to the leaders of IGHV (n = 14), IGKV (n = 7), and IGLV (n = 8).

Conclusions: We have developed openPrimeR, a new computational tool for designing, evaluating, and comparing primer sets for mPCR. Using openPrimeR, we constructed primer sets that might have the potential of boosting the further investigation of bnAbs against HIV-1 by enabling researchers to identify previously undetectable bnAbs. To validate the quality of the primer sets, we are currently performing mPCR using cDNA from germline B cells. Our tool is very general and could be applied to design primers for many other interesting sequence regions such as genetic regions of HIV or HCV.

Abstract 97

Evaluation of the Vela DX system for determining HIV-1 genotypic resistance and comparison with direct sequencing and next generation sequencing platforms

Raymond S1,2, Nicot F1, Jeanne N1, Lefebvre C1, Carcenac R1, Saune K1,2, Delobel P1,2, Izopet J1,2

1Toulouse University Hospital, 2INSERM U1043

Background: The aim of this study was to evaluate the diagnostic performance of the Vela Sentosa next-generation sequencing system in conjunction with the Sentosa SQ HIV Genotyping Assay for sequencing and genotyping HIV-1 samples.

Materials and methods: Plasma samples were extracted and templates prepared on the Sentosa SX instrument. Reverse transcriptase (RT), protease (PR) and integrase genes were sequenced on the Sentosa SQ 301 Sequencer.
(PGM Ion Torrent). Sequence analyses and lists of mutations were provided by the Vela software. HIV-1 specimens of various subtypes harboring resistance-associated mutations were used to validate all analyses. Methods were compared using 46 clinical samples (mean viral load: 6.15 log copies/mL) that had been characterized by direct sequencing and on two NGS platforms, 454 GS-Junior Roche or MiSeq Illumina.

Results: The analytical sensitivity for detecting major resistance mutations in HIV-1 subtype B samples was 200 copies/mL; it was 500 copies/mL for CRF02-AG specimens. Minor variants were detected with a sensitivity of 5% at 100,000 copies/mL. The sequences of reference HIV-1 strains (A, B, C, D, F, G, CRF01-AE and CRF02-AG) were concordant with those obtained by direct sequencing. Reproducibility was measured on a mixture (10,000 copies/mL) containing 20% mutated variants; the within-run coefficient of variation was 13% and the between-run coefficient was 33%. No sample cross-contamination was observed. The Vela DX system detected 100/103 mutations identified by direct sequencing (concordance 97%). Vela DX identified 3/17 mutations, accounting for 1-20% of the quasispecies identified by MiSeq and 50/50 mutations >20% (23 clinical samples). Vela DX also identified 3/27 mutations, accounting for 1-20% of the quasispecies identified by the 454 GS-Junior and 61/64 of the >20% mutations (43 clinical samples). The Vela DX and 454 GS-Junior quantified 66 mutations (Spearman correlation ρ=0.6898; p<0.0001; mean difference: 2.3% by Bland Altman plot). The Vela DX and MiSeq quantified 53 mutations (Spearman correlation ρ=0.4060; p=0.0026; mean difference: 1.1%).

Conclusion: The Vela DX system and HIV Genotyping Assay accurately identified HIV-1 genotype resistance mutations. Nucleic acid extraction, PCR reagent distribution, library preparation and bio-informatic analysis are all automated. Vela DX identified the same resistance-associated mutations as those found by direct sequencing. The Vela DX seemed less sensitive for detecting minor variants than were the other NGS platforms. However, the three NGS platforms, Vela DX, 454 GS-Junior and MiSeq, all detected variants accounting for more than 20% of the quasispecies.

Evaluation of the Aptima® HIV-1 Quant Dx Assay for HIV-1 RNA Viral Load Detection and Quantitation in the Real-Life Clinical Practice

Longo S1, Bon I1, Musumeci G1, Bertoldi A1, D’Urbano V1, Re M1
Microbiology Section of the Department of Experimental, Diagnostic and Specialty Medicine, School of Medicine, University of Bologna, Italy.

Background: HIV-1 RNA Viral Load (VL) along with CD4 T lymphocyte (CD4) cell count are the front line laboratory tests that play a main role as markers of initial and sustained response to antiretroviral therapy (ART). With currently available ART, most HIV-infected patients are able to achieve and maintain HIV viral suppression with a consensus VL value persistently below 50 cp/ml. High-sensitive real-time PCR assays are routinely used to monitor HIV-1 infected subjects. Nevertheless, inter-assay discrepancies have been described at the low VL end (Blips), where clinical decisions regarding possible virological rebound are based. The clinical performance of the recently approved Hologic Aptima® HIV-1 Quant Dx assay was compared to the test routinely used in the S.Orsola-Malpighi Hospital in Bologna for HIV-1 VL assessment (Roche CAP/CTM HIV-1 v2.0). Discordant results was resolved based on the follow-up data including CD4+ count, CD4+/CD8+ ratio and VL as clinical indicators.

Materials and methods: The study included plasma samples obtained from HIV-1-infected patients of the “S.Orsola-Malpighi” Hospital in Bologna from March to June 2016. A total of 335 clinical samples (248 B-subtype and 87 non-B subtypes) were selected, spanning the full range of HIV-1 viral load values. Demin Regression and Bland-Altman plot have been used for clinical correlation analysis. The Aptima HIV-1 Assay (Hologic, Inc.) was performed on the fully automated Panther system. Aptima HIV has a linear quantification
range of 30–10,000,000 copies/ml (with 0.5 ml plasma) with a LOD = 13 copies/ml (3rd HIV-1 WHO-IS). Roche testing (Roche Molecular Systems, Inc., Pleasanton, CA) was performed on the CAP/CTM platform. The linear quantification range of the assay is 20–10,000,000 copies/ml (1.1 ml plasma) with a LOD = 20 copies/ml.

Results: Out of 335 tested samples, 164 samples had quantifiable results in both assays using the Aptima LLoQ of 30 cp/ml. Demin Regression demonstrates a high correlation with an R2=0.97. The mean values obtained with the Aptima HIV assay were 0.22 log10 copies/ml higher than the mean values from the Roche assay. Out of 65 discordant samples, we further investigated 6 samples with possible clinical implications that were quantified by Roche and Detected or Not Detected by Aptima. Follow up clinical data (CD4+, CD4+/CD8+ and VL), quarterly collected during the next one year follow up, confirmed the higher accuracy of Aptima results in the side-by-side discordant comparison, demonstrating a significant reduction in Blips events.

Conclusions: This study evaluated the clinical utility of the Aptima HIV-1 Quant Dx Assay in the clinical practice by using 335 plasma samples obtained from HIV-1-infected patients. Along with the outstanding performance, it is important to highlight the full automation, ease of use, and improved workflow of the Aptima assay on the Panther system. This system allows random access testing of various analytes, processing up to 275 samples in an 8-hr shift. This enables high flexibility to adapt to low or high throughput testing. Combined with the clinical performance data, these characteristics make the Aptima HIV-1 Quant Dx Assay an excellent choice for routine monitoring of HIV-1 VL in clinical laboratories.

Abstract 99

New HIV Genotyping Assay based on Next Generation Sequencing for HIV Drug Resistance Testing

Heger E1, Hille J1, Löber A2, Knops E1, Thielen A2, Däumer M2, Görtz A2, Ariyaratne P4, Lee C4, Michel G4, Kaiser R1

1University of Cologne, Institute for Virology, 2Vela Diagnostics, 3Institute for Immunology and Genetics, 4Vela Research Singapore

Background: Since live saving treatment with antiretroviral HIV drugs became possible in 1987, the emergence of resistance to antiretroviral drugs has become a major life threatening concern. Although HIV treatment has improved dramatically with respect to number, potency and availability of drugs the analysis of resistance mutations remains of high clinical importance. Since the first commercially available HIV drug-resistance tests were launched in-house assay underwent significant improvements. The emergence of next generation sequencing (NGS) platforms allowed parallel deep sequencing of clinically relevant regions with high accuracy. Here we present validation data from the first commercially available NGS-based HIV genotyping assay specifically developed for routine diagnostic use in comparison to in-house genotyping analysis (Sanger and NGS).

Methods: We used the Sentosa® SQ HIV Genotyping Assay (Vela Diagnostics) covering the HIV protease (PR), reverse transcriptase (RT) and integrase (IN) genes. The system comprised of 1) a robotic liquid handling system for RNA extraction and NGS library preparation; 2) Ion Torrent-based NGS system; 3) kits for RNA extraction, HIV NGS library preparation and sequencing, and 4) data analysis and reporting software. The Vela system allows sequence data export for usage of alternative data interpretation systems. For comparison all samples were analysed with our in-house HIV genotyping system using Sanger and NGS for genotyping. Subtype prediction of HIV Vela system was compared to our in house system using the COMET HIV-1 subtyping tool. Resistance
interpretation of the HIV VELA system as well as the exported sequencing data were compared to an in-house Sanger and NGS-based analysis using the HIV-GRADE interpretation system.

**Results:** We analysed 122 plasma samples with a viral load of more than 1000 copies/mL of therapy naive and therapy experienced patients. Not all samples were successfully analysed with all three methods. With Vela, we successfully analysed 117 PR-RT and 117 IN samples; with Sanger we generated 114 PR-RT and 114 IN products, with our in-house NGS method 113 PR-RT and 110 IN were generated. For the PR-RT region one hundred samples could be analysed in total with all 3 methods. The integrase region was successfully analysed in 97 samples with all 3 methods.

With regard to subtype prediction between Vela versus Sanger we saw a concordance of 98.2% within PR-RT and 99.1% within IN. The detected resistance mutations were highly concordant between Vela and Sanger and between Vela and in-house NGS.

**Conclusion:** Vela Diagnostics provides a highly automated HIV genotyping platform based on next-generation sequencing with impressive fast sample analysis in only 3 days. The concordance of the results between Vela, in-house Sanger and NGS was very high. Discordant results were due to samples with rare subtypes or due to low viral load.

**Abstract 101**

**TILDA assay for the evaluation of the amount of HIV competent virus in cell reservoir**


1Microbiology Section, Department Of Experimental, Diagnostic And Specialty Medicine, University Of Bologna, 2Department of Biochemistry, Erasmus Medical Center

**Background:** Despite the success of antiretroviral therapy (ART) in suppressing viral replication, HIV can persist in patients in presence of effective treatment. During long-term viral suppression, integrated forms of HIV persist in a silent, but replication-competent state in resting CD4+ T cells, constituting the major viral reservoir. A small latent reservoir is associated to a higher likelihood of controlling viral replication after ART withdrawal and, thus, reduction of reservoir size is one of the major goals of the new strategies aimed at curing HIV. TILDA assay, a new assay specifically developed to measure the frequency of presumably replication-competent provirus, will be used to assess the reservoir size in samples from long-term treated HIV patients. Furthermore, TILDA was specifically developed for the clade B but we slightly adapted it to detect also the subtype C.

**Materials and methods:** Clinical study: blood samples were collected from HIV subjects receiving ART and aviremic for at least 2 years. TILDA assay: after 16h stimulation with a potent mitogen, CD4+ T cells isolated from blood samples of HIV-infected patients were serially diluted. Presence of cells harbouring active HIV was evaluated using a nested PCR assay targeting the tat/rev mRNA. The frequency of latently infected cells was calculated with the maximum likelihood method. Sensitivity of the PCR method was estimated using serial dilutions of RNA standards. Performances of the method were further evaluated on cell suspensions containing the latently infected J-Lat11.1 cells (harboring a latent copy of the HIV genome) and Jurkat cells at known ratios.

**Results:** The first part of the project evaluated the performances of the assay using RNA standards, and cell lines models of latency. The PCR method showed good sensitivity, with a limit of detection of 20 RNA copies/reaction. Furthermore, TILDA proved to be able to correctly estimate the frequency of latently infected cells in mixtures containing 10, 50 and 100 J-Lat cells/106 Jurkat cells. Finally, using TILDA, we were able to quantify the reservoir size in long-term treated HIV patients with a good inter-assay reproducibility. Moreover, we could successfully perform an adapted protocol of TILDA on patients samples infected with the C clade.

**Conclusions:** The quantification of latently infected cells is essential to evaluate the efficacy of available regimens against cellular reservoirs and a rapid achievement of complete virological
suppression is essential to prevent the seeding of a large HIV reservoir. Here, we evaluated the sensibility and the reproducibility of TILDA and we successfully performed it on patient samples infected either with the subtype B or with the subtype C, observing a good reproducibility inter-assay.

Abstract 102

Update on NGS in routine HIV-1 resistance diagnostic - frequency of additional resistance relevant mutations in 2% and 1% population proportions correlated to viral load and additional patient follow-ups

Ehret R1, Schuetze M1, Obermeier M1
1MVZmib AG

Background: The relevance of mutations detected by NGS technologies in low frequencies is still a subject of debate. Clinical data is rare. We here report the frequency of additional mutations in population-proportions of greater than 2% and 1% in routine laboratory testing and correlate them to viral load. Therapy implications for patients with relevant minor viral populations were monitored.

Materials & methods: 645 HIV-1 resistance tests (reverse transcriptase/protease) performed between 10/2014 and 04/2016 with an in house PCR followed by NGS (Illumina MiSeq, sequences reported with >100 reads only) were analyzed. Sequences were interpreted by HIV-GRADE (http://www.hiv-grade.de) for resistance mutations using 10%, 2% and 1% minority cut-offs. Besides the overall increase in mutations, a specific focus laid on differences in reported resistance associated mutations and resistance levels (e.g. additional drug class or further drugs same class). The proportion of subpopulations harbouring additional mutations with greater than 1000 and 2000 c/mL (= mutational load) were calculated, therapy data and follow up for those patients was monitored as far as available.

Results: 483 (74.9%) samples were identified as subtype B. No drug resistance associated mutations were reported by HIV-GRADE for 44% with a 10% cut-off, 29.5% and 19.7% with 2% and 1% respectively. This also correlates with an increase of resistance level in the interpretation, especially for NNRTIs. With a cut-off of 10% in 148 samples (105 non-B subtype) only PI relevant mutations were detected. We found mutations only relevant for NRTIs in 21 samples and for NNRTI in 100 samples. Additional mutations could be detected in 94 of the samples using a 2% minority cut-off. This corresponds to an additional mutational load of >2000 c/mL in 76 cases with a 2% minority cut-off and additional 134 mutations at 1% cut-off. Consequences on treatment concepts and regimen were found rarely in therapy data.

Conclusions: A relative high proportion (56%) of investigated sequences showed resistance mutations at a minority cut-off of 10%. 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. Relevance of mutations in these low percentages is often discussed. The concept of "mutational load" tries to correlate the viral load with the proportion of mutation in the whole viral population. Despite the low percentage these viral quasispecies can be detected in relevant absolute quantities which increases the probability that these mutations represent viable resistant virus. There is a clear need for clinical evaluation of the relevance of mutations in the low percentage range for resistance interpretation due to its broader use in clinical routine.
Abstract 103

Development of a more sensitive integrated HIV-1 DNA quantification assay

Ruggiero A1, Malatinkova E2, De Spiegelaere W3, Rutsaert S2, Geretti A1, Vandekerckhove L2, Pollakis G1, Paxton W4

1University Of Liverpool, Department of Clinical Infection, Microbiology and Immunology, 2Ghent University, Department of Internal Medicine, 3Ghent University, Department of Morphology

Background: During HIV-1 replication reverse transcribed double stranded DNA is stably integrated within the host genome, thereby generating a cellular pool refractory to treatment and which fuels viral rebound following treatment interruption. Different assays have been described to quantify the integrated HIV-1 DNA reservoir, including the Alu-gag assay. This method includes a multi-sampling approach (40 independent replicates needed for integrated HIV-1 DNA amplification) in a two step protocol: PCR1 that allows amplification of the integrated HIV-1 DNA by targeting the human Alu DNA repeat and the HIV-1 gag region; a qPCR2 that quantifies the HIV-1 products obtained in PCR1. The assay’s rate of detection averages 10%. There is the need for a more sensitive assay for quantification of the viral reservoir, especially in the context of eradication studies. Here we describe the development of a more sensitive Alu-HIV-1 PCR assay.

Materials and methods: We developed a new HIV-1 integrated DNA quantification assay based on designing a primer targeting the HIV-1 LTR region and which could amplify between this region and Alu (Alu-5LTR assay). The primer is 845 nucleotides closer to the Alu region than gag, allowing for more efficient amplification. Results obtained in qPCR2 were used to calculate integrated HIV-1 DNA copies/replicate by using Poisson statistics that corrects to reflect the 10% sensitivity of the original assay. Patient PBMC samples included those receiving antiretroviral therapy and showing full suppression of viral load for at least one year. Cellular DNA was isolated from PBMCS by using commercial kits (Qiagen).

Results: Eighteen individuals were selected to determine integrated HIV-1 DNA levels using the Alu-5LTR and Alu-gag assays and results were compared. Integrated HIV-1 DNA was measured with both assays in 17/18 (94%) patients, 1/18 (6%) patient failed with the Alu-gag assay but not the Alu-LTR assay. Among the 17 patients with results obtained with both assays, median integrated HIV-1 copies/replicate were 3.1 (range: 1.1-12) and 11.6 (4.7-25.9) with the Alu-gag and Alu-5LTR assays, respectively. Ratio of integrated HIV-1 DNA measured with Alu-gag vs Alu-5LTR was median 3.5 (1.4-6.3). Overall, ratios were distributed in the individuals as follow: 3/17 patients had ratios<2, 11/17 had ratios between 2 - 4, and 4/17 patients had ratios>4.

Conclusion: We identify that the newly designed Alu-5LTR assay possesses the capacity to quantify integrated HIV-1 DNA in patients receiving suppressive ART. We demonstrate that the assay is quantitatively superior to the Alu-gag assay with better detection rates and higher median copies of integrated HIV-1 DNA being determined. This improved assay will aide in the analysis of future HIV-1 persistence and eradication studies.

Abstract 104

Comparative Precision Analysis of the Aptima HIV-1 Quant Dx (Hologic) and the RealTime HIV-1 (Abbott) Viral Load Assay

Wiesmann F1, Braun P1, Naeth G1, Haase B1, Knechten H1

1PZB Aachen

Background: The precise quantification of HIV viral load is crucial for monitoring of HIV-infection and therapy. Viral load results exceeding specific cut-offs may lead to clinically relevant decisions by the physician and the patient before and during treatment. A success of therapy is defined as a maximal viral load of 50 copies/ml. A viral breakthrough with an elevated risk of viral
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resistance is considered when HIV RNA is repeatedly quantified above 200 copies/ml. The Aptima HIV-1 Quant Dx represents an assay of the more recent generation for the use with the fully-automated Panther-System (Hologic). It received CE-IVD approval in Europe in 2014 for diagnostic use and therapy monitoring. Beside this newer assay, multiple viral load quantification assays, such as RealTime HIV-1 by Abbott, are currently approved for monitoring of HIV RNA in blood plasma. They all differ in assay precision particularly at the lower limit of quantification. The subject of this evaluation was to assess the precision near the lower limit of quantification for both assays.

Methods: Within this comparative evaluation frozen plasma samples of HIV-1 positive, therapy-naive patients were diluted to two concentrations representing clinically relevant cut-offs at 50 copies/ml and 200 copies/ml. Four different clinical specimens, representing the four most prevalent HIV-1 subtypes worldwide (B, C, AE and AG) were selected for this procedure. All samples were tested in triplicates in five independent runs with both systems. The limits of quantification are specified as 30 for the lower end and 10.000.000 copies/ml for the upper end of quantification. All samples underwent the same thawing cycles and treatment conditions.

Results: Coefficients of variation were assessed based on logarithmic results. They ranged from 6.8 to 8.3% (RealTime) and 7.6 to 9.2% (Aptima) for nominal 50 copies/ml and 3.3 to 4.7% (RealTime) and accordingly 4.1 to 6.5% (Aptima) for nominal 200 copies/ml. The precision of both systems were on a comparable high level. In total 9 samples were detected but not quantified with both assays and therefore excluded from precision analysis. This applied to 4xsubtype B, 1xC, 1xAE and 3xAG for RealTime and 2xB, 5xC and 2xAG for Aptima, respectively. Results of both assays did not differ significantly from each other and showed a comparable distribution near the nominal viral load concentrations.

Conclusion: Both assays showed convincing data concerning precision at the lower limit of quantification. Coefficients of variation were comparable low despite the low viremic setting without major outliers. This is of major importance since cut-offs at 50 copies/ml and 200 copies/ml help to differentiate between BLIPs (short-termed residual viremia) or therapy failure. All four analyzed HIV-1 subtypes were reliably detected with both systems with a comparable precision. Consequently, the Aptima assay represents an appropriate alternative to currently well adapted HIV viral load assays in clinical routine with a less time-consuming fully-automated sample handling approach.

Abstract 105

A novel Next Generation Sequencing assay as an alternative to currently available methods for Hepatitis C virus genotyping

Dirani G1, Paesini E1, Farabegoli P1, Dalmo B1, Bartolini B2, Garbuglia A2, Capobianchi M2, Sambri V1

1Ausl Romagna O.U Microbiology, 2INMI "L. Spallanzani", Virology

Chronic HCV infection is one of the leading causes of liver-related death and in many countries it is a primary reason for having a liver transplant. HCV genotype identification has long been used in the clinical practice, since different genotypes have different response rates and required different doses and durations of IFN/RBV treatment; moreover both the frequency and the pattern of resistance to different Direct-Acting Antivirals (DAAs) classes are subtype specific. Hence the necessity to make an accurate HCV subtyping becomes a fundamental tool to optimize current and future clinical management of HCV infected subjects. In the present study we evaluate the performance of a next generation sequencing (NGS: based on the Ion Torrent Platform -Vela Sentosa SQ 301 sequencer) HCV genotyping assay, targeting a region of the NS5B gene. As a comparative method a commercial method based on the detection via reverse hybridization of 5’UTR and core regions (Versant HCV Genotype 2.0 Assay,
LiPA, Siemens) was selected. A total of routinely not selected 207 plasma samples were used. The results show a better performance of NGS assay. Concerning genotype 1 and 3, the discrepancy of assigned subtypes for LiPA assay with respect to Vela NGS assay is not relevant (1.8% and 2%, respectively); in contrast, the difference of assigned subtypes for genotypes 2 and 4 is very high (96.6% and 100%, respectively). The NGS based assay, in addition to a more efficient subtyping, also allows the detection of SNPs related to DAAs resistance. Unfortunately, the resistance mutations data, except for 1a and 1b subtypes, remain scarce; the future relevant challenge will be to identify subtypes-specific drug resistance mutations, which are essential to create highly personalized therapeutic pathways.

Abstract 106

Evaluation of serum Golgi Protein 73 in patients with HCV infection before and after DAA treatment

De Murtas V1, Pisano C1, Babudieri S1, Maida I1

1University Of Sassari, Department of Clinical and Experimental Medicine

Introduction: Chronic HCV infection is a condition favoring the progression to fibrosis / cirrhosis. The guidelines for chronic HCV treatment identify patients with fibrosis > F3 as a priority for the increased risk of progression to cirrhosis, hepatic decompensation and HCC. Several studies show overexpression of Golgi membrane glycoprotein 73 (GP73) in a variety of acute and chronic liver disease, including chronic hepatitis HCV. It is known that an overexpression of GP73 can cause a significant increase of HCV virions secretion, while its inhibition causes its suppression. A correlation was observed between serum levels of GP73 and liver fibrosis stage, although this association is still unclear.

Aim: The study was designed to evaluate differences in GP73 serum levels in HCV patients before and after antiviral therapy with DAA. Moreover, to check the correlation between GP73 protein levels and liver fibrosis.

Methods: Serum samples from 19 subjects, 11 Male/8 Female (mean age: 58.9±14.1 years; range 25-84), with Chronic Hepatitis C (CHC), were analyzed in the present study. Quantitative determination of GP73 in serum was performed using commercially available sandwich enzyme-linked immunoassay (ELISA) (Cloud-Clone Corp., Houston, USA) according to the manufacturer’s protocol. Liver stiffness was determined by FibroScan (FibroScanH, Philips, France), based on manufacturer’s protocol. Results were expressed in kilopascal (kPa).

Results: Nineteen patients were evaluated (11 M/8 F), mean age 58.9 ± 14.1 years, of which 1 with F2 fibrosis stage, 2 with F3 and 16 with F4 measured by FibroScan. HCV genotyping showed genotype-1a, 1b, 3a and 4, respectively in 16%, 42%, 16% and 26%. Preliminary results have revealed that there is no significant difference between GP73 serum levels before and after therapy, GP73 serum levels and HCV viral load before treatment. Moreover, a weak correlation was observed between GP73 serum levels and liver fibrosis stage (Pearson Coefficient: -0.14).

Conclusions: Literature reports that high levels of GP73 values can be considered as valid marker for evaluating the progression of fibrosis in patients with liver disease, and could help to understand which patients have higher priority treatment than patients with normal levels of GP73. Although the sample size does not allow us to draw conclusions yet, our results seems to suggest a weak correlation between fibrosis and GP73.

Abstract 107
Assessment of liver stiffness after sustained virological response to DAAs therapy for chronic hepatitis C – a central Italy multicenter study using transient elastography

Di Biase V1, Castelletti S2, Pellicelli A3, Cacciatore P4, Schimizzi A5, Puoti C6, Furlan C7, D’Alonzo A8, Falconi Di Francesco L1, Di Giammartino D9, Tarquini P10

1Infectious Diseases Unit, G. Mazzini Hospital, 2Infectious Diseases Clinic, Azienda Ospedaliera S. Camillo-Forlanini, 3Infectious Diseases Unit, S. Spirito Hospital, 4Internal Medicine, 5Liver and Transplant Unit, Azienda Ospedaliera S. Camillo-Forlanini, 6Hepatology Unit, INI Institute Grottaferrata, 7Hepatologic Unit, La Sapienza University

Background and aims: The Food and Drug Administration approved Transient Elastography (TE) FibroScan® for non-invasive testing to measure liver fibrosis with results comparable to biopsy. The primary endpoint of our study is to investigate quantitative changes in Liver Stiffness (LS) in a large Italian cohort of patients after treatment with DAAs and Sustained Virological Response (SVR).

Methods: We are presenting a retrospective study to assess, in terms of quantity, the reduction of LS in HCV patients treated with DAAs between January and October 2015, in 7 Central Italy Hepatological centers. All patients achieved SVR12. Patients included, had LS measurement 3 months prior to beginning therapy (T0) and within 24 (T1) and 48 (T2) weeks after the end of treatment (EOT).

LS measurement was performed using TE. Results were recorded in kPa, range 3-75; values assessed by 10 consecutive measurements; to assure quality of data, TE exams with an IQR divided by mean value >30% or a success rate <60% were excluded, as recommended. The result above 12.5kPa was considered indicative for LS defined cirrhosis.

Results: A total of 1006 consecutive patients were screened, of which 290 had LS controls assessed by TE, and were enrolled in the study. Median age was 68 years [IQR 52-70]; 67% were male; median BMI was 26[IQR 23-28]; 2% had HIV coinfection. Patients were stratified into 2 groups based on LS measurement: cirrhotic (n=192) 66% and non-cirrhotic (n=98) 34%.

Pre-treatment LS measurement median was 16,9kPa [IQR 12-26,7]. LS median score was 21,5 [IQR 16,6-34,8] vs 10,7kPa [IQR 9,2-11,8] in cirrhotic and non cirrhotic group respectively (T0). Median LS score at 24 weeks EOT (T1) was 17[IQR 13-25,7] for cirrhotic group and 7,4kPa [IQR 5,9-9] for non-cirrhotic group. In both groups the median LS decrease is considered to be statistically significant (p=0,0001). Median LS score at 48 weeks EOT (T2) is available only for n=56 patients from both groups. LS was 10,6kPa [IQR 7,5-15,2] vs 17.1 at baseline in this group; LS decrease was significant (p=0,0038).

Conclusions: The use of TE to measure fibrosis regression is still to be defined and validated. Our data series have shown a statistically significant and sustained decrease in LS at 24 (for both cirrhotic and non-cirrhotic patients) and 48 weeks EOT. Our work is ongoing and we need further knowledge about fibrosis regression to assess the impact of SVR on long term liver-related morbidity and mortality.

Abstract 108

HCV RNA detection in finger prick capillary blood for point-pf-care testing (POCT)

Beloukas A1, Hopkins M2, Austin H1, Villa G1,3, Papadimitropoulos A1, Davies P1, Williams J1, Railton E3, Geretti A1,3

1Institute Of Infection And Global Health, University Of Liverpool, 2Liverpool Clinical Laboratories, 3Royal Liverpool University Hospital

Introduction: The rapid diagnosis of HCV infection is hampered by slow antibody responses and lack of reliable point-of-care tests (POCT). In addition, patients often report preferring blood collection by finger-prick rather than by phlebotomy. The Cepheid GeneXpert platform...
Abstract 101

GeneXpert for Quantitative HCV RNA Detection in Finger-Puncture and Venous Blood Samples

Montoya V1,2, Olmstead A1,2, Tai V3, Chiu C1, Dong W, Grebely J4, Applegate T4, Dore G4, Harrigan R1,5, Joy J1,5, Howe A1

1BC Centre for Excellence in HIV/AIDS, 2BC Centre for Disease Control, 3Department of Biology, University of Western Ontario, 4The Kirby Institute, University of New South Wales, 5Department of Medicine, University of British Columbia

Background: People who inject drugs (PWIDs) are likely to have multiple exposures to HCV as a result of on-going high-risk behaviors. Repeated exposures can result in infection with multiple virus strains. Given many of the currently approved regimens are genotype and subtype specific, patients infected with multiple HCV strains may respond poorly to treatment. It is estimated that the prevalence of HCV reinfection, and mixed infections ranges from 5-39% and 1–5% per year, respectively, depending upon the study population. How these types of infections influence key aspects of HCV pathobiology such as HCV evolutionary rate, immunologic escape, and resistance to direct acting antiviral therapies is unknown. Complicating the matter further,
criteria used to identify mixed infections have not yet been established.

**Methods:** NGS was performed in artificially mixed HCV genotype samples (Gt 1a/1b, 1a/2, 1a/3, 1a/6) in ratios of 10:90, 50:50, 90:10 and 95:5. HCV sequencing libraries were prepared either using a random priming (RP) approach that non-selectively amplifies sequences in a sample, or using target-capture probes (TCPs) from Illumina to enrich HCV genetic material following RP amplification. Percent coverage and the average depth for the NS3, NS5A, and NS5B regions were quantified and used as criteria to identify mixed infections. Accuracy assessment for mixed infection classification using percent coverage and average depth was performed with receiver operating characteristic (ROC) curves. We subsequently applied our criteria to HCV infected plasma samples obtained from two cohorts of PWID: VIDUS (n = 79), and ACTIVATE/DARE-CII (n = 140).

**Results:** The RP method yielded an average 2.2x10⁵ HCV reads/sample (16% of total reads; average read depth 5.50 x10³) while the TCP approach yielded 1.1x10⁶ HCV reads/sample (66% of total reads; average read depth 4.0x10⁴). On average the read depth was 4.7 fold-greater for both the most abundant and less abundant HCV genotypes in a sample using TCP compared to RP. Optimal genotype classification was observed when at least a coverage of 90% and a minimum average depth of 2% was reached for each of the genotype-specific NS3, NS5A, and NS5B regions. These criteria correctly classified experimentally mixed samples in all cases except for the 98/2% admixtures, where in each case one out of three regions was found to be below the designated thresholds. When applied to the ACTIVATE/DARE-CII cohorts, four samples (2.8%) with potential mixed infections were identified, whereas in the VIDUS cohort 10 samples were identified (12.7%).

**Conclusions:** Random priming NGS assay provides an unbiased, agnostic approach to evaluate potential mixed infection. Addition of the target capture probes can further enhance sensitivity. Our criteria successfully classified minor genotypes when present in at least 2% of the viral population.

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**Abstract 110**

**Distribution of different HBV DNA forms in plasma and PBMCs of chronically infected patients**

**Vicenti I¹, Rossetti B²,³, Mariano S², Zazzi M¹, De Luca A²**

¹Medical Biotechnology Department, University of Siena, ²Infectious Diseases Unit, Azienda Ospedaliera Universitaria Senese, ³Clinic of Infectious Diseases, Catholic University of Sacred Heart

**Background:** The molecular basis of Hepatitis B virus (HBV) DNA persistence and its pathogenic role in extra-hepatic sites still remain unclear. Peripheral blood mononuclear cells (PBMCs) could play a role as reservoir and facilitate HBV escape from immune surveillance. However, findings in this field are heterogeneous, due to different patient populations analysed and methods used. The aim of this study was to evaluate the distribution of total (tDNA) and covalently closed circular (cccDNA) HBV DNA in PBMCs of low viremic patients, as a possible HBV reservoir.

**Materials and methods:** We developed real-time PCR methods for differential amplification of the cccDNA and tDNA. Briefly, cccDNA was preferentially amplified with specific primers flanking the gap region and the incomplete strand of HBV DNA while tDNA primers detected all forms of HBV DNA, including relaxed circular forms and cccDNA. The specificity of the method was increased by treatment with plasmid safe DNase and the Beta-Globin gene was targeted to estimate the number of cells in each PCR reaction, where 500 ng of spectrophotometrically measured DNA were used.

**Results:** Thirty-seven patients with low or undetectable HBV plasma viremia were recruited: 73% Caucasian, 70% males, 68% with genotype D, 54% treated with nucleos(t)ide analogues, 43% inactive carriers, 3% HBsAg-negative but HBeAg-positive. Median (IQR) age and years from HBV infection diagnosis were 48.4 (37.8-60.5) and 7.3 years (4.9-13.8), respectively. Overall, 4 patients were HBeAg-positive, 33
HBeAb-positive; 2 were HIV-1 coinfected. Plasma HBV DNA quantified with the certified Abbott RealTime HBV Viral Load Assay was undetectable in 15 samples, detectable but below the limit of detection in 5 and above 10 IU/ml in 17; median HBV DNA levels in viremic patients was 336 IU/ml (IQR 69-793). The in-house tDNA assay was highly sensitive (detection limit 10 IU/ml in plasma and 60 copies per 10⁶ cells in PBMC), linear over a 6-log range and with excellent inter and intra-assay reproducibility. Plasma tDNA levels measured by the Abbott reference system and the in-house assay correlated well (r = 0.81; P<0.0001), with mean difference + 0.20 log10 IU/ml (SD ±0.62) and 6 (16.2%) samples differing of >1 log. There was no apparent association between plasma tDNA levels and HBeAg status or HBV genotype. tDNA was detected in 4 PBMC samples, all from patients with detectable plasma viremia (range 633-6406 IU/ml), and cccDNA was not detected in any PBMC sample despite the high sensitivity of assay (30 copies per 10⁶ cells).

**Conclusions:** A few previously published studies have documented PBMC tDNA and occasionally cccDNA in a proportion of patients with chronic and occult HBV infection. We detected no cccDNA and few cases with tDNA, all in the presence of measurable plasma HBV DNA. The discrepancy with previous literature could be attributable to the high diversification of HBV status: inactive/active carriers or occult infection, plasma viremia levels, HBeAg status, proportion of treated patients. Analysis of a larger and comprehensive HBV population with a standardized method is required to clarify the prevalence and role of PBMC tDNA and cccDNA.
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Abstracts # 58, 90 and 95 were withdrawn from publication after the abstract review.