Abstract Book
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Abstract: O_01

Clinical Challenges in HIV Therapy

Impact of pre-HAART viral load on virological rebound in HIV-1 infected patients starting their first line regimen

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Background: The concept of 'high viral load' (VL) (today arbitrarily set at >100,000 copies/mL) is discussed in most guidelines, as a factor to be considered in both the starting of therapy and the use of some drug regimens. This study aims at characterizing the impact of high pre-HAART VL on virological rebound (VR) after achieving virological suppression (VS) in a large population of HIV-1 infected patients starting their first line HAART.

Material & Methods: HIV-infected individuals enrolled either in the ICONA Foundation Study or in other clinical centers in Italy who started their first line therapy over the period 2000-2011 and who achieved VS (VL<50 copies/mL) after HAART starting were selected on the basis of the following criteria: i) therapy based with ≥3 drugs; ii) age ≥18 years; iii) VL at the time of starting therapy >500 copies/mL. Patients were grouped according to levels of pre-HAART VL (<100K, 100-300K, 300-500K, >500K copies/mL). The association between pre-HAART VL and the risk of VR (defined as the first of two consecutive VL>50 copies/mL and/or two consecutive VL>200 copies/mL, after VS) was evaluated by Kaplan-Meier curves and Cox regression analyses. The following variables were used as potential confounders in multivariable analyses: age, gender, calendar year, risk factor, type and number of drugs administered as part of the first HAART regimen, CD4 at time of VS.

Results: 2569 patients that achieved VS were included. Their median pre-HAART VL was 4.9 (IQR:4.4-5.3)log10 copies/mL; 56%, 25%, 8%, and 11% had values in the ranges of <100K, 100-300K, 300-500K and >500K copies/mL, respectively. Within 24 weeks after starting therapy, the 71%, 57%, 46%, 42% of patients achieved their VS, according to pre-HAART VL of <100K, 100-300K, 300-500K, >500K copies/mL, respectively (p<0.001). Overall, by 96 weeks after achieving VS, the probability of VR was 14% (with VL>50 copies/mL) and 7.8% (with VL>200 copies/mL). After stratifying these Kaplan-Meier estimates by pre-HAART VL groups, the highest 96 week probability of VR (VL>50 copies/mL) was estimated for VL>500K copies/mL (19%, 17%, 15% and 11% for >500K, 300-500K, 100-300K, and <100K copies/mL respectively, p<0.001). By Cox univariable analyses, compared to patients with VL<100K copies/mL, patients with pre-HAART VL>500K copies/mL showed the highest risk of VR (using both the VL>50 copies/mL [RH (95CI%): 1.81 (1.40-2.36), p<0.001] and VL>200 copies/mL [RH (95CI%): 1.68 (1.28-2.20), p<0.001]). Cox multivariable estimates confirmed this result (for VR=VL>50 copies/mL, RH [95CI%]: 1.82 [1.40-2.36], p<0.001); for VR=VL>200 copies/mL, RH [95CI%]: 1.52 [1.10-2.20], p=0.023).

Conclusions: Patients with pre-HAART VL>500K copies/mL are at highest risk of experiencing VR, compared to patients starting HAART with VL<100K copies/mL.

No conflict of interest
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Clinical Challenges in HIV Therapy

Low level viremia and HIV-1 drug resistance in patients with virological rebound after suppression with a first line antiretroviral regimen


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Background: Low-level virological rebounds (LLVR) may be observed in HIV-1 infected patients after achieving virological suppression and they may be associated to virological failure (VF) in the long term. Aim of this study was to evaluate the association of LLVR with appearance of resistance mutations at VF in patients on a first line antiretroviral regimen.

Patients and Methods: Viroimmunological parameters and antiretroviral treatment of patients with a genotypic resistance test (GRT) available after starting a first line regimen based on NNRTI or boosted PI (bPI) and achieving at least one viral load (VL) below 50 copies/mL were retrieved from the ARCA database. The first available GRT after virologic suppression was chosen as a proxy of VF. LLVR were defined as a detectable (>50 copies/mL) VL below 1000 copies/mL. In patients with more than 1 LLVR the highest VL was considered. Univariate and multivariate regression analysis was used to define the predictors of genotypic resistance (any major IAS mutation fall 2012) at VF.

Results: Sequences and data regarding 222 patients were retrieved; 71.8% of patients were male, median (IQR) age was 40 (34-47) years, median baseline VL was 4.87 (3.72-5.36) log10 copies/mL, median baseline CD4 cell count 190 (71-3615) cells/μL. First-line therapy was ritonavir bPI-based for 55.9% and NNRTI-based for 44.1% of patients; the NRTI backbone was 3(F)TC/TDF for 35.1% of patients, d4T+ddI for 2.3%, d4T+3TC 11.7% and other for 7.2% of patients. No LLVR was detected in 45.9% of patients, 13.1% showed a single LLVR between 50 and 100 copies/mL (group A), 34.2% between 101 and 500 copies/mL (group B) and 6.8% between 501 to 1000 copies/mL (group C). Median VL at LLVR was 2.3 (1.9-2.6) log10 copies/mL, median VL at failure 3.7 (3.4-4.4) log10 copies/mL. At multivariate analysis, the detection of any resistance mutation (38.3%) was predicted by LLVR group B (OR versus no LLVR 2.92, 95%CI 1.47-5.80, p=0.002) and C (OR 16.9, 3.87-74.31, p<0.001) and use of NNRTI-based therapy (OR 2.11, 1.13-3.94, p=0.020) after adjusting for type of NRTI backbone and calendar year of sequencing. The detection of any NNRTI resistance mutation (22.5%) was positively predicted by the occurrence of a “group C” LLVR (OR versus no LLVR 2.92, 1.53-48.25, p=0.005), use of EFV (9.47, 3.23-27.78, p<0.001) or NVP (9.47, 3.23-27.78, p<0.001) and associated with the detection of the NRTI mutations K70R (58.3, 1.80-1883.60, p=0.022) or M184V (10.3, 3.58-29.60, p<0.001), after adjusting for calendar year of sequencing and NRTI backbone. The detection of any major PI resistance mutation (10.8%) was positively associated with the presence of the NRTI mutations K65R (OR 9.23, 0.97-88.09, p=0.05), T215Y (OR 16.34, 1.74-153.02, p=0.014) or M184V (OR 4.46, 1.18-16.86, p=0.028) after adjusting for LLVR category, year of sequencing, NRTI backbone and use of bIDV.

Conclusions: LLVR are associated with an increased risk of resistance, particularly to NNRTI and when NNRTI-based therapies are employed, independently from the type of backbone, suggesting the necessity for a closer virological monitoring with these regimens.
Abstract: O_03

Clinical Challenges in HIV Therapy

Viral and host factors associated with persistent low-level viremia under ART.

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Background: Since the introduction of HIV-1 viral load assays with a lower quantification limit of 20 copies/ml, some patients consistently show low but detectable viremia under antiretroviral therapy (ART). The causes and clinical consequences of persistent low-level viremia (PLLV) are still unknown. The aim of this study was to identify host and viral factors associated with PLLV in HIV patients on ART.

Material & Methods: Two patient groups were compared, 58 PLLV patients with viral loads between 20 and 250 c/ml for half of minimum 6 consecutive measurements collected at least 6 months after starting ART, and 109 control patients with uninterrupted viral load results of <20 c/ml over a period of at least 29 months starting 6 months after ART initiation. Host and viral characteristics were collected from the patient records. Quantification of total HIV DNA was done using real-time PCR, presence of a defective CCR5 allele was defined using PCR and co-receptor tropism was determined after V3 sequencing and geno2pheno prediction. Parameters were compared using Chi² tests and Mann-Whitney U nonparametric tests in SPSS (v.20). Significance was set at p < 0.05.

Results: Plasma HIV viral RNA load and cellular HIV DNA load at the time of initiation of ART were significantly higher in PLLV patients compared to control patients (mean log 5.1 RNA copies/ml vs. 4.6 c/ml; p < 0.001 and mean 3672 copies/10⁶ PBMC vs. 798 c/10⁶ PBMC; p < 0.001). Other characteristics that differed between the PLLV and control group were the higher representation of men (88.4% vs. 68.6%; p = 0.003), the shorter treatment period (59 months vs. 70 months; p = 0.006), the higher representation of subtype B infections (73.9% vs. 57.8%; p = 0.031) and the higher number of PI-based regimens (77.3% vs. 61.9%; p = 0.038). The CD4 count at ART initiation or the CD4 nadir did not differ between both groups (mean 241 cells/mm³ for the PLLV group vs. 262 cells/mm³ for the control group; p = 0.428 and mean 193 cells/mm³ vs. 211 cells/mm³; p = 0.263, respectively). Also no influence of the patients’ origin, age, CCR5-genotype or of the virus co-receptor tropism, was observed.

Conclusions: Viral persistence under ART was strongly associated with a higher pre-ART viral RNA load and a higher pre-ART viral DNA load. This finding, combined with the observation of a lack of association between PLLV and the CD4 count at ART initiation or the CD4 nadir, led us to hypothesise that PLLV does not result from a more compromised immune function at the time that the ART is started, but may be due to the presence of virus with higher replicative capacity. Evidence is also given for the hypothesis that patients with higher viral RNA and DNA load may need longer time to achieve full viral suppression.

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Clinical Challenges in HIV Therapy

HIV-1 RNA and HIV-1 DNA persistence during long-term suppressive ART

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Background: Whether HIV continues to replicate during apparently successful ART remains controversial. Growing evidence indicates that plasma HIV-1 RNA and cellular HIV-1 DNA remain detectable long-term in treated patients but the cause-effect relationship between these two viral parameters is unclear. This study aimed to investigate residual plasma HIV-1 RNA detection and cellular HIV-1 DNA burden in patients receiving first-line ART and showing a plasma HIV-1 RNA load (VL) persistently <50copies/ml.

Methods: Eligible patients started first-line ART with either efavirenz or nevirapine (no change allowed) plus two NRTIs, achieved a VL <50 copies/ml within 6 months, and during subsequent follow-up showed all VL results <50 copies/ml (≥2 measurements per year) without blips or treatment interruptions. Patients were recruited into 10 groups according to ART duration (1 to ≥10 years). Plasma HIV-1 RNA and cellular HIV-1 DNA levels were measured by real-time PCR; the assays 50% and 95% detection thresholds were 1 and 3 HIV-1 RNA copies/ml and 20 and 40 HIV-1 DNA copies/10⁶ PBMC, respectively.

Results: The study recruited 104 adults (median age 47 years; range 27-76) equally distributed across the 10 ART-duration strata, with a median pre-ART VL of 4.9 log₁₀ copies/ml (range 2.8-6.9) and nadir CD4 count of 201 cells/mm³ (range 3-800); 81/104 (78%) patients were male, 59/104 (57%) white, and 53/104 (51%) MSM. Patients started ART in 1997-2011, most commonly with efavirenz (87/104, 84%). Considering all groups combined, plasma HIV-1 RNA was detected in 52/104 (50%) patients at a median level of 4 copies/ml (range 1-35; IQR 2, 7). Cellular HIV-1 DNA was detected in 102/104 (98%) patients at a median level of 2.5 log₁₀ copies/10⁶ PBMC (range 0.9-3.5). Over 10 years there was a mean HIV-1 RNA decrease of -0.62 log₁₀ copies/ml (95% CI: -1.37, 0.12; p=0.10) and a mean HIV-1 DNA decrease of -0.22 log₁₀ copies/10⁶ PBMC (95% CI: -0.63, 0.19; p=0.283).

Conclusions: Plasma HIV-1 RNA and cellular HIV-1 DNA remain detectable in a large number of patients receiving apparently successful ART. There was a trend suggestive of reduced HIV-1 RNA detection in patients with the longest duration of suppressive ART. The source and significance of residual HIV-1 RNA detection during ART warrant further studies.

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Transmission & Evolution of Drug Resistance

Impact of transmitted resistance to antiretroviral drugs on predicted HIV-1 susceptibility to single-tablet regimens (STR)

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Background: STR combine a full antiretroviral regimen in one tablet taken once daily potentially improving adherence, treatment satisfaction and virologic outcome. Two STR based on non-nucleoside reverse transcriptase inhibitors (NNRTI -STR) are currently licensed in Europe, while two STR based on integrase inhibitors (INI-STR) will be available soon.

Patients & Methods: We analyzed treatment-naive adult patients diagnosed and genotyped from 2008 to 2012 with at least a low-level resistance (Stanford 6.2.0 algorithm) to one component included in STR (tenofovir [TDF] + emtricitabine [FTC] + efavirenz [EFV] + PIs; TDF + FTC + rilpivirine [RPV]; TDF + FTC + cobicistat / elvitegravir [EVG], abacavir [ABC] + lamivudine [3TC] + delavirdine [DTG]). Resistance to DTG was conservatively defined as the presence of Q148H/K/R. Cases were selected from the Italian ARCA database when an HIV-RNA measured ≤12 weeks before reverse transcriptase and protease genotyping was available. Contemporary integrase (IN) genotype was included when available. Differences in the prevalence of resistance to the different STRs were assessed by chi-squared test. Logistic regression was employed to detect predictors of resistance to TDF/FTC/EFV and TDF/FTC/RPV.

Results: We included 1850 cases, with IN genotype available for a subset of 174. 69% carried a B subtype, 71.9% were males, 31.7% heterosexuals, 22.9% MSM, 8.1% IDU, median age was 39 years (IQR 31-47), CD4 221 cell/μl (9-424), HIV RNA 4.8 log10 cp/ml (4.2-5.3), 48.1%, 37.8%, 14.0% of cases were from North, Centre and South Italy, respectively. The prevalence of any TDR mutation (Bennet 2009) was 8.9% (NNRTI 4.4%, NNRTI 4.6%, PI 1.5%). The most frequent NRTI mutations were A62G (29.2%), 184IV (20.8%), 210W (17%) and 215FY (11.3%), for NNRTI 103N (47.2%), 101E (12.2%), 106AM (6.5%). Only 1 of 174 (0.6%) sequences carried resistance to INI. At least low-level resistance was predicted for TDF/FTC/EFV in 7.5%, for TDF/FTC/RPV in 4.6% (p<0.001 vs. TDF/FTC/EFV), for TDF/FTC/EVG in 4.0% (p=0.08) and for ABC/3TC/DTG in 4.0% (p=0.08). After adjusting for risk factor, gender, baseline viremia, calendar year of genotyping and geographic area, independent predictors of at least low-level resistance for TDF/FTC/EFV were age (p=0.01, +10 years OR 1.28, 95% CI 1.06-1.56) and viral subtype (non-B vs. B, p=0.001 OR 0.30, 0.15-0.60); the same factors predicted low-level resistance to TDF/FTC/RPV (age p<0.01, +10 years OR 1.36, IC 95% 1.09-1.71; subtype non-B vs. B p<0.001, OR 0.12, IC 95% 0.03-0.38).

Conclusions: TDR may limit the use of STR in a small proportion of cases in Italy. TDF/FTC/EFV seems more affected by TDR as compared to the other STRs. Non-B subtype carriers and younger patients seem less affected by TDR to STR. Primary resistance to INI remains uncommon.

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Abstract: O_06


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Aim: To compare two different approaches to evaluate TDR in CoRIS, a cohort with wide territorial representation in Spain, from 2007-2011.

Methods: CoRIS is an open, prospective, multicenter cohort of adult patients with confirmed HIV infection and naïve to antiretroviral treatment at entry, established in January 2004. By the end of 2011, 23 sites from 11 of the 17 regions conforming Spain provided fasta sequence collection together with epidemiological data. TDR associated mutations were evaluated following two different approaches: first the WHO surveillance drug resistance mutation list updated in 2009; and second, the current version of the Stanford HIVdb resistance algorithm (STAN) drug resistance to first line drugs (Tenofovir-TDF, Abacavir-ABC, Lamivudine-3TC, Emtricitabine-FTC, Efavirenz-EFV, Nevirapine-NVP, Lopinavir-LPV, Atazanavir-ATZ, & Darunavir-DRV). Low-level resistance, intermediate resistance or high-level resistance were all pooled into resistant for calculations. Prevalence and temporal trend of TDR were estimated globally and for each antiretroviral (ARV) class. Resistance to first line regimens (February 2012, Spanish Guidelines) was also evaluated following STAN.

Results: Overall, 2827 subjects were studied. Using STAN the number of patients that showed any relevant resistance was 188 [prevalence of 6.8% (95%CI 5.8-7.7)], compared to the 221 patients that showed any mutation from the WHO list [prevalence of 7.9% (95%CI 6.9-9.0)]. Prevalence of resistance to first line ARVs (STAN) was also lower than WHO TDR for NRTIs [2.3% (95%CI 1.8-2.9) vs 3.6% (95%CI: 2.9-4.3)] and PIs [0.8% (95%CI 0.4-1.1) vs 1.7% (95%CI: 1.2-2.2)], while it increased in the case of NNRTIs [4.6% (95% 3.8-5.3) vs 3.7 (95%CI: 3.0-4.7)]. A significant decrease in resistance to first line ARVs (STAN) was found from 2007 (8.1%) to 2011 (4.7%), explained by a borderline statistical significance decrease in both NRTIs (from 2.3% in 2007 to 1.7% in 2011) and NNRTIs (from 5.4% to 2.8%). Using WHO, no significant trend in TDR was found over time, although TDR to NNRTIs decreased from 5.2% in 2007 to 2.8% in 2011. Any resistance (STAN Genotypic Sensitivity Score-GSS- <3) to first line regimens decreased from 5.6%-5.7% (EFV based) and 6.1%-6.2% (NVP based) for NNRTI based regimens, to a range of 2.2% to 2.7% for PI based regimens. When the STAN GSS threshold was lowered to <2.5, again PI based regimens were less affected (0.7-0.9%) than NNRTI containing regimens (3.4% to 3.9%).

Conclusions: Evaluation of TDR using the WHO surveillance list results in lower NNRTI resistance but higher PI resistance than the STAN algorithm. While results by WHO list are very relevant to evaluate the emergence of resistant strains in Spain, STAN results are more informative for recommendations and decision making in the clinical practice. Baseline resistance to NNRTI containing regimens remains a problem of concern in Spain, through the period 2007-2011. Resistance to first line PI containing regimens is less frequent, and very rare if a GSS <2.5 is considered. Testing naïve patients for protease resistance may not be cost-effective in our setting.

No conflict of interest
Abstract: O_07

Transmission & Evolution of Drug Resistance

Inter-country mixing in HIV transmission clusters: a pan-European phylodynamic study

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Background: Tracking HIV-1 transmission patterns on an epidemic scale is of increasing social relevance as the WHO reports a steady level in the incidence of newly diagnosed infections. Sequence data from fast evolving pathogens allows the reconstruction of inter-patient transmission linkage and the study of epidemic dynamics. Recent individual country studies have shed light on the epidemic dynamics and the extent of spatial segregation present in transmission clusters. To our knowledge, the present work is the largest pan-European study to date which aims to quantify the extent of geographical segregation within transmission clusters and to compare these trends to country-specific studies.

Materials and Methods: Study data comprises 21844 HIV-1 subtype B pol gene sequences collected by the EuResist consortium. Sequences are sub-typed using COMET and aligned using ClustalW against an HIV-1 HXB2 reference alignment taken from the Los Alamos database. Nucleotide positions under immune and antiviral drug selective pressure are removed resulting in 705 nt long sequences. A transmission graph is built by thresholding evolutionary divergence as estimated by the log-det. The log-det threshold which captures true transmission edges while minimizing false edges is determined based on graph coagulation. Phylogenetic tree reconstruction is done using FastTree with the GTR + gamma model of nucleotide substitution. A majority rule consensus tree is constructed from 1000 bootstrap replicates. Transmission clusters are defined as clades with a bootstrap support > 90% and a minimum size of 10. Graph analysis has been performed using the R package igraph and tree analysis using the R and python packages ape and DendroPy.

Results: 5514 sequences (25%) are linked to at least one other sequence in the transmission graph constructed at the log-det threshold. Inter-country mixing in this graph is very low at stricter thresholds as given by high assortativity coefficients of 0.89±0.012. In the phylogenetic tree built using these log-det selected sequences, 4775 sequences show a strong phylogenetic support to at least one other sequence (BS >90%). The clade size distribution is right-tailed with 794 clades of size 2, 429 between 3 and 9 and 78 clades of size 10 or more. Within transmission clusters, most sequences come from one country (median= 90%, IQR= 77%-100%), indicating high country-wise segregation. Within cluster transmission-wise segregation is also high (median= 88%, IQR= 71%-100%). Missing information (25% for country, 43% for transmission) adds some uncertainty to these Results:

Conclusions: High country-wise assortativity in the transmission graph and low country-wise mixing within transmission clusters are indicative of low inter-country transmission. Geographic segregation seems to be a general trend in HIV transmission with recent studies focusing on Germany, Italy and Switzerland also reporting high region wise assortativity.

No conflict of interest
**Abstract: O_08**

**Transmission & Evolution of Drug Resistance**

Large scale ancestral route reconstruction shows a multiplicity of single point introductions and spread within male clusters in Luxembourg.

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**Background:** Large scale phylogenetic analyses using powerful bioinformatic tools can provide new insights in the dynamics of epidemic infectious diseases. Here we investigated the dynamics of HIV infection in Luxembourg by determining its geographical origin, route of transmission, of entry into the country and expansion of foci within Luxembourg among different risk groups.

**Material & Methods:** A codon corrected multiple alignment was generated from 31430 HIV-1 prot-RT sequences from the LANL database and 601 sequences from the Luxembourg (lux.) cohort (minimum length >1245 bp). The lux. cohort is composed of 159 female and 442 male patients. The contamination routes were 277 heterosexual (46.09%), 241 MSM (40.1%), 62 IVDU (10.32%), 11 unknown (1.83%) and 10 other (1.66%). Sequences from the lux cohort were labeled with the contamination route. Sequences from the LANL database were labeled 'external'. A first tree including all Luxembourg and LANL sequences was generated with FastTree. The lux. cluster distribution was analyzed with a custom script using the biopython library. In a second analysis, 100 bootstrapped phylogenetic trees were inferred from the initial alignment with FastTree. The ancestral states, i.e. the contamination routes, were reconstructed with Mesquite. The trees mapping the ancestral routes were exported and analyzed with in-house scripts. Transitions are inter-group infections within the lux. cohort.

**Results:** The cluster analysis showed that the majority of infections are single point introductions (343 out of 601, 57.07%) that do not spread further within the lux. cohort. Clusters of more than 5 patients were: 1) one cluster of 37 patients infected with CRF42_BF through heterosexual and homosexual contact, 2) 8 clusters of mainly men, 7 including only MSMs and 1 including MSM and heterosexuals, 3) one cluster of IVDUs (9 patients) and 2 small heterosexual clusters. In 39 cases (13.64%) the virus spreaded within likely couples (cluster size of 2) (23 male/female, 16 MSM). Reconstruction of the ancestral route showed that the HIV epidemic in Luxembourg is impacted by substantial external inputs: 362 (average: 100 bootstrap values, standard deviation (std) 4.61, 60.17%) new infections were derived from an 'external' ancestor vs. 239 (average: std 4.61, 39.83%) from internal routes. Most infections with an 'external' ancestor occurred through heterosexual contact, immediately followed by homosexual contact and then by IVDU. When the analysis was restricted to patients born in Luxembourg only, the distribution shifted to 97.29 (std: 2.81, 52.59%) external vs. 87.71 'internal' routes (std: 2.81, 47.41%). The average number of transitions between 'internal' transmissions was 9.92% for the whole cohort and were mainly from MSM to heterosexual and from heterosexuals to MSMs.

**Conclusions:** Large scale ancestral reconstruction of the contamination routes using viral sequences showed that the lux. cohort is mainly driven by single-point external introductions through heterosexual and homosexual contacts leading to a dead-end. Only few large clusters exist in the lux. cohort, due mainly to homosexual transmissions. This study shows that fine-grained analyses of infectious diseases are possible and can be exploited to target prevention strategies.

No conflict of interest
Transmission & Evolution of Drug Resistance

HIV-1 V3 diversity can be increased by Apobec3-editing, predominantly at residues involved in co receptor usage and immunity: a refined UDPS analysis

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Abstract: O_09

Background: Cellular Apobec 3G (A3G) restriction factor is responsible for the majority of G-to-A mutations in HIV DNA. For this reason it may increase HIV genetic diversity and facilitate drug resistance emergence. Here, we investigate A3G contribution in modulating the extent of genetic variability in the env V3 loop critical for HIV-1 co-receptor usage.

Methods: The rate of A3G-induced GG-to-AG and GA-to-AA mutations was assessed both in vivo and in vitro. For the in vivo analysis, we defined the rate of A3G-induced GG-to-AG and GA-to-AA mutations in 238 HIV B subtype V3 Bulk proviral sequences from PBMCs. The analysis was also confirmed in 2,920 HIV-1 B V3 Bulk sequences obtained from plasma. B subtype V3 consensus sequence was used as reference. For the in vitro experiment, the rate of GG-to-AG and GA-to-AA V3 mutations was assessed by using NL4-3 wt and NL4-3 Vif K22H mutant, known to have a suboptimal activity against A3G (NL4-3 K22H) to infect both MT-2 cells (A3G⁺) and Sup-T1 cells (A3G⁻). After 2 rounds of infection, the frequency of V3 proviral mutations in HIV quasi-species recovered from MT2 and SupT1 cells was analyzed by ultra-deep sequencing (UDPS) (GS Junior). After Shorah correction, only mutations detected in both forward and reverse primers in >5 reads were considered.

Results: By analyzing the V3 proviral sequences, 38.6% (92/238) of samples showed at least 1 GG-to-AG or GA-to-AA mutation (median number[1QR]:2[1-2]). These mutations occur at 10 V3 positions (prevalence range: 0.4%-14.6%), and include the mutation G24E (GGA-to-GAA), associated with a preferential use of CCR5-coreceptor, and the mutations G24R (GGA-to-AGG) and E25K (GAA-to-AAA), associated with a CXCR4-usage. In V3 Bulk plasma sequences, their prevalence ranges from 0.2% to 22%. In in vitro experiments, no GG-to-AG and GA-to-AA mutation is detected in Sup-T1 cells (A3G⁻) infected with either NL4-3 wt or NL4-3 K22H (0/15,133 and 0/19,652 UDPS reads). Only 1 mutation (prevalence, 0.04%[6/28,656]) is detected in MT-2 cells (A3G⁺) infected with NL4-3 wt. A different situation is observed in MT-2 cells (A3G⁺) infected with NL4-3 K22H in which 6 GG-to-AG or GA-to-AA mutations are identified (prevalence range: 0.1%[33/25,743]-5.0%[1,296/25,780]). Among these, 5 V3 mutations result non-synonymous. They include G26E/R (corresponding to G24E/R in B subtype V3 consensus sequence), correlated with CCR5/CXCR4 usage, and G19R (corresponding to G17R) localized in the tip of V3 loop. The acquisition of positive charge in this region is known to modulate HIV recognition by neutralizing antibodies.

Conclusions: By analyzing V3 proviral sequences from PBMCs and by in vitro experiments, we demonstrated that Apobec3 can drive editing in V3 region and promotes the acquisition of positively or negatively amino acids at positions involved in co receptor usage and HIV sensitivity to humoral immunity. This can have implications in favoring HIV escape from CCR5 antagonists and immunity.

No conflict of interest
Abstract: O_10

Clinical Challenges in HCV Therapy

Early detection and persistence of resistance in HCV patients treated with BOC/TPV-based therapy: analysis by population- and ultra-deep sequencing

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Background: The utility of performing a baseline genotypic resistance test in chronic HCV infected patients treated with triple therapy including the protease inhibitors (PIs) boceprevir or telaprevir in clinical practice is still discussed. The aim of this study was to analyze the selection, emergence and persistence of NS3-protease resistance-associated-variants (RAVs) at baseline and during triple therapy.

Methods: NS3-protease sequences of 26 patients treated with boceprevir or telaprevir + pegIFN/ribavirin were analyzed by population-sequencing at baseline, at early time-points (6h-24h-48h-1week-2weeks-4weeks) and, afterwards, every 4 weeks. Ultra-deep-454-Pyrosequencing (UDPS) was performed in all patients at baseline and during treatment in a subgroup of patients. HCV-RNA was assessed (detection-limit= 12/15 IU/ml) at all time-points.

Results: Eight out of 26 patients failed triple-therapy (boceprevir=5, telaprevir=3), all with resistance associated variants (RAVs) at failure. Failing-patients were infected with HCV-1a (N=4) and HCV-1b (N=4). All of them were treatment-experienced: 1 breakthrough, 2 relapers and 5 non-responders. At baseline, 2 out of 26 patients (7%) presented linear-PI RAVs detected by both population-sequencing and UDPS (V36L=100% [3278/3278-reads] in HCV-1a; T54S=100% [4352/4352-reads] in HCV-1b infected patients). The patient with V36L experienced viral-failure within 4-weeks of telaprevir-based therapy, with the additional emergence of R155M. Differently, the patient with baseline T54S experienced a rapid virological response. He is still under triple-therapy. Among the other 24 patients without linear-Pis-RAVs at baseline, 7 (29.2%) failed triple-therapy and 17 (70.8%) achieved HCV undetectability (median[IQR] of follow-up: 12[8-24] weeks). Regarding telaprevir-failures, one patient showed, at 48h, early emergence of V36A and R155K as minor-species by UDPS (V36A=2.5% [88/3504-reads]; R155K=4.1% [136/3291-reads]). At 18-weeks, V36M (100% [3115/3115-reads]) and R155K (99.9% [4792/4793-reads]) were detected as major quasispecies (without the presence of V36A). The same resistance pattern (V36M+R155K) was detected at failure in another HCV-1a patient. In both cases, these mutations remained detectable, by both population-sequencing and UDPS, up to 20 weeks after telaprevir-discontinuation (end of observation). Among the 4 boceprevir-failing HCV-1b infected patients, two relapsed with T54A (UDPS prevalence range: 30.9-33.1%) plus V170A (prevalence range: 46.3-94.9%). Another patient experiencing virological breakthrough at 21-weeks, showed at failure the presence of many RAVs T54A (0.8%, [24/3051-reads]), T54S (0.8% [25/3051-reads]), V55A (3.8% [116/3051-reads]), A156T (6.5% [71/1085-reads]) and V170A (34.2% [355/1037-reads]). Lastly, a non-responder patient showed after 4w of boceprevir-administration T54A RAV (38.9% [468/1204-reads]). In this patient, T54A prevalence increased up to 100% (3252/3252 reads) at week-8, in association with a 0.9log HCV-RNA increase. Eight weeks after boceprevir-interruption, the T54A was no longer detected at population-sequencing, but at UDPS was detectable a minority T54S variant (16.2% [155/959-reads]). In the only HCV-1a boceprevir non-responder patient, the typical V36M (91.7% [5069/5530-reads]) plus R155K
(74.5% [2096/2814-reads]) resistance-pattern was observed with the development of RAVs as major quasispecies. In addition, V36A (3.7% [205/5530-reads]) and R155T (25.1% [707/2814-reads]) were observed as minority variants.

Conclusions: In these cases, early detection of RAVs, either at baseline or at 48h, was associated with PI-failure in PI-treated patients. The clinical relevance of early resistance testing, as well as the mutations persistence after therapies discontinuation, deserve further analyses.

No conflict of interest

Abstract: O_11

Clinical Challenges in HCV Therapy

Prevalence of drug-resistant HBV in antiviral therapy experienced patients in Europe: Reports from the CAPRE study.

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Introduction: Drug resistance to nucleos(t)ide analogues (NUCs) in chronic hepatitis B (CHB) has important clinical consequences. However, Europe-wide data on the prevalence of resistance in clinical settings are lacking. We present results on the prevalence of drug resistance in NUC-treated patients in the framework of the CAPRE study (Combined Analysis of the Prevalence of drug-Resistant HBV in antiviral therapy Experienced patients).

Methods: We performed a multi-centre retrospective survey on genotypic resistance test results performed during routine clinical assessments of CHB patients from 21 centers in 17 European countries. Clinical data and genotypic resistance patterns from HBV-DNA-positive NUC-experienced patients, analyzed using Sanger sequencing or INNO-LiPA were included. Mutations rtA181T/V, rtA194T, rtM204V/I/S, rtN236T, rtM250V were interpreted as primary resistance mutations, while rtL80V/I, rtI169T, rtV173L, rtL180M, rtT184G, rtS202I were considered secondary mutations. Univariate statistical analyses were performed using t-tests or χ²-tests. Multivariate logistic regression was performed on variables of interest.

Results: 2,379 samples from 1,640 European patients were analyzed. 70.1% of patients were male, mean age was 46.5 years. 11.1% harbored a co-infection with HCV or HIV. Median HBV viral load was 4.5E³-8.0E⁵ [IQR: 1.6E³-8.0E⁵], median serum-ALT 51 IU/ml [IQR: 33-96.75]. At least 1 primary resistance mutation was detected in 50.8% [833/1640]. Of these, 6.7% concern ≥2 primary mutations. The mutation rate differed significantly (p<0.001) between samples analyzed using Sanger (44.3%, [415/936]) and INNO-LiPA (59.1% [416/704]). In patients harboring resistance mutations, rtM204 was observed in 89%, followed by rtA181 (6.2%), rtN236 (4.8%), rtM250 (3.8%) and rtA194 (0.5%). Secondary mutations concerned rtL180 (57.5%), rtL80 (21.2%), rtV173 (10.8%), rtB184 (5.8%), rt202 (1.9%) and rt169 (0.7%). Rates of drug resistance were highest in patients exposed to TBV (72% [13/18]), followed by LAM (56.9% [679/1194]), ETV (41.6% [47/113]), ADV (13.7% [30/219]), TDF (1.7% [1/59]). Interestingly, ETV resistance was noted in (14% [8/57]) of patients using ETV without prior exposure to TBV/LAM. All ETV-resistant isolates harbored the L180M±M204V/I/S LAM resistance signature. The A194V/T mutation associated with resistance to TDF was seen in 4 cases. Three were TDF naïve and LAM experienced, carrying associated resistant mutations (L180M+204V/I/S±M250V). In one case, the mutation was solitary, and TDF had been reportedly used as first-line therapy. In univariate analysis, factors associated with resistance were: age (p<0.001), LAM (p<0.001) or TBV (p<0.05) exposure, genotype D (p<0.05) and use of INNO-LiPA (p<0.001). TDF and ADV exposure (p<0.001), and genotype B, C (p<0.05) and E (p<0.001) were negatively associated with resistance. In multivariate analysis, risk factors were genotype A and D (p<0.001), previous LAM use (p<0.05) and use of INNO-LiPA (p<0.001).

Conclusions: In this largest-to-date European clinical cohort of 1640 NUC-experienced
patients, drug resistance is observed in half of cases. This high prevalence is largely due to LAM use. Frequent cross-resistance for second-line ETV also indicates prolonged LAM use. Prevalence of resistance varies between different genotypic tests. This can be explained by differences in sensitivity or by different rates of LAM exposure over time. High prevalence of resistance supports the use of genotypic testing in selecting the most effective anti-HBV drug for second-line therapy.

No conflict of interest

Abstract: O_12

HCV Drug Resistance

Characterization of two distinct HCV genotype 1a clades: association with primary resistance mutations to HCV protease inhibitor

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Background: HCV genotype 1a is more prone to resistance to NS3 protease inhibitors (NS3i) and reduced response to triple HCV therapy regimens including NS3i as compared with 1b has been observed. We previously described segregation of HCV genotype 1a in two distinct clades based on sequencing of the NS3 region. We aimed at further characterizing this phylogenetical segregation and testing its association with epidemiological factors and with natural resistance to linear and macrocyclic NS3i.

Patients and Methods: Plasma samples were obtained between 2003 and 2012 from NS3i-nàïve patients chronically infected with HCV genotype 1a and followed at 6 sites throughout Italy. RNA was prepared by spin column or automated extraction. The whole NS3 region or a shorter region including the first 181 aminoacids (i.e. the protease domain) were amplified by reverse transcription nested PCR and sequenced. Linear and macrocyclic NS3i resistance mutations were retrieved from reference lists [HCV Phenotype Working Group, HCV Drug Development Advisory Group, 2012; Wyles 2012] and used to build a prototype 5-level genotypic susceptibility scoring (GSS) system ranging from 1 (maximal activity) to 0 (no activity). HCV genotype was confirmed using the REGA HCV Subtyping Tool. Maximum likelihood (ML) phylogenies were employed. The best-fitting nucleotide substitution model was tested with a hierarchical likelihood ratio test and ML phylogenies were then re-estimated with the selected model, using a neighbour-joining tree as starting tree, and the TBR algorithm for branch swapping. Robustness and reliability of the trees were confirmed with a 1000-replicate bootstrap analysis. The tree was rooted by midpoint rooting. Calculations were performed with PAUP* 4.

Results: A total of 131 patients were examined: 72% were males, 77% HIV co-infected; transmission groups were 70% IDU, 18% sexual, 4% blood products, 8% unknown; median (IQR) age was 47 years (43-50), time from HCV diagnosis 16 years (10-19), HCV RNA levels 6.14 log10 IU/mL (5.45-6.69). Phylogenetic relationships among the different HCV1a isolates were supported by bootstrap values >70%. Two main clades were confirmed: 58 sequences (44%) segregated in clade “1” and 68 (52%) in clade “2”, while 5 could not be assigned. Within each clade, statistically supported clusters and subclusters were identified. Gender, age, transmission group, HIV status, geographical region, HCV RNA levels and time from HCV diagnosis were not associated with clade classification. Patients from the same site tended to be more often associated in clusters and subclusters. The distribution of NS3i resistance mutations was significantly different. Clade “1” showed 80K in 53.4%, 36L in 3.4% and 54S, 122G, 155Q, 156S each in 1.7%,
resulting in simeprevir GSS<=0.5 in 53.4% and boceprevir/telaprevir GSS<=0.75 in 3.4%. Clade “2” showed 122G in 36.8%, 36L, 55A and 168E each in 4.4%, 54S in 2.9%, 80K, 132V and 170T each in 1.5%, resulting in simeprevir GSS<=0.5 in 4.4%, boceprevir GSS<=0.75 in 8.8% and telaprevir GSS<=0.75 in 4.4%.

Conclusions: The presence of two distinct HCV1a clades is confirmed. While they are not associated with any detectable epidemiological pattern, clearly distinct natural NS3i resistance patterns occur with respect to linear and macrocyclic NS3i.

No conflict of interest

Abstract: O_13

HCV Drug Resistance

Influence of HIV coinfection in natural variability of HCV NS3 protease and resistance-associated mutations assessed by Next Generation Sequencing

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Background: HCV quasispecies variability represents the Background: for the selection of mutations and for the development of drug resistance. Natural aminoacid changes in NS3, associated with reduced protease inhibitor susceptibility, have been observed in treatment-naïve patients. The relevance of naturally occurring mutations with respect of resistance development and probability of success of antivirals is an hot issue. It is expected that impaired immunity due HIV coinfection provides reduced selective pressure for HCV quasispecies evolution, affecting heterogeneity of HCV quasispecies. The influence of HIV-coinfection on natural variability of NS3 has been addressed in few studies so far, based on standard sequencing, hence unable to go deep into the quasispecies population. We used next generation sequencing (NGS) to assess the NS3 heterogeneity in patients with genotype 1 HCV infection, naïve to anti-HCV treatment, with/without HIV coinfection. The two groups presented similar HCV viral load. HCV subgenotype 1a was more frequent in HIV-coinfected patients, as expected. All but one HIV-coinfected patients were under cART (including a protease inhibitor in nine cases), with undetectable (<50 cp/ml) HIV viremia in most cases.

Methods: Plasma RNA was amplified by RT nested-PCR using two sets of subtype-specific barcoded primers. Two overlapping amplicons (residues 1-211, subgenotype 1a; 6-256, subgenotype 1b) were generated. A total of 161578 and 127049 reads for amplicons 1 and 2, respectively (mean coverage 8017, range 1977-22543) were obtained.

Results: The extent of NS3 diversity was not significantly correlated with HCV viral load (r=0.346, p=0.106 in Spearman correlation test). Mean nucleotide diversity was similar in 1a and 1b (0.0172±0.0105 and 0.0188±0.0102 nt substitutions/site, respectively, p=0.55). Nucleotide diversity in monoinfected patients was about twice that observed in HIV-coinfected patients (0.0249±0.0125 and 0.0135±0.0054 nt substitutions/site, respectively, p=0.0145). In HIV-coinfected patients NS3 diversity was independent from CD4 number, HBV-coinfection and presence of HIV protease inhibitors in the cART regimen. Among the aminoacid residues included in the NS3 catalytic triad (H57, D81 and S139), only position 57 presented a single variant (H57R) in 2 patients, at very low intra-patient frequency (≤0.4%), confirming the high conservation of these positions. Twenty aminoacid positions, known to harbour mutations associated with resistance to either linear or macrocyclic protease inhibitors, were analysed in details (16; 36; 39; 41; 43; 54; 55; 56; 80; 107; 109; 132; 138; 155; 156; 158; 163; 168; 170; 175). Overall, at least one polymorphic site was observed in twenty-two patients (91.7%); nineteen patients (79.2%) carried known resistance-associated mutations, mostly, but not exclusively, as minority variants. Multiple mutations (up to five in one case) were observed in eleven (45.8%) patients.
Conclusions: Due to the fast evolving therapeutic landscape, and to the high plasticity of HCV quasispecies naturally occurring mutations may be relevant with respect of resistance development and probability of success of direct acting antivirals. More studies are necessary to fully evaluate the impact of HCV mutations naturally occurring in individual quasispecies, their baseline frequency, their combination and their dynamics, on the emergence of clinical resistance to NS3 inhibitors, particularly for people with concomitant HIV infection, where probability, pathogenesis, and clinical implications for development of resistance are much more complex and less analysed.

No conflict of interest

Abstract: O_14
HCV Drug Resistance

Deep sequencing analysis of liver and plasma HCV quasispecies of treatment naïve patients

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Background: The HCV genome exhibits significant genetic heterogeneity due to accumulation of random mutations during viral replication. This variability can be attributed to the limited fidelity of the RNA-dependent RNA polymerase, and generates a dynamic population of heterogeneous but closely related variants designated as quasispecies. The composition of viral quasispecies may influence the outcome of anti-viral therapy and variants’ distribution could be important in the selection of drug resistance. Deep sequencing technologies allows for the identification of minority variants bearing drug resistance mutations. In this study, we performed deep sequencing of HCV liver biopsies and plasma samples from twenty three treatment naïve patients to compare the overall quasispecies distribution between plasma and liver and estimate the prevalence of naturally occurring variants within the polymerase gene (NS5b).

Materials & Methods: Plasma and liver samples were obtained from 23 treatment naïve patients infected with HCV genotype 1b. Population and 454 deep sequencing was performed on the HCV polymerase gene spanning positions 8245 to 8645 from 23 plasma samples and 23 liver samples. The region included amino acid residues known to confer resistance to antiviral drugs acting as polymerase active site inhibitor. Sequencing data analysis was performed by alignment against reference sequence. Major and minor variants occurring in the populations were determined and the frequency of distinct haplotypes in the viral population estimated.

Results: We analysed samples from 23 chronically infected patients with HCV plasma levels that ranged between 1.2 x 10^4 to 7.0 x 10^6 IU/ml. For both the liver and plasma samples a minimum of 16000 sequence reads was generated. In a detailed analysis, the frequency of the most dominant sequence was shown to be identical in liver and plasma. Comparing the overall quasispecies distribution between plasma and liver samples showed a remarkable overlap in each patient; unique sequences for either plasma or liver were rare. In twenty three plasma and liver samples no codon changes were detected at position 282 from Serine and at position 320 from Leucine, whereas one patient had mutant V321I both in plasma and liver.

Conclusions: The HCV quasispecies in plasma can be very similar to the quasispecies in liver tissue (down to 0.5%). In both compartments the same dominant sequence was encountered and given that very few compartment unique sequences were obtained it suggests a constant and dynamic flow of viruses between the two compartments and a deterministic evolution selecting for the most fit (dominant) strain. The absence of variation in polymerase codon use at positions 282, 320 and 321 not only in plasma but also in liver may indicate that variation at this position may lead to fitness loss resulting in out competition of these mutants by wild type virus.

No conflict of interest
Abstract: O_15

Round Table Discussion: Cost effectiveness

Cost-effectiveness of baseline genotypic testing in HIV infected patients in the Netherlands

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Background: About 10% of patients newly diagnosed with HIV-1 in Europe become infected with a virus containing drug resistance associated mutations. Transmission of drug resistant HIV-1 is associated with an increased risk of virological failure. Treatment guidelines therefore recommend performing a genotypic resistance test as soon patients enter into care. Epidemiological studies, however, report that 50% of patients infected with drug resistant HIV carry a virus that contains thymidine associated mutations (TAMs). Importantly, TAMs confer resistance to drugs such as zidovudine and stavudine that are no longer recommended in first-line regimens. Resistance to tenofovir and emtricitabine that are popular in first-line treatment generally occurs in <2% of all newly diagnosed patients. An expensive baseline genotyping test (cost of more than 300 Euro’s) may therefore only be of benefit to a small number of patients. The aim of this study is to determine the cost-effectiveness of baseline-genotyping in the Netherlands.

Methods: We designed a probabilistic state-transition model to project clinical and cost outcomes in a hypothetical cohort of antiretroviral-naïve HIV-infected patients in the Netherlands. In 2010, 8.7% were infected with a virus containing drug resistance associated mutations; transmission of resistance most frequently involved nucleoside reverse-transcriptase inhibitors (5.7%), followed by protease inhibitors (2.1%) and non-nucleoside reverse-transcriptase inhibitors (1.9%). Rates of efficacy of treatment, virological failure, opportunistic infections, mortality and health-related costs were derived from published randomized clinical trials, observational cohort studies, and data from a Dutch HIV care centre. Cost-effectiveness was calculated as the difference in costs (comparing a scenario with and a scenario without baseline genotyping) for the two scenario’s per quality adjusted life year (QALY, a QALY of one means a year lived in perfect health). A cost of less than € 40,000 per QALY is considered to be cost-effective.

Results: The cost-effectiveness was calculated using different assumptions regarding the impact of drug resistance on virological failure. We first assumed that 50% of patients infected with a drug resistant virus would experience virological failure in case treatment could not be optimized using a baseline genotype test. This resulted in a cost-effectiveness ratio of €1.6 million per QALY. We then assumed that in the absence of baseline genotyping, all patients infected with a drug resistant virus would experience virological failure in case treatment could not be optimized using a baseline genotype test. This resulted in a cost-effectiveness ratio of €740,000 per QALY. Nonetheless, genotypic testing showed to be more cost-effective in patients with a CD4 count below 200 cells/mm3 compared to patients with a CD4 count of >200 cells/mm3, with cost-effectiveness ratios of €65,000 and €330,000 per QALY gained, respectively, at an absolute reduction in failure rate of 5%.

Conclusion: Baseline genotyping is not cost-effective in the Netherlands. Possibilities for optimizing cost-effectiveness could be to target baseline genotypic testing to a particular populations (e.g. only patients with a CD4 of <200 cell/mm3) and to reduce the costs of resistance testing.

No conflict of interest
Efficacy of Tenofovir and Efavirenz in combination with Lamivudine or Emtricitabine in antiretroviral-naïve patients in clinical practice in Europe


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Abstract: O_16

Clinical Management

Efficacy of Tenofovir and Efavirenz in combination with Lamivudine or Emtricitabine in antiretroviral-naïve patients in clinical practice in Europe


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Background: The combination of Tenofovir, Efavirenz and either Lamivudine or Emtricitabine (TELE), in particular the single tablet regimen Atripla, has proven highly effective in clinical trials for first-line treatment of HIV-1. However, limited data are available on the efficacy in clinical practice and on resistance profiles following virological failure (VF).

Abstracts

Materials & Methods: A retrospective data-analysis of all naïve patients starting TELE before the 1st of June 2009 in 13 European centres. Efficacy was studied according to intention-to-treat (missing or switch = failure,ITT) and on-treatment (OT) analysis. Treatment success was defined as a viral load (VL) < 50 cp/ml. VF was defined as a rise of VL > 200 cp/ml following previous suppression < 50 cp/ml or a rise of 1 log following previous suppression of at least 1 log, not resuppressed at the next assessment. Baseline genotypic susceptibility scores (GSS) were determined using the Stanford HIVdb-algorithm. Efficacy of 2nd line therapy was assessed after 1 year. Data were collected until July 2010. Factors associated with VF were analysed using multivariate logistic regression.

Results: 1622 patients were included, 81.1% were male, 49.5% MSM, 33.6% HSX and 5.4% IDU. A baseline genotype was available for 54.9% of patients, 65.9% were infected with subtype B virus. At 48 weeks 70.6% (ITT) and 91.3% (OT) of patients had a VL< 50 cp/ml. 22.8% of patients discontinued TELE at week 48, mainly due to CNS-toxicity or psychiatric side-effects. Only 3.3% (n=53) patients experienced VF within 48 weeks. In multivariate analysis lamivudine-containing regimens, a baseline CD4- count below 200 cells/mm³ (both p<0.001) and a GSS<3 (p=0.001) were associated with a higher risk of VF. In 47.2% (25/53) patients experiencing VF genotypic resistance testing was performed. Most frequent observed mutations were K103N (60%, of which 67% were selected during a lamivudine containing regimen), K65R (48%, 75% during lamivudine) and M184I/V (44%, 45% during lamivudine). Following toxicity, 53.7% switched to another NNRTI-based regimen. Following VF 77.4% switched to a boosted PI, mostly lopinavir or atazanavir. 76% of patients who switched because of VF had a GSS<3 for the 2nd line regimen. Still, following VF on TELE, after 1 year of 2nd line therapy, VL was resuppressed to < 50 cp/ml in 73.5% and to < 200 cp/ml on 85% (OT).

Conclusions: In Europe, treatment failure on TELE-regimens is relatively frequent in clinical practice due to toxicity. Virological failure is rare, and more often observed with lamivudine than emtricitabine. Intermediate baseline resistance to one drug in an otherwise fully active regimen, significantly increases the risk of virological failure on TELE. Following virological failure on TELE, PI-based 2nd line
therapy was often successful despite a GSS<3.

No conflict of interest

Abstract: O_17

Clinical Management

Prevalence of mutations and determinants of genotypic resistance to rilpivirine in B and the most prevalent non-B HIV-1 subtypes in Italy

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Background: Rilpivirine (RPV) is a second generation non-nucleoside reverse-transcriptase inhibitor (NNRTI) recently approved for HIV-treatment. Here, we evaluated the prevalence and the patterns of RPV-resistance mutations (RRMs) in the most common HIV-1 subtypes in Italy (B, C, F, and CRF02_AG).

Material & Methods: Primary RRMs (L100I-K101E/P-E138G/Q-Y181C-Y188L-G190A/S-H221Y-F227C-M230I/L) were examined in plasma-samples of 7,444 RPV-naïve patients infected by HIV-1 subtypes B (n=6,287), CRF02_AG (n=547), C (n=332), or F (n=278). These patients belonged to 3 categories: a) drug-naïve (n=4,974); b) HAART-failing-patients, never experiencing NNRTIs (NNRTI-naïve; n=1,211); c) NNRTI-failing-patients (NNRTI-treated; n=1,259: 664 efavirenz-treated, 578 nevirapine-treated, 17 etravirine-treated). To identify patterns associated with RPV-resistance, other potential RRMs V90I-K10IT-V106A/I/M-V179I-Y188C/H, as well as K103N-M184I/V, were also analyzed. Logistic-regression analyses were performed to define factors associated with ≥1RRM.

Results: Patients with ≥1RRM and ≥2RRMs were found with the following proportions: drug-naïve-patients: 6.5% and 0.5%; NNRTI-naive: 11.0% and 2.5%; NNRTI-treated: 56.8% and 22.6%. Among NNRTI-treated patients, the presence of ≥1RRM was more prevalent in those treated with efavirenz (70.6%) or nevirapine (64.2%) than those treated with efavirenz (50.0%; p<0.001). The E138A was the most common primary RRM both in drug-naive (4.5%) and NNRTI-naïve (5.2%) patients; its prevalence was similarly maintained in NNRTI-treated population (6%). The primary-RRMs (L100I-K101E/P-E138G/Q-Y181C-Y188L-G190A/S-H221Y) were <2% in both drug-naïve and NNRTI-naïve-patients, while in NNRTI-treated-patients their prevalence was in the range of 2%-19%. The other RRMs (K101T-E138K/R-V179L-Y181I/V-Y188C-G190E-F227C-M230I) were present with a prevalence ≤1% in all 3 groups. Interestingly, a strong correlation was found between L100I and the mutations K103N, V90I, K101T (0.11>phi<0.30; p<0.001). No patient carried the typical RPV-failure pattern E138K+M184I, while 9 patients carried the E138K+M184V: 6-NNRTI-treated (0.5%) and 3 NNRTI-naïve (0.3%). According to NNRTI-usage, the mutations L100I-K101P-G190E were more frequently found in patients failing efavirenz than nevirapine (11.7% vs. 0.2%; 3.5% vs. 0.5%; 1.7% vs. 0%: p≤0.002). Conversely, Y181C-G190A-H221Y were more prevalent in nevirapine than efavirenz-failures (32.4% vs. 7.5%; 25.3% vs. 13.7%; 10.6% vs. 5.6%; p≤0.001). As expected, E138K/Q mutations were mostly found in etravirine-failing patients (both 11.8%), while their prevalence was ≤2% in efavirenz/nevirapine-failing-patients. No statistical association was found between RRMs and subtype. The only exception was the mutation E138G, mostly found in the overall F-subtype-population (2.5%) in comparison to subtypes B (0.5%), C (0.0%) and CRF02_AG (0.7%; p=0.001). By logistic-regression multivariable analysis, the
presence of ≥1RRM was associated with high viremia at genotypic-resistance-test (Odds Ratio [95%CI]: 1.21[1.11-1.34], p<0.001) and the NNRTI-usage (NNRTI-naïve vs. NNRTI-treated patients: 0.08[0.07-0.11], p<0.001; drug-naïve vs. NNRTI-treated patients: 0.04[0.03-0.05], p<0.001). HIV-1 subtype did not result as predictor for the presence of ≥1RRM in none of the regression models built.

Conclusions: Analyzing more than 7,000 HIV-1 infected RPV-naïve-patients, the prevalence of at least one RRM is relatively low in drug-naïve and NNRTI-naïve patients, while it is recognized in nearly half of NNRTI-treated patients (mainly treated with etravirine or nevirapine than those with efavirenz). Specific patterns of RPV-mutations were found in HAART-treated patients. Different HIV-1 subtypes (among B, C, F and CRF02_AG) do not significantly influence the presence of RRM.

No conflict of interest

Abstract: O_18

Clinical Management


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Introduction: Antiretroviral regimen simplification improves quality of life and medication adherence while reducing the risk of HIV virologic failure and long-term drug-related toxicities. GS-US-264-0106 and GS-US-264-0111 were 48-week, prospective, open-label studies that evaluated switching virologically-suppressed, HIV-1 infected subjects from a ritonavir-boosted protease inhibitor regimen (PI+RTV+2NRTIs) or efavirenz / emtricitabine / tenofovir DF (EFV / FTC / TDF), respectively, to the single tablet regimen (STR) of rilpivirine / emtricitabine / tenofovir DF (RPV / FTC / TDF). Here, we present integrated resistance analyses from these 2 studies.

Methods: Study GS-US-264-0106 was a phase 3b study evaluating the safety and efficacy of switching from PI+RTV+2NRTIs to RPV/FTC/TDF in virologically-suppressed subjects in North America and Europe. Subjects were randomized 2:1 to switch to RPV/FTC/TDF at baseline or maintain their current PI+RTV+2NRTIs regimen with a delayed switch to RPV/FTC/TDF at Week 24. Study GS-US-264-0111 was a single-arm, phase 2b study designed to evaluate switching from EFV/FTC/TDF to RPV/FTC/TDF in virologically-suppressed subjects who desired a change in their regimen due to EFV intolerance. Historical genotypes were analyzed to confirm sensitivity to RPV/FTC/TDF at study entry. Confirmed virologic failures through Week 48 or discontinuation samples with ≥400 copies/mL HIV-1 RNA were analyzed for emergent resistance by PhenoSense GT (Monogram Biosciences).

Results: A total of 366 virologically-suppressed subjects from these 2 studies were included in the Week 48 analysis (GS-US-264-0106: immediate switch at baseline n=317; GS-US-264-0111: n=49). Of these subjects, 58% had HIV-1 subtype B, 35% were undetermined, and 7% were non-B subtype. Through Week 48, 89.3% of subjects who switched from PI+RTV+2NRTIs at baseline and 93.9% of subjects who switched from EFV/FTC/TDF maintained virologic suppression by FDA snapshot analysis. Through 48 weeks in these 2 studies, a total of 8 subjects treated with RPV/FTC/TDF were analyzed for resistance development with data available for all subjects (7 switched from PI+RTV+2NRTIs and 1 switched from EFV/FTC/TDF). Of these, 4 subjects developed primary NRTI or NNRTI resistance mutations and reduced susceptibility to FTC and/or RPV (4/366, 1.1%). Two subjects who switched from a PI+RTV+2NRTIs regimen at baseline developed resistance by Week 24 and 2 subjects developed resistance between Weeks 24 and 48. No subjects who switched from EFV/FTC/TDF developed resistance through Week 48. All 4 subjects with emergent resistance developed the M184V/I mutation
and 3 of the 4 also had emergent resistance mutations to RPV (L100I and K103N with pre-existing V90V/I; E138K; and V108V/I and E138K with pre-existing K103N and V179I). All 4 subjects had reduced susceptibility to FTC and lamivudine. Two out of 3 subjects with RPV resistance mutations also developed phenotypic resistance to RPV and cross-resistance to at least one other NNRTI.

Conclusions: The rate of resistance development was low (1.1%) in virologically suppressed subjects switching from a stable regimen of PI+RTV+2NRTIs or EFV/FTC/TDF to RPV/FTC/TDF in studies GS-US-264-0106 and GS-US-264-0111 through Week 48. Emerging mutations were similar to those seen in previous studies with RPV. A high level of virologic suppression was maintained after switching to RPV/FTC/TDF in both studies by FDA snapshot analysis through Week 48.

Conflict of interest

financial relationship(s): All co-authors are employees and shareholders of Gilead Sciences, Inc.

Abstract: O_19

Clinical Management

HIV resistance to dolutegravir is conferred by integrase mutations R263K and H51Y that jointly diminish viral fitness

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Introduction/Background: Dolutegravir is an HIV integrase strand transfer inhibitor ( INSTI ) that has been extensively studied in phase 3 clinical trials. No previously INSTI-naïve patient has yet developed resistance against DTG, although patients who have previously failed other INSTIs may sometimes fail a DTG-containing regimen, due to cross-resistance that seems to be principally conferred by mutations at positions 148 and 140 in the integrase gene. In order to characterize the resistance profile of Dolutegravir, we selected for resistance in tissue culture against this compound.

Materials and Methods: We propagated HIV-1 of different subtypes in both MT-2 cells and in peripheral blood mononuclear cells over protracted periods, with the concentration of DTG being incrementally increased from 0.05 nM, i.e. 4 times less than the EC50. After a total of 6 months, a final drug concentration of 50-100nM was achieved, beyond which virus could no longer be grown. Viral DNA was sequenced and the biological relevance of any mutations was confirmed by site-directed mutagenesis.

Results: The most frequent integrase resistance mutations to arise in subtype B and recombinant A/G viruses were R263K followed by H51Y, while, in subtype C viruses, G118R was followed by H51Y. The presence of R263K alone conferred an approximate 2-5-fold level of resistance to DTG in culture, a 30% drop in levels of recombinant integrase strand transfer activity, as well as an approximate 20-30% loss in viral replicative capacity. In contrast, H51Y alone did not significantly affect either strand transfer activity or resistance to DTG, but the combination of R263K together with H51Y led to an increase in levels of DTG resistance to about 12-fold accompanied by a ≈70% loss in both viral replication capacity and integrase strand transfer activity. Moreover, the combination of R263K together with H51Y resulted in an approximate 80% drop in the ability of viral DNA to become integrated into host cell DNA. Similar results were observed in the case of subtype C viruses and recombinant enzymes with the combination of the G118R and H51Y mutations. No other mutations were seen over more than one year.

Conclusions: R263K or G118R together with H51Y can augment levels of resistance to DTG yet result in a more severe attenuation of viral replication capacity and integrase strand transfer activity than either of the primary mutations alone, in spite of the fact that the secondary mutation H51Y did not by itself affect either of these activities. This is in contrast to the situation usually observed in drug resistance studies, whereby secondary mutations usually act to increase overall levels of drug resistance while simultaneously serving a compensatory function in regard to
restoring viral replication capacity and enzymatic activity to near wild-type levels. These data suggest that viruses containing both the R263K/G118R and H51Y mutations may be at a severe replicative disadvantage and help to explain why primary resistance to DTG has not yet arisen in clinical studies performed to date.

No conflict of interest

Abstract: O_20

Clinical Management

Clinical validation of hiv-1 genotypic resistance to Raltegravir by parallel genotypic and phenotypic analyses: the GEPHERAL study


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Background: Raltegravir (RAL) has provided a novel option to treat HIV-1 infected subjects, including those who were failing previous anti-HIV-1 regimens. However RAL resistant variants are selected in patients failing RAL-including regimens with primary resistance mutations in three key positions in the viral integrase: 143, 155 and 148. However, a complex pattern of primary and secondary mutations always occur that modifies the phenotypic profiles of selected variants. Moreover, the availability of novel drugs within the class of integrase inhibitors (INIs) with only partially overlapping resistance profiles underlines the need for a correct genotypic evaluation of RAL resistance to guide patients' management.

Material & Methods: A prospective study was conducted on treatment-experienced, HIV-infected patients failing a RAL-containing regimen (namely with two consecutive HIV RNA viremia over 50 copies/ml). The genotypic analyses of the integrase sequence were performed at the beginning of RAL-including regimen and at failure. From patients' samples where RAL-associated mutations were observed, clonal ex-vivo phenotypic evaluations where performed in purified primary CD4+ T cells.

Results: Among 567 patients under RAL-based regimens, ninety-five patients failed HIV-1 treatment. In 34 patients the pol gene could not be amplified due to low plasma viremia. In 61 patients genotypic evaluation showed that only 36 patients had selected viral variants with primary mutations: Y143H/R/C (8.2% of all failures), N155H (11.3%) and G140S+Q148H/R (32.7%). In 27 patients no major mutations were observed at failure. In all patients that failed with the G140S+ Q148H mutations, recombinant viruses showed an IC50 fold change (FC) increase >200 and a reduction of viral replication capacity (RC) between 0-25%. Viruses with mutations in position 155 or 143 showed a variable level of resistance to RAL (between 12 and 60 FC and reduction of RC between 60 and 20%) partially explained by the selection of secondary mutations (L74M, E92Q, T97A and/or T66AI and G163R). In 14 patients, RAL was maintained despite failure due to lack of available regimens and evolution of genotypic and phenotypic profiles were also analysed. A progressive increase in FC and a partial recovery of RC due mainly to selection of secondary mutations (up to three secondary mutations) and less frequently to novel combinations of primary mutations (N155H+Y143R, 1 patient). No polymorphisms observed at baseline could predict the failing pattern. However, 2 patients were carrying viruses with the T97A polymorphic secondary mutation (no difference in FC to RAL was observed when T97A was present alone) before RAL treatment, later selected variants with T97A+Y143R mutations.

Conclusions: Although the almost 40 different combination of primary or secondary mutations were observed, the increase of FC resistance was primarily due to the selected primary mutation and in all cases the FC increase was always much above the clinical cut-off. These and recent data on the cross-resistance profile
of second generation INIs suggest that a prompt evaluation of integrase genotype at RAL-failure will eventually limit the accumulation of complex combinations of secondary mutations and may still allow to plan future savage therapies in a significant proportion of patients that failed a RAL-based regimen.

Conflict of interest
financial relationship(s): Merck partially supported this study.

Abstract: O_21
Monitoring Technologies
Effects of sequencing errors on geno2pheno[coreceptor] predictions
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Background: The validity of HIV-1 genotypic coreceptor tropism determination with geno2pheno[coreceptor] has been corroborated by several studies. However, the effects of inaccurate sequencing of the V3 Loop on geno2pheno[coreceptor] have not yet been systematically studied. The goal of this work is to analyze the effects of inaccurate sequencing on predictions with geno2pheno[coreceptor].

Materials and Methods: A dataset of 70,644 unique HIV-1 nucleotide V3 sequences (fasta files) were extracted from the Los Alamos National Laboratory Sequence Database. This dataset was analyzed by using two procedures: (1) from every sequence in the dataset, 1470 sequences were generated by in-silico mutation of its 105 nucleotides with each of the 14 possible nucleotide and nucleotide ambiguity code alternatives. The geno2pheno[coreceptor] false-positive rate (FPR) was computed for each of the resulting 111,264,300 sequences as well as the unmodified sequences. Sequences producing alignment errors in geno2pheno[coreceptor] were discarded; alignment errors in original sequences triggered the deletion of all of its in-silico mutants. (2) Six sets of 1000 sequences were randomly drawn from the original dataset of 70,644 sequences. Each of the datasets were used for a separate experiment involving the introduction of one to three mutations into each sequence by using either unique nucleotides or ambiguity codes at a random position. After evaluation with geno2pheno[coreceptor], proportions of X4 and R5 misclassifications were quantified. Classification cutoffs of 10% and 20% FPR were used.

Results: (1) Filtering out sequences with alignment errors resulted in a dataset of 85,840,504 sequences with predicted FPRs for 53,862 sequences in the non-mutated dataset (NM). FPRs of NM presented a tropism distribution of 66% R5, 24% X4 and 10% undefined. In contrast, the mutated set of sequences showed a tropism distribution of 59% R5, 26% X4 and 15% undefined. Position-wise switch probabilities ranged from 0.00 to 0.41 and were similar for unique nucleotide (UNM) and ambiguity code mutations (ACM), albeit UNM tended to have higher switch probabilities (P=0.10 vs. P=0.07). X4-missclassification hotspots (P > 0.20) could be found around nucleotide positions 21, 33 and 70. Nucleotide-wise analysis showed a relatively uniform distribution of switch probabilities across UNM and ACM (min(P) = 0.07; max(P) = 0.12). (2) For each of the six 1000 sequence pair datasets between 18 and 92 sequences were removed due to alignment errors. The experiment introducing 3 UNM resulted in the highest proportion of switches from R5 to X4 (7.5%; n=68), while the experiment introducing 3 ACM resulted in the lowest proportion of such switches (4.5%; n=36). The highest proportion of switches from X4 to R5 resulted from introducing two UNM per sequence (3.7%; n=35), while introducing two ACM yielded the lowest proportion (1.5%; n = 15)

Conclusions: Low misclassification probabilities corroborate geno2pheno[coreceptor]’s robustness in coreceptor tropism determination (CTD). In a low number of cases, even single sequencing errors might result in 6% overcalling of X4 and 2% overcalling of R5. This highlights the importance of accurate sequencing and editing, especially at misclassification hotspots. Furthermore, errors involving unique nucleotide mutations have a
higher probability of causing misclassification than those involving mixtures.

No conflict of interest

Abstract: O_22

Monitoring Technologies

Sensitive detection of minor variants and viral haplotypes using single-molecule, real time (SMRT) sequencing

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Background: Genotypic testing of chronic viral infections is an important part of patient therapy and requires assays capable of detecting the entire spectrum of viral mutations. Most genotypic assays are CE-based and offered as CLIA-validated Lab Developed Tests (LDTs). Single Molecule, Real-Time (SMRT®) sequencing offers several advantages to CE, including superior resolution of mixed populations and long read lengths capable of spanning entire viral protein coding regions.

Methods: We examined detection sensitivity of SMRT sequencing using amplicon mixtures of various coding regions of both HCV and HBV. For HCV: Large fragments spanning NS3/4a (2,055 bp) and NS5a (1,341 bp) were amplified from plasmids containing either Con1 HCV genotype strain 1b or the H77 HCV genotype 1a strain. Amplicons were purified, concentrations normalized, and reciprocal mixtures made by serial 1:2 dilution from 20% to <0.1% minor variants. For HBV, we examined sensitivity of SMRT sequencing to detect very low level mixtures of known resistance-associated mutations in the Polymerase Gene (P) of HBV. Three mutations were introduced into an HBV plasmid by site-directed mutagenesis. Two amplicons (575 bp and 1,389bp) were generated for both wild type and mutant plasmids; amplicons were purified, normalized, and mixed by serial 1:2 dilution from 20% to 0.08% minor variant frequencies. SMRTbell™ libraries were constructed and sequenced using standard Pacific Biosciences® chemistry and protocols.

Results: We used both a reference-based approach and de novo approaches for variant detection and haplotyping. Average read lengths were +3,500 bp across samples, with >5% of reads longer than 9,000 bp. From a single SMRT® Cell, minor variants were accurately and reliably detected down to 0.1% with simple analyses.

Conclusions: The random error profile and long read lengths make it possible to call minor variants at <0.1% frequency from as few as 10 molecules, while sequencing individual molecules allows phasing of mutations hundreds of bases apart. SMRT® sequencing can identify species comprising a mixed viral population, with granularity and low cost of consumables allowing for smaller multiplexing of samples and first-in-first-out processing.

Conflict of interest

financial relationship(s): I am an employee of Pacific Biosciences

Abstract: O_23

Monitoring Technologies

Performance of HIV-1 PR-RT genotyping on clinical samples with low viremia levels

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Background: Resistance testing in HIV-1 infected patients is recommended to guide the choice of antiretroviral therapy in clinical practice. In this regard, genotypic testing is the most common method used for detecting drug-resistant strains of HIV. This assay is not usually recommended in persons with a plasma viral load (VL) <500 copies/mL (DHHS, March 2012). Aim of this work was to evaluate the efficiency of the test, and the reliability of the sequences, obtained from plasma samples with HIV-1 RNA levels of 50-500 copies/mL.

Materials & Methods: A retrospective analysis was performed on 11,741 HIV-1 clinical samples that were genotyped over the years 1999-2011 in the two clinical centers in Rome, Italy, by means of a of a homemade system or the commercially available ViroSeq HIV-1 Genotyping System (Abbott Molecular). For each clinical sample, VL value at genotyping was known. The retrieved 11,741 samples were stratified in 8 groups according to different VL levels (<50, 50-100, 101-200, 201-300, 301-400, 401-500, 501-1000, >1000 copies/mL). Genotyping success rate (GSR) was determined in total and per each VL-group, independent of the genotyping platform (equipment, kits and reagents) upgrades that occurred during this period. Moreover, in order to test genotyping reliability with VL levels ≤500 copies/mL, a phylogenetic analysis was performed on 1613 sequences from 470 patients having at least 1 sequence with contextual VL <500 copies/mL and at least 1 sequence with VL >1000 copies/mL.

Results: Samples analyzed were 80% from patients carrying a subtype B virus, and 20% from patients carrying non-B subtype viruses. The following non-B subtypes were the most prevalent: C(4.4%), CRF02_AG(4.1%), F(3.0%), A(1.2%), G(1.2%) and CRF12_BF(1.2%). Two thousands fifty-one (17.5%) samples had VL≤500 copies/mL. Overall, GSR was of 89.9%; according to the 8 groups, GSR was of 17.7% (163/953 samples; VL<50 copies/mL), 62.4% (217/348; VL=51-100 copies/mL), 78.3% (239/324; VL=101-200 copies/mL), 89.0% (162/182; VL=201-300 copies/mL), 92.9% (121/146; VL=301-400 copies/mL), 98.8% (392/381; VL=501-1000 copies/mL), 98.8% (920/9318; VL>1000 copies/mL). GSR was independent from subtype in all VL-groups. Phylogenetic analysis revealed a high homology within the sequences performed from the same subject for 457/470 (97.2%) patients analyzed, also for sequences with VL <100 copies/mL.

Conclusions: Reliable HIV-1 genotypes can be obtained from plasma samples with a high rate of success (>70%) also in patients with viremia levels in the range of 100-500 copies/mL. Therefore, genotyping resistance test could help in the individualization of therapy also for failing patients with low viremia levels.

No conflict of interest

Abstract: O_24

Monitoring Technologies

Detection of non-R5 HIV by ultrasensitive genotypic testing is associated with CD4+ count decreases also after the initiation of ART

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Background: The prognostic value of HIV-1 tropism to predict CD4 and clinical outcomes in HIV-1-infected subjects receiving ART is unclear.

Methods: We conducted a nested case-control study within the EuroSIDA cohort, where people with an AIDS diagnosis or who died for any causes for whom there was a stored plasma sample available in the time...
window of 3 to 12 months prior to the event were identified. Two controls were selected for each case matched for age (+/- 5 years), viral load (+/- 0.5 log) and HCV status at the time of sampling. Controls were still event-free after a matched duration of time from the date of sampling. HIV tropism was estimated using 454 sequencing of the V3-loop (non-R5 HIV defined as ≥2% of sequences with a Geno2Pheno FPR≤3.75%). In this analysis we compared the CD4 slope in people with R5 and non-R5 HIV over the 12 months following the date of sampling. A linear mixed model with random intercept and slope was used to estimate the difference in the two groups by fitting an interaction term. Analyses were controlled for gender, age, race, HCV co-infection, current viral load, use of ART and calendar year of sample, and were performed using all CD4 values, as well as after restricting to those determined while subjects were ART naïve, or to those obtained following ART initiation.

**Results:** Tropism estimates were available for 113 patients (39 cases and 74 controls) tested on a sample stored on average in 2004 (IQR:2002-2008). 20% of subjects had non-R5 HIV. There were 23% women, 45% MSM, 93% Caucasians, 21% co-infected with HCV. At the time of sampling 50% were ART-treated, 39% were ART-naïve and remained untreated and 11% were ART-naïve and started ART within the year. Median age, CD4 and viral load was 35 (IQR:31-42) years, 352 (182-548) cells/mm$^3$ and 4.82 (4.41-5.19) log c/mL, respectively. Baseline characteristics were well balanced by tropism. Subjects contributed 347 CD4 measurements (70 X4 and 277 R5) over the year after the genotypic test (5% over 3 months; 11% over 3-6 months; 20% over 6-9 months, and 64% over 9-12 months). In the multivariable analysis controlled for gender, age, race, HCV co-infection, current viral load, calendar year of sample and use of ART (the latter only in the model including all counts), the mean (95% CI) non-R5 - R5 difference in CD4 count change/year was (p-value for interaction): -133 (-266,0) (P=0.05); -191 (-491,+109) (P=0.21), and -164 (-295,-33) (P=0.01), in the overall analysis (n=347 counts), after restricting to CD4+ counts obtained while subjects were ART naïve (n=117 counts), and after restricting to CD4+ counts obtained after ART initiation (n=230 counts), respectively.

**Conclusions:** Non-R5 HIV estimated using 454 sequencing was associated with steeper CD4 count decreases also after ART initiation, which suggests a higher risk of clinical complications for subjects with non-R5 HIV even in time periods following the initiation of treatment. Ongoing analyses will verify if this observation may result in a different risk of progression to AIDS or death.

No conflict of interest
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Abstracts
Poster presentations
Abstract: P_01

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

HIV-1 strain and Primary HIV-1 drug resistance among treatment naive Cameroonian

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Background: Cameroon is the country harbouring the highest reported Human Immunodeficiency Virus (HIV) genetic diversity. Since the introduction of antiretroviral (ARV), there has been a steep increase in the number of individuals initiating antiretroviral therapy (ART). Highly Active Antiretroviral Therapy (HAART) has dramatically improved survival and quality of life in people living with HIV and AIDS. However, drug resistant mutations of HIV are a great challenge to the benefits of HAART. Development of drug resistance to antiretroviral medication can occur in persons on antiretroviral therapy, by acquisition of an already resistant strain in persons who have never taken medication or by natural polymorphism of the virus in vivo. This leads to treatment failure hence complicating management of HIV patients. This study was carried out to determine the prevalence of primary HIV drug resistance among newly-diagnosed, treatment naïve Cameroonian prior to initiation of antiretroviral treatment, and harbouring diverse HIV variants.

Material and Methods: Samples from 33 ARV drug-naïve HIV-positive individuals were collected from the Dermatological Outpatient Clinic at the University of Yaoundé Teaching Hospital. Viral RNA was extracted from patients’ plasma and RT-PCR of the viral genomic region spanning pol PR-RT-RNase H was conducted. Samples were sequenced and phylogenetic analysis was performed for identifying HIV-1 subtype. Drug resistance analysis was inferred by sequence submission to the Stanford HIV Drug Resistance Database.

Results: Of the 33 samples analysed, the most prevalent HIV-1 subtype was CRF02_AG (23; 70%), followed by CRF11_cpx, CRF01_AE and subtype C (2; 6% each), and CRF18_cpx, CRF16_A2D, subtype F2 and group O (1; 3% each). Nineteen samples were analysed for major mutations conferring HIV drug resistance, and in only one case (5%) the Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) mutation K103N was found. No Nucleoside Reverse Transcriptase Inhibitor (NRTI) or Protease Inhibitor (PI) major mutations were observed, but secondary PI mutations L10I/V and L33I, were found in 4 and 1 case, respectively.

Conclusions: Primary drug resistance mutations in treatment naïve Cameroonian are present in Cameroon, but the prevalence is low in this study, although this finding is controversial in view of recent reports. Transmission of HIV drug resistant strains was, however, documented in this community, where the administration of ARV is increasing and may represent a serious setback for public health policies.

No conflict of interest

Abstract: P_02

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

Drug resistance testing of blood-borne viruses in Poland

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Introduction: With the introduction of telaprevir and boceprevir for hepatitis C
therapy, directly acting drugs became accessible for three major blood-borne viruses: HIV, HBV and HCV. However, since the therapy is either long-term or - in case of HIV - lifelong, the appearance of antiviral resistance is a serious issue in proper management of infection. The drug resistance doesn’t have to develop de novo: it can also be acquired with preexisting drug resistant mutants (transmitted drug resistance - TDR). Because of this drug resistance (DR) testing is crucial not only in case of viral failure but also before the initiation of therapy.

Material and Methods: In our center we perform genotypic DR testing based on sequencing for three leading viruses: HIV (since 2001), HBV (2011) and HCV (2012). For HIV we use one of commercially available assays (Trugene or ViroSeq) and for HBV and HCV - in-house Methods: All sequencing is carried out on ABI Prism 3130 Genetic Analyser. Raw data is verified with chromatograms and manually corrected with manufacturer’s software or SeqScape. Interpretation of sequencing data from in-house sequencing geno2pheno algorithm is used; in case of HBV additionally genotype is determined; HCV assay is optimized for genotype 1.

Results: HCV drug resistance testing was implemented for evaluation of naturally occurring protease inhibitors (PIs) resistant variants among naïve patients genotype 1 infected. Until now 79 samples were analyzed. Sixty eight (86.1%) of samples had wild-type HCV. In 11 (13.9%) samples mutations associated with decreased PIs susceptibility were detected. Five (6.3%) out of these mutants harboured the D168E substitution, 2 (2.5%) A87T, 2 (2.5%) T54S, 2 (2.5%) R117H. None of these strains carried more than one DR substitution. In 2011 370 naïve HIV-positive patients were screened for TDR defined as presence of any mutation on the WHO 2009 list of mutations for surveillance. Any mutations associated with TDR were detected in 19 out of 370 patients (5.1%). The number of sequences with PI-resistance associated mutations was 8 (2.2%) and with resistance to NRTI and NNRTI 9 (2.4%) and 3 (0.8%), respectively. Only one person was infected with a variant harboring mutations for two drug classes (NRTI and NNRTI). The most common subtype was subtype B found in 329 persons (89%). Subtype A and CRF01_AE was detected in 5% of the samples each and CRF02_AG in 1%. The serum samples from 411 patients [154 (37.5%) women and 257 (62.5%) men] with chronic hepatitis B were tested to determine HBV drug resistance. The prevalence of HBV drug resistant variants was as follow: in 249 (60.6%) of cases-susceptibility, 111 (27%) -lamivudine resistance, 7 (1.7%) -possible resistance to entecavir, 35 (8.5%) - entecavir resistance, 9 (2.2%) -adefovir resistance. Additionally 129 escape mutations in SHB protein were detected. Among tested samples the following variants were predominant: 128V 35(8,5%), 120T 11(2,7%) and 133T 11(2,7%).

Conclusions: Drug resistance testing becomes crucial point in the planning and efficiency monitoring of modern antiviral therapies. In house sequencing protocols, combined with geno2pheno algorithms, create cost-effective diagnostic tools

No conflict of interest

Abstract: P_03

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

Analysis of HIV drug resistance in naïve patients in Armenia

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Background: First case of HIV-infection in Armenia was registered in 1988. The epidemic evolved as in other FSU countries since first case was registered in 1990 in IDU-group but no data were obtained concerning the molecular epidemiology and HIV drug resistance prevalence in this country. In this work we present the preliminary data of molecular genetic analysis of HIV-1 samples from Armenian naïve HIV-infected patients and estimate the prevalence of HIV-resistance to different classes of ARV drugs.
Material & Methods: 89 samples of extracted chromosomal DNA from Armenian naïve HIV-infected individuals were studied. Analysis of drug resistance mutations in gag (from 1576 to 2040 nucleotide), pol (Pro, RT and IN regions) and env (from 6556 to 7792 nucleotide) gene was made by in-house method. HIV-drug resistance analysis of pol gene regions was carried out by HIVdb Program v.6.2.0. Geno2pheno was used for viral tropism determination (V3-loop analysis, FPR=10%) and for gag-gene drug resistance analysis. Genotyping analysis was carried out by COMET HIV-1/2 & HCV v.0.2 and REGA HIV-1 Subtyping Tool v.2.0.

Results: We obtained 46 sequences of Pro region (1-99 amino acids), 26 sequences of RT region (17-346 amino acids) and 7 sequences of IN region (29-254 amino acids) from the same group of samples. We obtained 33 sequences of env gene and 28 sequences of gag gene as well. Genotyping analysis of Pro region found 43/46 (93.5%) of subtype A1, 2 (4.3%) CRF02_AG samples and 1 (2.2%) subtype B sample. These results were confirmed by RT and IN regions analysis. We didn’t find any major resistance mutations in Pro region but 4 (8.7%) Pro-sequences harbored minor mutation L10I/V. Four samples had K20I substitution. In RT region M184I mutation was found in 1/26 sample (3.8%). Five A1-samples (19.2%) harbored A62V which is a polymorphic mutation for A1-variant dominating in Russia. All 7 samples of IN region (6 of subtype A1 and 1 of CRF02_AG) harbored L74I substitution which is usually present in almost 100% of samples of A1-variant dominating in Russia. One sample had also H114Y. Viral tropism analysis in 33 samples showed that 5/33 (15.2%) samples had X4-tropic viruses. We have found 4/28 (14.3%) samples harboring maturation-inhibitor-associated mutation V370A in gag-gene and one sample with Q369H substitution.

Conclusions: Our results showed that HIV-1 subtype A1 dominated in Armenia as it did in other former USSR countries. The presence of A62V and L74I in RT and IN regions, accordingly, may be explained by polymorphic features of this variant. Anxious data about X4-tropic HIV-variants in 13.9% samples lead the necessity for further tropism analysis in Armenia prior maraviroc treatment.

No conflict of interest

Abstract: P_04

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

Temporal trends of transmitted HIV drug resistance in Romania

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Introduction/Background: Transmitted drug resistance (TDR) is an important predictor of virological response to therapy. Recent epidemiological studies have showed discordant trends in the frequency of transmitted HIV variants over time: while in Western Europe, a relative stable or declining trend of primary resistance was recorded, in United States, the prevalence of TDR rate seems to increase, especially associated with non-nucleoside reverse transcriptase inhibitors mutations. However, reported data are available mainly for HIV-1 subtype B viruses. In Romania, a steady predominance of subtype F was reported among both long-term survivor children and newly infected adults. In this context, our goal was to assess the prevalence of transmitted drug resistance mutations in a group of treatment-naive Romanian patients, newly diagnosed with HIV infection between 1997-2012 and to evaluate the temporal trend of TDR.

Material & Methods: The pol gene of 70 drug-naïve, HIV-infected patients was sequenced using the ViroSeq HIV-1 Genotyping System (Abbott Laboratories, USA). Mutations associated with transmitted drug resistance were defined according to the Stanford University HIVdatabase and for subtypes determinations, all sequences were submitted to the REGA HIV-1 subtyping tool.
Results: Subtype F1 was prevalent (87.14%, 61/70 patients), however, other HIV-1 clades are increasingly identified, especially in the group of recently infected individuals: subtype C (5.71%; 4/70), subtype B (4.28%; 3/70), CRF 06_CPX and CRF 14_BG each in 1 patient (1.42%). The overall prevalence of TDR was 12.85%, mainly associated with NRTI-resistance (11.42%). Most of the subjects harboured HIV strains resistant to only one drug class; triple class resistance was not observed. Thymidine analogue resistance mutations (TAMs) and M184V were the most common type of TDR mutation. All HIV-1 strains carried minor mutations in the protease and RT genes, often detected at polymorphic positions. A declining trend of transmitted drug resistance to any antiretroviral drug was recorded from a rate of 25% in 1997-2003 to 12% in 2004-2008 and to 4% in 2009-2012. Moreover, a decrease frequency of TDR was observed for all drug classes. No primary resistance was identified among recent seroconvertors.

Conclusions: The high efficacy of highly active antiretroviral therapy (HAART) and the increasing number of treatment experienced persons with virological success who have a low risk of transmission can explain the declining rates of transmitted drug-resistant HIV-1 in Romania. Further and continuous TDR surveillance is necessary to gain more knowledge on the incidence and spread of TDR patterns in Romania, and to confirm the observed trend.

No conflict of interest

Abstract: P_05

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

The acquired HIV resistance to antiretroviral drugs in HAART treated patients in Belgrade, Serbia

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Background: Despite advances in antiretroviral therapy that have revolutionized HIV management and the control of the spread of regional epidemics, resistance to antiretroviral drugs has emerged in all locales in which such drugs are used. This study was conducted to analyse acquired HIV resistance among HAART treated patients who experienced virologic failure.

Patients and Methods: The retrospective study included 32 unselected patients, aged 14 years or above, treated in Belgrade School of Medicine Infectious Diseases Hospital Department for HIV/AIDS, from September 2010 until December 2012, who experienced virologic failure, defined by the viral load greater than 2.6 log10 copies of HIV RNA / ml of plasma. Genotypic resistance testing was done on a plasma sample taken at the time when virologic failure was recorded, by using commercial genotypic resistance assay.

Results: In a series of 32 patients, who were HAART treated for 6.2±3.7 years, with mean 6.8±2.5 antiretroviral drugs, genotypic resistance testing was performed. In 26 patients resistance associated mutations were found, while in six no such mutation were recorded. Of these, mutations associated with resistance to NRTIs and NNRTIs, emerged in 26 and 25 patients, respectively. Only 3 patients exhibited PI associated mutations, and in all of them triple class resistance was recorded. Even though the mean duration of HAART was 8.3 years in those with triple class mutations in comparison with 5.1 years in the subgroup with both mutations associated with resistance to NRTIs and NNRTIs, this difference did not reach statistical significance. Pre-treatment clinical AIDS did not affect resistance. The number of drugs taken during HAART did not differ between these two subgroups. However, the emergency of triple class mutations was associated with poor compliance (below 75% of drugs taken, P=0.007).

Conclusion: In this small number of HAART treated patients who experienced virologic
failure, we demonstrated that emergency of resistance was primarily associated with compliance, while the duration of HAART / mean number of drugs taken, nor the pre-treatment AIDS affected the emergency of HIV drugs resistance.

No conflict of interest

Abstract: P_06

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

Detection of integrase-gene-HIV-1 mutations based on proviral DNA: an alternative to RNA genotyping method.

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Background: Integrase resistance mutations have been observed in both in vitro and in patients experiencing virologic failure on Raltegravir (RAL), Elvitegravir (EVG) or Dolutegravir (DTG). Identifying such mutations can optimize and guide antiretroviral scheme selection. Currently, the HIV-1 integrase-genotype-tests designed to detect mutations associated with resistance-integrase-inhibitors are based on viral RNA. However, for patients with low viral load or undetectable, it is not possible to carry out this assay and few data are available on such mutations in patients with low viremia. In this study, integrase genotyping is performed simultaneously on RNA and proviral DNA samples from HIV patients under antiretroviral treatment with detectable or low viremia.

Material & Methods: We performed integrase genotyping simultaneously on RNA and proviral DNA samples from four HIV patients. Two patients treated with RAL and low viral load (patients A and B), and two patients treated without RAL and with detectable viral load (patients C and D). All patients had virologic failure. Viral RNA was extracted using an automatic system from plasma and proviral DNA was extracted using a column system from 200 uL of PBMC. For RNA genotyping we used a RT-PCR nested assay. For proviral DNA method we used the same mixes and temperatures programs, only the reverse transcription was omitted. PCR products were sequenced by Sanger’s method. The sequences obtained were analyzed and assembled with REcall software (provided by PR Harrigan, BC Centre, Vancouver, Canada). All the sequences approved by REcall were analyzed in three different databases for integrase resistance mutations reports: Stanford, Geno2pheno and REcall. Then, the obtained mutations were compared for both nucleic acids and evaluated the susceptibility or resistance to integrase inhibitors for each patient.

Results: For patient A, for RNA genotyping the mutations D10E, A23V, V31I, V72I, I113V, G123S, A124N, T125A, R127K, G140GS, Q148HQ, V165I, V201I, K215N, N232E were detected. For patient B the mutations D10E, K14KR, V72I, G123S, R127K, E138T, G140S, Q148H, I203IL, Q216PQ, N232D were detected. When analyzed these samples for proviral DNA genotyping the same number and kind of mutations were obtained in both patients. Patients with RAL treatment showed the integrase resistance mutations G140S, Q148H with clinical relevance which may explain the virologic failure due to RAL exposure. For patients without RAL therapy, the RNA and DNA genotyping showed the same sequences and did not detect integrase resistance mutations and they were susceptible to RAL, EVG or DTG.

Conclusions: These results demonstrate that exits a good correlation between the oligonucleotide sequences for integrase’s gene obtained for RNA and Proviral DNA. The number and kind of integrase resistance mutations were the same in two patients with RAL treatment. This evidence offers a promising approach for integrase genotyping in clinical practice, particularly for the assessment of under-treatment-patients with low or suppressed viraemia. Further research using deep sequencing tools and including a higher sample number is needed in order to corroborate these results.

No conflict of interest
Abstract: P_07

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

Drug resistance as a determinant of virological success in a cohort of HIV-infected patients receiving a nevirapine-based HAART

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Background: There are four non-nucleoside reverse transcriptase inhibitors (NNRTIs) approved for clinical use: nevirapine and efavirenz are first-generation NNRTIs, while the next generation is represented by etravirine and rilpivirine. Since their approval, nevirapine and efavirenz have been a milestone for treatment of HIV-1 infection. However, the clinical use of these antiretroviral drugs may be limited by their relatively low genetic barrier to resistance. The aim of our study was to describe the genotypic resistance before starting nevirapine as a factor related to response and durability of nevirapine-containing HAART. We analyzed differences between subjects with a virological failure and those who continued a nevirapine-containing regimen or discontinued this regimen for other reasons.

Material and Methods: We conducted a retrospective study among HIV-1 infected patients, attending our Infectious Diseases Unit in Milan from 1995 to nowadays. We selected 85 out of 531 patients undergoing an ART-regimen composed by a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs) plus nevirapine as third drug. Patients had a genotypic resistance test, CD4 count, HIV-RNA and complete therapeutical history before starting nevirapine (baseline). We described two groups of patients: ongoing or discontinued and virologically failed a nevirapine-containing regimen or discontinued this regimen for other reasons.

Results: Patients' characteristics were as follows: males 58.8%, median age 41 (IQR 35-46), IVDUs 27.1%, MSM 12.9%, heterosexuals 50.6%; median CD4+ count 317 cell/µL (IQR 195-476), median log10 viral load 3.8 (IQR 1.9-4.7). We observed a difference between the two populations in the median number of resistance mutations for NRTIs plus NNRTIs at baseline (p .008). An increased risk of virological failure when resistance for NRTIs and NNRTIs (analyzed together) were present at baseline (OR 3.5 [1.32-9.42], p .010) was noted, but the risk was not directly proportional to the increase of number of mutations. We observed a statistically significant risk of virological failure when more then five protease mutations were present at baseline (OR 3.9 [1.15-13.11], p .045). PIs administration before starting nevirapine was associated with a significant risk of virological failure (OR 7.1 [2.13-23.54], p .001). Durability of nevirapine-containing regimens was longer for subjects who did not have a previous PIs exposure (p .048) and without mutations in the RT region (p .013).

Conclusions: Nevirapine-containing regimens demonstrated to be effective although some factors have to be taken into account, such as resistance to NRTIs and NNRTIs, previous PIs exposure and degree of PIs resistance. Drug resistance of companion drugs plays a major role in virological success of nevirapine-containing HAART.

No conflict of interest
Abstract: P_08

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

Substitutions in RNaseH and Connection domain of HIV-1 CRF06_cpx viruses in treatment naïve and treatment experienced populations

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Background: HIV-1 drug resistance mutations (DRM) have been extensively studied in protease and N-terminal part of reverse transcriptase (RT). The knowledge about pol gene connection and RNaseH domains is limited. In addition, majority of studies considering DRMs have been focused mainly on HIV-1 subtype B viruses. Current study investigates pol gene RT C-terminal region, connection domain and RNaseH substitutions in treatment naïve (TN) and in treatment experienced (TE) patients infected predominantly by HIV-1 CRF06_cpx viruses in Estonia.

Methods: The viral genomic RNA was extracted, reverse transcriptase amplified and sequenced in protease, RT N-terminal region [amino acid (aa) 10-250] and in RT C-terminal, connection domain and RNaseH region (aa 286 – 554) in 78 treatment naïve and 118 treatment experienced patients. For statistical analysis the TE population was subdivided according to the type of treatment (main treatment groups were EFV + 3TC + ZDV or EFV + 3TC + ddI) or according to the possession of different DRMs in RT N-terminal region (M184IV, K103N and L74IV). The differences in distribution of DRMs and substitutions in RT C-terminal, connection domain and RNaseH region were determined using published data in literature and comparing the TN and TE populations of the current study.

Results: Phylogenetic analysis of TN and TE populations revealed that in both populations more than 90% of viruses clustered together with Estonian CRF06_cpx reference sequences. In TN population polymorphisms in 13 positions were recently described of being associated with antiretroviral treatment (ART). Five of them were consensus aa in CRF06_cpx: R356K, G359S, T377M, K390M and A400T. In comparison of TN and TE populations eight substitutions associated with ART in other subtypes (I326V, G333E, T366R, T369IV, E399D, Q509L, K527N and Q567K) were found to be out selected in TE population (all p<0.05). In addition the RT C-terminal region substitution K287T and connection domain substitution V437A were enriched in all TE subpopulations independent of their treatment regimen or DRMs compared to TN population. After multiple comparison following differences remained significant: the K287T was overrepresented in subjects with K103N and M184IV DRM and in those treated with EFV + 3TC + ZDV as compared to TN population (65%, 61% and 66% vs 22%, respectively; all p<0.05). The frequency of V437A was higher in M184IV DRM possessing and in those treated with EFV + 3TC + ZDV as compared to TN population (90% and 97% vs 59%, respectively; both p<0.05). The widely spread connection domain DRM N348I was not found in TN nor any TE populations sequences in CRF06_cpx viruses.

Conclusions: New RT C-terminal, connection domain and RNaseH region substitutions associated with ART in TE HIV-1 CRF06_cpx viruses were described in this study. It also found several polymorphisms as consensus aa in CRF06_cpx viruses which have been associated with ART experience in other subtypes. Whether these substitutions influence the ARV therapy in HIV-1 CRF06_cpx viruses in subtype specific manner needs to be cleared.

No conflict of interest
Abstract: P_09

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

Development of distinct protease-inhibitor resistance in a patient with Lopinavir in first line monotherapy

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Background: A boosted protease inhibitor (PI) single regimen can maintain plasma HIV-1 RNA suppression in a large proportion of patients. However, in comparison to patients receiving standard triple regimens, more patients on mono-therapy did experience episodes of low level viremia (50-400 copies/mL). In the majority of cases, this low level viremia did not result in protease inhibitor-associated resistance mutation.

Methods: Herein, we report the case of a 45 years old woman with a failing LPV/r mono-regimen: Tested HIV positive in 08/1988 she did not accept any therapy until she experienced generalized CMV - and oral Candida - infection in 12/2007. She consented to start treatment but only accepted LPV/r -monotherapy (BID). Resistance analyses, viral load (VL), CD4 counts and therapeutic drug monitoring (TDM) are documented.

Results: Before starting therapy in 2007 the patient had a viral load of 22,045 copies/mL and 340 CD4 cells /µl (33%). The result of the resistance analysis showed a wildtyp HIV-1 subtyp B virus. A TDM in 02/2008 showed sufficient LPV concentrations (9,803 ng/mL). Due to side effects the patient first reduced the dose and later stopped the virologic successful medication completly in 02/2009. In 01/2010 she restarted (VL: 21,357 copies/mL, CD4: 420 cells/µL, 39%) LPV/r together with Abacavir and Lamivudin co-medication for two months followed by LPV/r-monotherapy, which again was virologically successful. After some adherence problems with fluctuating drug concentrations a viral break through was observed in 10/2011. The detected resistance mutations 02/2012 were: 10F, 20R, 24I, 46I, 54V, 82A.

Conclusions: In general, LPV/r- monotherapy should not be considered as a preferred treatment option in antiretroviral-naive patients, especially in patients with suboptimal adherence, due to the higher risk for resistance mutation.

No conflict of interest

Abstract: P_10

Late Breaker abstracts

Virological and immunological response of Enfuvirtide plus OBR after 48 weeks of therapy in treatment-experienced patients

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Background: Enfuvirtide (ENF), is the first entry inhibitors to be approved for the treatment of HIV infection in treatment-experienced patients with evidence of HIV replication despite ongoing antiretroviral therapy. The intensification with ENF has suggested improvement in virological responses.

Objective: We report 48 weeks effectiveness and safety data on real life situation of enfuvirtide plus optimized background regimen (OBR) in mexican population.

Material and Methods: We conducted an observational prospective study. Patients were included if they had a multidrug resistant HIV-1 infection and enfuvirtide was part of the OBR. Demographic characteristics data were obtained. Virological, immunological and safety
Abstracts

outcomes were collected at 48 weeks of treatment.

**Results:** A total of 11 patients were included in our study. The median age was 42 years (IQR 39 - 51), 10 (90%) were men. OBR included TPV/RTV plus RAL in 63% of patients and DRV/RTV + RAL in 27%. TDF was included in 45% of OBR. The median of antiretroviral regimens was 6 (IQR 4 – 7). The median of CD4+ cells count at the beginning of the study was 255 cells/mL (IQR 100 – 339) and RNA HIV-1 viral load was 71,600 copies/mL (IQR 5567 – 191667), cholesterol and triglycerides were 173 mg/dL (IQR 145 – 192) and 182 mg/dL (IQR 159 – 286) respectively. At 24 weeks of treatment the median CD4+ cells count was 320 cells/mL (IQR 218 – 549) and RNA HIV-1 viral load was 152 copies/mL (IQR 40 – 400). At week 48, the median CD4+ cells count was 453 cells/mL (IQR 285 – 641) and RNA HIV-1 viral load was < 50 copies/mL in 63% of patients and < 400 copies/mL in 81%. At the same time, the median of cholesterol was 188 mg/dL (IQR 173 – 221) and triglycerides were 264 (IQR 174 – 317). Nodules, induration and pruritus were present in 5 patients. All of them had pain in site injection. One patient stop treatment at 16-week associated to ENF adverse events.

**Conclusions:** The addition of enfuvirtide to an OBR provided significant antiretroviral and immunologic response through 48 weeks in patients who had previously received multiple antiretroviral drugs and had multidrug-resistant HIV-1 infection; reactions at the site of the injections were very common in patients receiving enfuvirtide.

**Abstract: P_11**

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

**Clinical evaluation of Rega 8: An updated genotypic interpretation system that significantly predicts HIV-therapy response.**


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**Background:** Clinically evaluating genotypic interpretation systems is essential in order to provide optimal guidance in designing potent individualized regimens. The aim of this study was to investigate the ability of the Rega algorithm to predict virological response.

**Materials & Methods:** 9231 treatment changes episodes were extracted from an integrated patient database. The virological response after 8, 24 and 48 weeks was dichotomized to success and failure. Success was defined as the achievement of a viral load of less than 50 copies/ml or alternatively, a 2 log decrease from the baseline viral load at 8 weeks. The predictive ability of Rega v8.0.2 was analysed in comparison with that of
previous versions (v5.5, v6.4.0 and v7.1.1) and two other algorithms (ANRS v2011.05 and Stanford HIVdb v6.0.11). In the univariate approach a logistic model based on the genotypic susceptibility score was used to predict virological response. In the multivariate approach additional confounding factors were added to the model such as: age, gender, risk group, baseline viral load and CD4 count, year of therapy start, introduction of a new drug class in the regimen, number of previous therapy switches and information on drug class experience (defined as more than 1 year on NRTIs, NNRTIs or PIs, respectively). Performance of the models was assessed using the area under the ROC curve (AUC) and compared using a Wilcoxon signed-rank test.

Results: Per unit increase of the GSS reported by Rega 8, the odds on having a successful therapy response on week 8 increased by 81% (OR = 1.81, 95%CI = [1.76-1.86], P<0.001), on week 24 by 73% (OR = 1.73, 95%CI = [1.69-1.78], P<0.001) and on week 48 by 85% (OR=1.85, 95%CI = [1.80 - 1.91], P<0.001). No significant differences were found between the performance of subsequent versions of the Rega algorithm. Inclusion of additional factors increased the performance with respect to the univariate model.

Conclusions: Rega 8 is a significant predictor for virological response and although there is no significant increasing trend in the performance, using the latest versions is recommended because of addition of recent drugs. Additional variables should be taken into account to ensure an effective regimen.

No conflict of interest

Abstract: P_12

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

Hepatitis C virus screening project of patients on current anti-HCV therapy

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Introduction/Background: Many patients suffering from chronic Hepatitis C Virus (HCV) infection do not respond to the dual treatment combination of the unspecific antiviral agents pegylated interferon alpha (IFNα) and ribavirin (RBV). Currently direct-acting-antivirals (DAA) are available. Therapy outcome depends on both host and viral factors, such as IL28B cytokine polymorphisms, viral genotype or presence of viral resistance mutations. The aim of this prospective, non-interventional study is to collect data about HCV baseline polymorphisms and resistance mutations in the NS3 protease gene (targeted by currently-approved DAAs), viral quasispecies distribution and, clinical outcome from patients on dual and triple therapies.

Material & Methods: Viral RNA was gained from blood samples. A conserved region within the NS5B was amplified, sequenced and subtyped using the geno2pheno[HCV] (http://hcv.bioinf.mpi-inf.mpg.de/). Subsequently, the protease domain of the NS3 region was amplified using subtype-specific primers, sequenced and also analysed with geno2pheno[HCV] with respect to resistance mutations to Boceprevir (BOC) and Telaprevir (TPV). If possible, host IL28B polymorphism was tested by sequencing. Patients were subgrouped in 3 cohorts. Cohort 1 included patients on dual (IFNα+RBV) therapy, cohort 2 patients on IFNα+RBV+BOC therapy and cohort 3 patients on IFNα+RBV+TPV therapy.

Results: Cohort 1 included 62 patients, whereof 19 were infected with 1a viruses, 16 with 3a and 16 with 1b. For 7 patients, host’s DNA was available: IL28B polymorphism distribution was: one CC, five CT, and one TT. The protease region from 56 samples could be amplified and sequenced. In 2 samples resistance mutations against protease
inhibitors were detected (117H; 168G). Cohort 2 included 5 patients. The genotype distribution was: four patients with subtype 1a and one 1b. IL28B polymorphism distribution in these patients was: one CC and two CT. No resistance mutations were detected at baseline, but the 1b virus from an IL28B CT-patient developed 36A after the lead-in phase although VL at week 8 was 18IU/mL. Cohort 3 included 16 patients. Twelve patients were infected with 1a viruses and four with 1b. The IL28 polymorphism was analysed in 12 patients: two CC, nine CT, and one TT. The protease region of all baseline samples could be amplified and sequenced. In 5 (four 1a, one 1b) samples from IL28B CC/CT-patients resistance mutations against protease inhibitors were detected (36A; 36M+155K; 36L; 54S+155K). All patients showed VL<12 IU/mL at week 4.

**Conclusion:** The current strategy of amplification and sequencing of a conserved region within the NS5B and interpretation with geno2pheno[HCV] allows genotyping/subtyping. Analysis of NS3 with geno2pheno[HCV] allows the prediction of susceptibility to the protease inhibitors Boceprevir and Telaprevir. By collecting additional viral and clinical data from patients treatment we will continuously improve the geno2pheno[HCV] for the interpretation of therapy failure.

No conflict of interest

**Abstract: P_13**

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

**Association between early development of Entecavir mutation and partial virological response in treatment-naive patients with chronic Hepatitis B**

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**Introduction/Background:** The emergence of Entecavir (ETV) resistance is rare in nucleoside-naive patients. The aims of our study investigate the incidence of ETV mutation and evaluate the clinical association between early development of ETV mutation and long term outcome in treatment-naive chronic hepatitis B(CHB) patients.

**Material & Methods:** 156 treatment-naive patients with CHB, who visited Chung-Ang University Hospital between January 2007 and January 2010, were enrolled in this study. The mean duration of treatment was 49 months (range, 24-73). Virological response(VR) was defined as undetectable HBV DNA by real-time PCR assay (<116 copies/mL) at year 1. Partial virological response(PVR) was defined as detectable HBV DNA(>116 copies/mL) at year 1. Stored serum samples from 20 VR patients and 22 PVR patients were analyzed antiviral resistant mutations of HBV reverse transcriptase region using direct DNA sequencing assay.

**Results:** Among 20 VR patients, 10 patients were HBeAg negative and mean initial HBV DNA levels were 7.7 log10 copies/mL (range, 5.8-9.4). In 7 patients, rtM204V/M, and rtT184L/T mutations on direct sequencing were detected at year 1. However, in only 1 patient, the level of HBV DNA was 5.1 log10 copies/mL at month 42. The others showed undetectable HBV DNA level. On the contrary, in 22 PVR patients, only 4 patients were HBeAg negative. Initial HBV DNA levels were 7.6 log10 copies/mL (range, 3.6-9.9). Among them 16 patients showed several mutations such as rtL180M, rtM204V/M, and rtT184L/T at year 1. Serum HBV DNA levels were detectable in 7 patients with ETV mutations during treatment period. However, the levels of serum HBV DNA were sustained to low titer (189-4,072). There was no biochemical breakthrough.

**Conclusions:** The ETV mutations can evolve with a relatively significant incidence in nucleos(t)ide-naive CHB patients. The emergence of genotypic resistance of ETV at 1 year, might be useful predictor for long term therapeutic efficacy in treatment-naive CHB patients

No conflict of interest

No conflict of interest
Abstract: P_14

The molecular variations an
genotypes of HBV in Southern
part of Iran

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Background: Hepatitis B virus (HBV) is an
agent of acute and chronic hepatitis, chronically infecting around 400 million
individuals worldwide. The morbidity and
mortality of per-sistient HBV infection are a
major public health concern. The aim of this
study was to determine the genotypes of HBV
in Southern part of Iran with and to
characterize the molecular variations of the
chronic patients.

Methods: This cross-sectional study has been
carried out on 12 blood donors from the
Khoozestan province, south Iran, who
diagnosed to be HBsAg positive. HBV DNA
was extracted by using a QIAmp DNA Blood
minikit (Qiagen, Hilden, Germany), according
to manufacturer’s instruction. PCR was carried
out in 100 µl of a mixture containing 5 µl of the
extracted DNA, using 2 pairs of specific primers
for complete surface gene amplification. A
quantity of the second round PCR products
were analyzed by electrophoresis in 1%
agarosegel, stained by ethidium bromide, and
visualized under u.v. light. The nucleotide
sequence of the S gene was determined with
anABI-3130 DNA Sequencer genomic
sequences obtained for the HBV S gene
were compared with all HBV genotype D
references sequences used on the NCBI
website as well as from the published and
unpublished Iranain sequences from the
Genbank. Sequences have been submitted to
GenBank, numbered from HM348682-
HM348693. The evolutionary history was
inferred using the Neighbor-Joining conducted
in MEGA4.

Results: The results of the phylogenetic tree
revealed that Iranian HBV isolates from
Khoozestan were of genotype D and
subgenotype D1. Overall, comparing with
reference sequence (Okamoto, 1988), at the
nucleotide level, of a total of 116 changes in 28
positions, 86 (74%) and 30 (26%) were silent
and misses. At amino acid level, 17
substitutions occurred. The average mutation
frequency of all sequences was 0.6 according
to the number of mutations per site. 15 (88%)
out of 17 amino acid mutations occurred in
different immune epitopes within surface
protein, of which 7 (46.6%) in B cell epitopes in
5 residues; 7 (46.6%) in T helper epitopes in 6
positions; 1 (7%) in inside CTL epitopes in 1
residue. 2 mutations were occurred in ‘a’
determinant: Y134D and S143P.

Conclusion. The proportion of deduced amino
acid changes in our chronically infected
patients was high, and that these proteins
were under a significant positive selection
pressure which had already been applied by
both arms of cytotoxic and humoral host
immune system: 15 (88%) out of 17 amino
acid mutations occurred in different immune
epitopes within surface protein. The
distribution of the mutations within known
surface protein immune epitopes reflects the
virus-host interaction with a prolonged infection
period. The consequence of selection pressure
posed by anti-S antibodies would be the
emergence of immune escape mutations in
this protein which no longer could be
recognized by the host immune system.
Viruses carrying such mutated T-cell epitopes
cannot be recognized by specific T-cells of an
individual, hence, will not enhance anti-HBs
production; this could be led to the progression
of chronic of hepatitis B virus infection.

No conflict of interest
Abstract: P_15

Spread of Drug Resistance

Baseline resistance to integrase strand inhibitors in newly diagnosed patients in Spain

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Background: Overall, a 30-40% prevalence of resistance mutations is currently detected in patients failing Raltegravir in Spain. Thus, potential transmitters may be circulating in Spain and transmitted INSTI drug resistance may be expected.

Aim: to estimate the prevalence of INSTI transmitted drug resistance (TDR) in all the newly diagnosed patients, submitted for protease (Pro) and reverse transcriptase (RT) TDR surveillance to the Hospital Universitario San Cecilio HIV Resistance Referral Laboratory, South of Spain, across 2011 and 2012.

Patients and Methods: we have performed a cohort observational study on 138 newly diagnosed patients. RT & Pro were sequenced with the Trugene HIV-1 genotyping Kit, and the integrase sequence, covering codons 45 through 287, was determined using an ‘in house’ protocol. For integrase TDR analysis, changes in E92Q, Y143RHC, Q148RHC and N155H were considered as primary mutations, and changes in T97A, L74M, E138AK, G140ACS, S147G, V151I, N155S, E157Q, I203M, S230NR, as secondary mutations; all polymorphisms in the integrase region were also analyzed, using a subtype B strain HXB2 as reference. For RT & Pro TDR, the WHO 2009 updated list was considered. Additional variables analyzed in the study were age, sex, and transmission way, country of origin, date of infection, CD4 count, viral load and Pro-subtype.

Results: mean age of the population was 37 years (IQR 30-45), 78.3% were male, and 13.8% were men having sex with men. Median viral load (log10 copies/ml) and median CD4 count (cells/ul) at diagnosis were 4.9 (IQR: 1.38-6.85) and 323 (IQR: 111-494), respectively. 71% of the patients were Spanish, 12.3% came from Africa, 8.7% from South America and 4.3% were from other European countries. 20.2% of the patients were infected by a non-B subtype. No INSTI primary resistance mutations were found, and only two patients (1.4%) carried the I203M secondary mutation. The most prevalent polymorphisms were G123S (76%), R127K (75.3%) V72I (57.9%), T125A (30.4%), L101I (27.5%), A124T (20.9%), T122I (17.4%) and M50I (11.5%). Protease, NNRTI and NRTI TDR was 1.4%, 5.1% and 1.4%, respectively, being K103N (3.6%), T215S (1.4%) and K219Q (1.4%) the most prevalent mutations.

Conclusion: Transmitted drug resistance to integrase strand inhibitors remains an unfrequent event in the South of Spain, so there is no need for routine testing of integrase baseline resistance. However, surveillance programs to monitor INSTI TDR should be implemented.

No conflict of interest

Abstract: P_16

Spread of Drug Resistance

Genetic characterization of HIV-1 strains and cellular HIV-1 DNA quantification of newly diagnosed antiretroviral naïve patients in Northern Greece

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**Background:** The molecular epidemiology of HIV-1 infection and the transmittance of antiretroviral drug resistance were studied in Northern Greece in the period 2000 to 2007 but the extent of HIV-1 diversity and the prevalence of transmitted drug resistance (TDR) have since remained elusive. In an effort to address this issue, the present study examined HIV-1 strains from 98 newly diagnosed untreated patients, representing 45.3 % of the total number of registered HIV-1-seropositive patients from the AIDS National Reference Laboratory of Northern Greece in the period 2009 to 2010.

**Materials & Methods:** DNA sequences encoding the **pol** (protease and reverse transcriptase), and **env** (C2-C5 region of gp120) were amplified from plasma RNA and peripheral blood mononuclear cells (PBMC) DNA, respectively. All amplified products were analyzed according to previously established genetic methodologies to determine the genetic subtype, the prevalence of treatment-associated polymorphisms in protease and reverse transcriptase to currently available antiretroviral drugs and the co-receptor tropism. Quantitative measurements of HIV-1 DNA in PBMC, were also determined in all patients in an effort to investigate further the association between cellular HIV-1 DNA levels and transmitted resistance to antiretroviral therapy in newly diagnosed patients across Europe.

**Results:** Analyses of the obtained viral sequences indicated that subtypes B and A1 were the most common subtypes present and accounted for 44.9 and 42.9% respectively, followed by subtype C (3.1%), CRF02_AG (4.1%), CRF04_cpx (2.0%), and subtypes CRF01_01, F1 and G (1.0%). The overall prevalence of TDR to current HIV-1 antiretroviral drugs was 17.34%, the prevalence of nucleoside reverse-transcriptase inhibitor (NRTI) resistance was 12.24% (12 of 98 patients), the prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance was 5.10% (5 of 98 patients) and the prevalence of protease inhibitor (PI) was 1.02% (1 of 98 patients). Three drug resistance mutation clusters were defined by the molecular phylogenetic analysis and all the HIV-1 strains were isolated from men were having sex with men (MSM). Cellular HIV-1 DNA load measurements resulted in a median of 3.309 log10 HIV-1 copies per 10^6 PBMC and demonstrated no correlation between cellular HIV-1 DNA load and transmitted drug-resistance. An absence of association between cellular HIV-1 DNA levels with plasma viral RNA load and CD4^+ T-cell counts was also reconfirmed. Co-receptor tropism for 96% of samples, whether or not they conferred resistance, was CCR5.

**Conclusions:** These newly found data demonstrate a heterogeneous epidemiological status of HIV-1 in northern Greece with subtype B and A1 being the dominant subtypes in relation to the other subtypes. The prevalence of antiretroviral resistance mutations is high among the newly diagnosed untreated patients in comparison with other European countries. The findings show no correlation between cellular HIV-1 DNA load and transmitted drug-resistance, suggesting that transmitted resistance does not impact disease progression in HIV-1 infected individuals. The CCR5 co-receptor tropism predominance implies that both resistant and non-resistant strains behave similarly in early infection.

No conflict of interest

**Abstract:** P_17

**Spread of Drug Resistance**

**Prevalence of HIV-1 transmitted drug resistance among patients diagnosed in 2011-2012 in Slovenia**

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**Background:** Two previous studies reported the prevalence of transmitted drug resistance (TDR) of 3.9% and 4.7% among therapy naïve Slovenian HIV-1 patients diagnosed in 2000-2004 and 2005-2010, respectively (Babic DZ...
et al. Virus Res 2006;118:156-163; Lunar MM et al. AIDS Res Hum Retroviruses 2013; in press.). Since the prevalence of TDR identified in Slovenia was lower than in other European countries, routine genotypic resistance testing in all newly diagnosed individuals was not implemented, except in selected patient groups (e.g. pregnant women, patients known to be infected abroad). Here we report results for 2011-2012.

**Material & Methods:** A total of 101 persons were diagnosed with HIV-1 in 2011-2012 in Slovenia. Among these 53 (52%) with an available baseline plasma sample and questionnaire were included for analysis of TDR. HIV-1 RNA was extracted from 400 µl of a patient’s plasma sample and partial pol gene sequences of approximately 1,000 bp were obtained by bidirectional Sanger sequencing. The presence of resistance mutations was determined according to the surveillance drug resistance mutation (SDRM) list, updated in 2009. Subtype of the sequences was assigned by using the REGA HIV-1 Automated Subtyping Tool, version 2.

**Results:** Mutations associated with TDR were detected in 3 out of 53 (5.7%) of patients, namely 2 mutations conferring resistance to nucleoside reverse transcriptase inhibitors (T69DGNS and T215D) and one to non-nucleoside reverse transcriptase inhibitors (K103N). All three patients reported homo/bisexual mode of infection, two of them were infected with subtype B virus and for one subtype was not possible to assign. Two patients were infected in Slovenia, whereas one was infected abroad. Subtype B was detected in 44 patients (83%), subtype A in 6% (3/53), subtype C in 4% (2/53) and G in 2% (1/53). For 3 patients (6%) subtype was not possible to assign using Rega subtyping tool.

**Conclusions:** A trend of increasing prevalence of TDR among treatment naïve patients in Slovenia in last decade was observed, affirming the need of continuing surveillance of TDR in our country.

The research leading to these results received funding from the European Community’s Seventh Framework Programme (FP7/2007-2013) under the project ‘Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)’ - grant agreement n° 223131.

No conflict of interest

**Abstract: P_18**

**Spread of Drug Resistance**

**Transmission of resistant HIV-1 in Utrecht, the Netherlands, in 2004-2011**

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**Background:** Transmission of drug resistance in HIV is associated with an increased risk of therapy failure. The prevalence of transmitted drug resistance in Europe is approximately 10%. Transmission of resistance to nucleoside reverse transcriptase-inhibitors (NRTIs) and protease-inhibitors (PIs) is declining, while the resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs) is increasing, in line with the increased use of NNRTIs in first line regimens. Our aim was to estimate the prevalence of transmitted drug resistance in newly diagnosed HIV-patients in Utrecht in the period 2004-2011.

**Methods:** In the period 2004-2011, 573 newly diagnosed therapy naïve HIV-infected patients entered care in the University Medical Center Utrecht. In 523 patients (91.3%) baseline genotyping was performed within 6 months of diagnosis and available for analysis. Prevalence of mutations associated with transmitted drug resistant HIV-1 was analyzed using the list of mutations for surveillance of transmitted drug resistance, defined by the WHO. Among those with a drug resistance mutation, we predicted the level of drug resistance as reported by the Stanford HIVdb Algorithm v6.2.0. Phylogenetic analysis was performed with the Tamura-Nei model of evolution, using bootstrap analysis with 1000 replicates (MEGA5).

**Results:** We included between 50 and 79 patients within each year. The majority was male (85.1%) and the mean age at time of diagnosis was 39 ± 11 years. 426 patients (81.5%) were from Dutch origin and 53 patients (10.1%) originated from Sub Sahara African countries.
Africa. Route of transmission was mainly sexual: 340 infections (65%) occurred through homosexual contact, 127 (24.3%) through heterosexual contact. Most patients were infected with subtype B virus (74.8%). The overall prevalence of transmission of resistance in 2004-2011 was 11.9%. The prevalence in 2011 was 7.3%. The prevalence of resistance by drug class was 6.3% for NRTIs, 1.3% for NNRTIs, 3.1% for PIs and 1.1% multiclass resistance. In 2007/2008, a prevalence of resistance of 22.9% was observed. Phylogenetic analysis revealed that this was the result of two large transmission clusters, one with an M41L and one with an M46L mutation. Excluding these transmission clusters, a stable prevalence of transmitted drug resistance against NRTIs was observed. Resistance against NNRTIs slightly increased over time. The most frequent observed mutation was M41L (19 cases), M46L (14 cases), T215 revertants (12 cases) and K103N (8 cases). A singleton M41L is not predicted to confer resistance to NRTIs. In 25 patients (4.8%) Stanford Algorithm predicted that the present resistance mutations generated decreased susceptibility to drugs used as first-line antiretroviral therapy. Predicted high-level resistance was mostly observed for NNRTIs, based on the presence of K103N.

Conclusion: The prevalence of transmitted drug resistance in Utrecht was 11.9% in the period 2004-2011. One out of twenty patients was infected with a virus conferring decreased susceptibility to drugs that are currently used as first line antiretroviral therapy in clinical practice.

No conflict of interest

Abstract: P_19

Viral Evolution & Genetic Diversity

Genetic analysis of HIV-1 variants circulating in the Russian Far East

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Background: Since 1996, Russia has been experiencing an explosive HIV-1 epidemic among IDUs and their sexual partners with predominance of variant IDU-A. Since 2005, variant IDU-A has been moving outside this group of risk and spreading through sexual contacts. This strain was registered in various regions of European territories of Russia, Urals and Siberia and caused all the largest outbreaks in Russia. HIV-1 subtype B variant is quite rare in Russia and distributing mainly among MSM. Numerous cases of infection caused by the recombinants CRF03_AB and CRF02_AG were registered in the surveyed areas of Russia. The genotyping of HIV-1 in the Russian Far East (administrative centers Blagoveshchensk, Vladivostok and Khabarovsk) has never been carried out due to remoteness of these regions from well-experienced research centers. Molecular-epidemiological studies in this region were of particular interest because this district borders with China and are close to Japan, where another than Russian genetic HIV-1 variants are circulating. The subject of this study was to characterize HIV-1 genetic strains currently circulating in administrative centers of the Russian Far East.

Material & Methods: Blood samples were collected, with informed consent, in 2012 in Khabarovsk Blagoveshchensk and Vladivostok from 169 HIV-infected IDUs and sexually-infected persons. All of them were analyzed in pol gene region with ViroSeq HIV-1 Genotyping System v. 2.0 (Celera Diagnostics, Alameda, CA) or in house-system and subtyped by REGA HIV-1 Subtyping Tool Version 2.0 and COMET HIV-1/2 (version 0.2).

Results: In Vladivostok the HIV-1 variants belonging to subtype A1 were found in 18/80 (22.5%) cases, to subtype B – in 43/80 (54%), to subtype C – in 18/80 (22.5%) and one person was infected by CRF02_AG. In Blagoveshchensk the HIV-1 variants belonging to subtype A were found in 37/40 (92.5%), to subtype B – in 1/40 (2.5%), and two cases of infection were caused by the recombinant CRF02_AG. In Khabarovsk 28/49 (57%) patients were infected by A1-variants, 9/49 (18%) by B-variants, 9/49 (18%) - by
CRF02_AG strains, 3/49 (6%) - by C-variants. According to phylogenetic analysis results, A1 sequences formed the common branch with nucleotide sequences of IDU-A strains found in other regions of Russia. Most of HIV-1 variants belonging to subtype C clustered together with nucleotide sequences of C variants from North-East Africa but not with strains from China, where this subtype dominates. HIV-1 variants of subtype B were quite heterogeneous and sub-clustered with sequences of East European B-variants and genetically distinct from strains associated with MSM transmission. The recombinant strains AG formed the common branch with sequences from Asian republics of former USSR.

Conclusion: The Russian Far East is characterized by the marked genetic polymorphism of HIV-1. Perhaps it is due to the fact that Vladivostok is the largest Russian port on the Pacific Ocean, and Khabarovsk is the port of the ‘river-sea’ ships and transportation links. So, the intensive human migrations can play an important role in the spreading of HIV-1. Vladivostok is the first region in Russia where the outbreak of HIV-1 infection is caused by HIV-1 strain of subtype B.

No conflict of interest

Abstract: P_20

Viral Evolution & Genetic Diversity

Ongoing influx of different HIV-1 lineages and low transmitted drug resistance among antiretroviral naive infected individuals in Cyprus (2010-2012)

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Background: A major characteristic of HIV-1 is its rapid evolution, which has resulted in substantial genetic diversity among different isolates, the majority of which are represented in areas in which multiple HIV-1 clades of the major group M co-circulate. Previous studies showed a very heterogeneous genetic pool of HIV-1 strains in Cyprus and this directed us to determine the dynamics of the local HIV-1 infection during 2010-2012 by characterizing HIV-1 strains isolated from 84 antiretroviral naïve HIV-1 infected individuals.

Materials & Methods: Nucleotide sequences of the pol (protease and reverse transcriptase) were amplified by RT-nested PCR using diluted RNA from all HIV-1 seropositives and sequenced using an in house designed assay. Detailed phylogenetic and bootscanning analyses were performed to determine subtype assignments, phylogenetic associations and to explore putative recombination patterns in the sequences, respectively. Drug resistance was determined according to the Genotypic Resistance Interpretation Algorithm guidelines of the Stanford HIV Drug Resistance Database.

Results: Phylogenetic analyses of the obtained viral sequences indicated subtype B as the main subtype (40.4%), followed by subtype A1 (19.0%), C (9.5%), CRF02_AG and F1 (4.8% each), A2 (2.4%), CRF01_AE (3.6%), and CRF06_cpx, CRF28_BF and CRF13_cpx (1.2% each). Eight HIV-1 isolates (9.5%), were not classified in any pure (sub)subtype or circulating recombinant form (CRF). Complete phylogenetic and bootscanning analyses revealed that each isolate had a recombinant pattern in pol (protease and reverse transcriptase) region and is distinct from any other known CRFs or unique recombinant forms (URFs). Three antiretroviral naïve HIV-1 infected individuals out of 84 (prevalence of 3.6%) showed resistance, two in nucleoside reverse-transcriptase inhibitors (NRTIs) and one in non-nucleoside reverse-transcriptase inhibitors (NNRTIs). No resistant mutations associated to protease inhibitors (PIs) were identified.

Conclusions: Analogous to the results of earlier epidemiological studies, this study expands on the HIV-1 sequence database and reveals the high degree of diversity of HIV-1 infection in Cyprus. In the newly diagnosed individuals, subtypes B and A are dominant in relation to the other subtypes and the fact that
only three patients had resistance in NRTIs and NNRTIs shows a low prevalence of transmitted drug resistance compared with most other European countries. Remarkably, the eight natural intersubtype recombinant forms need to be characterized through their whole full-length genome. The increasing cases of recombinant HIV-1 strains underline the significance of their contribution to HIV classification and may have important implications for HIV-1 disease control, vaccine development and surveillance.

No conflict of interest

Abstract: P_21

Viral Evolution & Genetic Diversity

Protease inhibitors and HCV NS3: reconstructing intra-host population dynamics

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Background: Not all hepatitis C patients respond to treatment with protease inhibitors such as telaprevir (2011), boceprevir (2011), and danoprevir (Phase III). Resistance mutations in the viral protease NS3 are believed to have a determining influence on the treatment's effectiveness. Serial sequencing lets us track the emergence of resistance mutations within a population. Next-generation technologies such as pyrosequencing make it possible to detect mutations at incidence ≥1%, offering unprecedented resolution of the population structure of the viral quasispecies. Long-term follow-up can tell us whether resistant strains revert to wild-type after treatment. Wild-type is defined here as the absence of resistance mutations.

Material & Methods: The study involves 15 patients infected with HCV subtype 1a, all non-responders; 2-6 time-points spanning a period of up to 5 years; and three protease inhibitors – telaprevir, boceprevir, and danoprevir. Protease inhibitor monotherapy lasts 2 weeks. 6 patients were sampled before the start of telaprevir monotherapy and again 3.5-5 years later; 3 more telaprevir patients were each sampled 5 times over a period of 8-18 months; 3 boceprevir patients were each sampled 3-5 times over a period of 22-51 months; 3 danoprevir patients were each sampled 5 times over a period of 15-22 months. For every sample of hepatitis C virus, the protease region of viral protein NS3 was sequenced using Roche/454 pyrosequencing, generating on the order of 1000 reads from each timepoint. Viral haplotypes are inferred using hierarchical clustering. A nearest-ancestor evolutionary network is reconstructed for each patient.

Results: Intra-host evolutionary networks demonstrate broadly coherent structure in terms of propagation of resistance mutations and variant abundances. Varied branching structures provide evidence for both strong and weak selection, consistent with the assumption that evolution will be weak over short time intervals and/or in the absence of protease inhibitor, and otherwise strong. Results suggest that reversion to wild-type (i.e. the extinction of resistant strains) does occur and takes as little as 13 months.

Conclusions: Next generation sequencing technologies have made it possible for the first time to directly observe molecular evolution at the intra-host population level. In this context, trials of HCV protease inhibitors have the potential to become valuable case studies of evolution with strong selection.

No conflict of interest

Abstract: P_22

Viral Evolution & Genetic Diversity

The prevalence of GBV-C and its associations with HIV infection among Estonian intravenous drug users
Background GB virus C (GBV-C) is a RNA virus of the Flaviviridae family that has not been associated with any disease. Previous studies have indicated a beneficial effect of GBV-C on HIV-1 disease progression. The frequency of GBV-C among HIV infected individuals varies between populations from 20% to 40%. We aimed to determine the prevalence of GBV-C and the distribution of it genotypes among Estonian injecting drug users (IDUs) and to evaluate associations between GBV-C, HIV-1 infection and co-infections.

Methods: The study included 344 IDUs from a syringe exchange program in Tallinn in 2011. The presence of GBV-C RNA was determined by reverse transcriptase-nested PCR in 5' non-coding region. PCR products were sequenced and thereafter genotyped by phylogenetic analysis. Differences in the distribution of GBV-C between infection status were compared by Fisher exact test. Uni- and multivariate logistic regression were used to determine associations between GBV-C and HIV infection.

Methods: Of total half (172/344) of subjects were HIV-positive, 88.7% (305/344) HCV-positive, 18% (63/344) HBV-positive and 33% (114/344) GBV-C-positive. Out of 114 sequenced GBV-C viruses 78% belonged to genotype 2a, 20% to genotype 2b and two viruses remained unclassified. The distribution of GBV-C between infection status were compared by Fisher exact test. Uni- and multivariate logistic regression were used to determine associations between GBV-C and HIV infection.

Conclusions: GBV-C infection is more common in HIV-positive IDUs than HIV-negative from Estonia and similar to other European countries and Russia the genotype 2a is prevailing.

No conflict of interest

Abstract: P_23

Viral Evolution & Genetic Diversity

HIV-1 subepidemic in the Romanian intravenous drug users (IDUs): a phylogenetic analysis

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Background: An increase in HIV-1 infections among IDUs was reported in Romania during recent years, from 3% in 2010 to 19% in 2011. The trend continued through 2012 when approximately 30% (159 persons) of the newly HIV-1 diagnosed cases were reported having this particular risk factor. Our objective was to analyze in details the characteristics of this new epidemic by using phylogenetic approaches.

Methods: A number of 68 IDUs newly diagnosed with HIV infection in 2011-2012 were included in this study. Epidemiological and demographic data were collected along with the blood samples. HIV-1 strains were genotyped in the protease and reverse transcriptase genes by using Viroseq™ HIV-1 Genotyping System and 3500 Genetic Analyzer (Applied Biosystems). HIV-1 subtyping was done using the publicly
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Available REGA HIV-1&2 automated subtyping tool version 2.0. HIV-1 sequences from IDUs and references available from public databases were aligned using ClustalW. Phylogenetic analysis was performed using maximum likelihood as implemented in PAUP, using the GTR (general time reversible) as nucleotide substitution model and gamma (Γ) distribution of rate variability among sites, calculated empirically from the data with 6 categories of rates. Bootstrapping was performed on the neighbor joining trees (1000 replicates) to assess the reliability of the obtained topologies.

Results: The majority (88%, 60/68) of the studied patients were young men (20-34 years), with secondary education level and unemployed, from Bucharest area. 16 of the 60 men were diagnosed while being in prison. More than 80% of the patients injected both heroin (median duration practice 10 years) and new psychoactive substances (in the last 1-3 years). No major resistance mutations were detected in the studied samples. Subtype analysis showed that 65 % of IDUs (n=44) were infected with subtype F1 viruses and 24% (n=16) with CRF14_BG. Subtype B was detected in 3 IDUs and the same number of patients were carrying subtype G strains. Phylogenetic analysis of the F1 sequences revealed 2 major transmission groups: one consisting 29 sequences, very closely related to each other and the second one 7 sequences; 3 of them sampled from men belonging to the same prison. CRF14_BG transmission was associated with traveling outside the country (p=0.02, OR 4.64), but these sequences cluster separately from those reported in Spain and Portugal suggesting local spread. More similarity was observed when compared with sequences coming from Greece, from the same risk category of patients.

Conclusions: Most of the HIV transmission events within the IDU community involve local F1 strains clustered in few transmission networks and an increasing number of CRF14_BG strains that circulate in Southern Europe.

No conflict of interest

Abstract: P_24

Viral Evolution & Genetic Diversity

Key patterns of mutations in HBsAg correlate with different levels of serum HBV DNA in the absence of therapy pressure

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Background: High serum HBV-DNA correlates with increased risk of cirrhosis and liver cancer. However, the risk of end-stage liver disease can still persist even with serum HBV-DNA <2,000IU/ml. Here, we define HBsAg genetic markers correlated with different levels of serum HBV-DNA in HBV chronically-infected drug-naive patients (pts).

Methods: This study includes 187 HBsAg + pts (134 genotype D, 53 genotype A, 37 are HIV-coinfected) stratified into the following ranges of serum HBV-DNA: 12-2,000IU/ml (N=58), 2,000-100,000IU/ml (N=60), and >100,000IU/ml (N=69). Association of HBsAg mutations with HBV-DNA was assessed by Fisher-test and multivariate logistic regression.

Results: Specific HBsAg mutations significantly correlate with HBV-DNA <2,000IU/ml. This is the case of Y206C, Y206H, and F220L occurring in 13.8% (8/58), 12.1% (7/58) and 13.8% (8/58) of pts with HBV-DNA <2,000IU/ml, in 3.3% (2/60), 3.3% (2/60) and 2.3% (2/60) of pts with HBV-DNA ranging from 2,000 to 100,000IU/ml, and in 0% (0/69), 1.4% (1/69) and 1.4% (1/69) of pts with HBV-DNA >100,000IU/ml (P<0.01). Y206C/H+F220L also form a tight cluster (bootstrap=0.99) observed only in patients with HBV-DNA <2,000IU/ml. Multivariate logistic regression analysis confirms the correlation of Y206C/H and F220L with HBV-DNA <2,000IU/ml after adjusting for patients' demographics, HBeAg, HIV co infection and HBV genotype (OR[95%CI]:6.5 [1.6-26.1], P=0.008 for Y206C/H, 7.2 [0.9-54.2], P=0.03 for F220L). The presence of Y206C/H or

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F220L also correlates with lower median [IQR] HBsAg levels (250 [115-840]IU/ml in pts with Y206C/H or F220L vs 4,300 [640-11,838]IU/ml in pts without them, P=0.023). Y206C/H and F220L localize in the C-terminal transmembrane domain critical for HBsAg-secretion and potentially involved in liver cancer development. An opposite situation is observed for Y100C occurring in 10.1% (7/69) of pts with HBV-DNA>100,000IU/ml, and in only 0.8% (1/118) of pts with HBV-DNA<100,000IU/ml (P<0.001). Y100C localizes in HBsAg domain involved in capsid interaction and in HLA epitope encompassing amino acids 97-106.

Conclusions: Key HBsAg mutations, localized in domains critical for HBsAg function or HBV immune control, modulate HBV replicative potential. The potential intracellular HBsAg retention associated with Y206C/H and F220L might explain why the risk of liver cancer can persist in patients with HBV-DNA<2,000IU/ml, and thus their role as predictive markers of disease progression deserves further investigation.

No conflict of interest

Abstract: P_25

Viral Evolution & Genetic Diversity

Quantifying an individual node’s spreading power in network epidemiology

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Introduction/Background: Network models are increasingly important for understanding the epidemic dynamics of diseases with heterogeneous transmission probabilities, such as HIV and HCV. Early research in network dynamics developed metrics for identifying network nodes which are highly influential to the spreading process, i.e. those with high degree, k-shell, or betweenness centrality. These measures, however, only rank nodes without quantifying the outcome, ignore the dynamics of the spreading process, and may considerably underestimate the spreading power of non-hub nodes. More recently, the physics community has proposed a number of measures which quantify individual node spreading power. To the best of our knowledge, these are all based on path counting approaches, and are derived within a discrete time framework. We here propose measuring a node’s spreading power using the greater resolution of a continuous-time framework by enumerating actual force of infection generated after two infection events. The measure, called the Expected Force (ExF), is shown to strongly predict the epidemic course in susceptible-infected (SI), susceptible-infected-susceptible (SIS), and susceptible-infected-recovered (SIR) models, in both continuous and discrete time.

Materials and Methods: The ExF is formally defined as the entropy of the distribution of the the number of infected-susceptible edges taken over all possible disease clusters which could arise after two infection events from a given seed node in an otherwise fully susceptible network, assuming no recovery. Extensive simulations are conducted on three classes of synthetic networks differentiated by their degree distribution. The first is a theoretical scale-free distribution (Pareto) and the second two mimic real-world social networks, the astrophysics collaboration network from ArXiv and Facebook wall posts from the New Orleans network. For each network, the ExF is measured for all non-hub nodes, along with the k-shell and the accessibility (a path-counting approach). Outcome measures include the time for an infection to cover half a network in a continuous time SI process, and a node’s probability of seeding an epidemic in both discrete and continuous time SIS and SIR models.

Results: The ExF exhibits a strong linear relationship to all outcome measures for all networks investigated. In the random scale-free networks, the adjusted r-squared values for ExF are over 0.90 for all models but the discrete time SIR, where the adjusted r-squared is 0.83. By comparison, the accessibility has adjusted r-squared in the range 0.70-0.80, with the lowest score also for the discrete time SIR. In the real world social networks, the ExF has adjusted r-squared of 0.71-0.82, while the access has 0.12-0.24. Akaike's information criterion also confirms the
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superiority of the ExF in all cases, in all types of networks tested.

Conclusions: The higher resolution offered by a continuous-time point of view allows extremely accurate quantification of the spreading power of individual nodes in network models of disease spread based only on the node’s local network topology.

No conflict of interest

Abstract: P_26

Viral Evolution & Genetic Diversity

Epidemiological networks and phylogeographic tracing of A1 subtype in Italy indicate three penetration events sustained by two distinct local variants

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Background: Subtype A accounts for around 12% of HIV-1 infections worldwide. A1 strains predominate in Russia and in Former Soviet Union (FSU) countries of Eastern Europe as a monophyletic variant of Central African origin. After an early and limited propagation via heterosexual contacts, this variant spread explosively among intravenous drug users (IDUs) and their sexual partners. A discrete A1 variant predominates also in Greece and

Albania as monophyletic strain that penetrated directly from Africa. Clade A1 accounts for 12.5% of non-B subtypes in Italy, being the most frequent after F1 subtype. Aim of this study was to investigate the features of A1 subtype circulation in our country and trace its origin and diffusion through phylogenetic and phylodynamic approaches.

Methods: We analyzed 113 A1 pol sequences included in the Italian ARCA database during the 1999-2011 period, obtained from both naïve and treated patients. The transmission networks were identified by Mr Bayes and Beast programs (GTR+I+Γ model). Geographical flows were identified using Bayesian approaches implemented in Beast and SPREAD programs.

Results: Italians, Europeans, Africans, South Americans and Asians accounted for 46.9% (n=53), 15.9% (n=18), 8.8% (n=10), 1.8% (n=2) and 0.1% (n=1) of patients with known country of origin, respectively. Known risk factors were distributed as follows: 64.3% (n=27) HEs, 19% (n=8) MSM and 16.7% (n=7) IDUs. Males accounted for 59% of patients (n=62). The phylogenetic analysis indicated that 71 patients (62.8%) clustered within 5 clades. A single network encompassed 59 isolates (83.1% of clustering sequences). A higher probability to be detected in clusters was found for patients from Eastern Europe and Italy (88.9% and 60.4%, respectively) compared to those from Africa (20%) (p<.001). The proportion of clustering sequences was slightly higher in IDUs (85.7%) and HEs (59.3%) compared to MSM (25%) (p=0.056). Clustering sequences harbored by women (n=35, 81.4%) showed a higher proportion compared to those carried by males (n=33, 53.2%) (p<.006). Among female HEs 80% were from Eastern Europe. Dated phylogeny indicated an African origin 30.1 years before 2011. Phylogeographical reconstruction highlighted 2 significant groups. One involved East European and Italian variants, the second encompassed some Italian and African strains and all sequences from Albania and Greece. Bayesian analysis of fluxes showed three main highly significant links (BF>3.0): a former introduction of African variants into Italy around 1989 (BF=5,651.0), and two subsequent albeit simultaneous fluxes from Greece/Albania and Moldavia/Ukraine (BF=28,267.6 and 28,667.6, respectively) around 2000. A subsequent analysis investigating local geographical A1 variants revealed that Moldavian and Ukrainian
strains segregated separately from African, Greek and Albanian ones.

**Conclusions:** As in previously documented A1 epidemics of East European countries, HIV-1 A1 subtype spread in part through IDUs in Italy. However, Eastern European females contributed to the penetration of such variant probably through sex work. Phylogeographical reconstruction suggests that A1 clade entered Italy through three distinct introduction events: a first getaway took place directly from Africa while two more recent and coeval events occurred from the Southern Balkan peninsula and the area encompassing Moldavia and Ukraine.

No conflict of interest

**Abstract: P_27**

**Detailed phylogenetic and phylogeographic analysis of HIV-1 in the Scandinavian region**

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The HIV-1 epidemic in the Scandinavian region is characterized by multiple subtypes and CRFs. Frequent travelling within Scandinavia may affect the migration patterns of HIV-1 and lead to outbreaks that span country borders. Here, we analysed 4,003 pol sequences (approximately 1,000 bp) from Sweden, Denmark and Finland collected 1982-2012 to determine the detailed molecular epidemiology of HIV-1 in Scandinavia. The dominating forms of HIV-1 were subtypes B (57%) and C (10%); and CRF01_AE (13%) followed by subtypes A, D, G and CRF02_AG, which were selected for further study. The most common transmission route was homo/bisexual transmission (74%) for subtype B, and heterosexual transmission for subtypes A (82%), C (83%), D (86%), G (81%), CRF01_AE (67%) and CRF06_cpx (58%).

To be able to identify local Scandinavian transmission cluster we used a Blast-approach to construct a reference sequence dataset consisting of (1) 5,788 patient-unique reference sequences from Genbank and (2) 2,268 non-overlapping, patient-unique reference sequences from a database within the SPREAD Programme, which contains 5,289 sequences that have been representatively obtained from patients in European countries during 2002-2010. Maximum-likelihood and Bayesian phylogenetic methods will be used to investigate phylogenetic clustering and phylogeography of HIV-1 in Scandinavia. Specifically, we will study (1) the number of HIV-1 introductions into Scandinavia, (2) the number of past and active transmission clusters and their demographic patterns within Scandinavia, and (3) HIV-1 spread within and between the Scandinavian countries. Preliminary data have shown small and larger Scandinavian clusters, especially among MSM and intravenous drug users. Some clusters involve patients in several countries. The results will be important for predicting the future HIV-1 spread in this region and therefore for HIV prevention.

**Abstract: P_28**

**Mortality and its predictors in HIV infected adults on first line antiretroviral therapy with and without tuberculosis in South West Ethiopia**

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Introduction: Data are inconsistent whether the mortality rate of HIV/TB co-infected patients on first line antiretroviral regimens is similar to that of HIV only infected ones and no such data is available in Ethiopia. Factors affecting mortality on treatment were also not fully identified.

Methods: All cause mortality was compared between 130 HIV/TB co-infected and 520 only HIV infected patients initiating antiretroviral therapy (ART) between 2008 and 2011 at Jimma University specialized hospital in South West Ethiopia using chi-square and fisher’s exact tests. The survival experience was compared by Kaplan Meier method. Predictors of this mortality were identified using Cox proportional hazard model.

Results: All cause mortality was significantly higher in patients with TB, 7.7% versus 2.9% at six months of ART initiation (p=0.040). Similarly, all cause mortality during the whole follow up period was higher in cohort with TB co-infection; 10.0% versus 3.5% (p=0.004). However, TB co-infection didn’t significantly increase the risk of death in multivariate analysis. Factors which independently increased the risk of death were ambulatory functional status [Adjusted Hazard Ratio, 5.53[1.110-27.520], P=0.037], bed ridden functional status [AHR, 6.25[1.400-14.473], P<0.001], BMI of 16-17 kg/m² [AHR, 4.47[1.386-14.399], p=0.012] and CD4 count <100cells/mm³ [AHR 2.95 [1.271-6.854], P=0.012] at the initiation of ART.

Conclusion: All cause mortality rate was higher in patients with TB co-infection. However, the presence of TB co-infection didn’t increase the risk of death on ART. Therefore, earlier presentation to care, early nutritional interventions and early initiation of ART such as before patient’s severely immunocompromised can reduce the risk of death on ART.

No conflict of interest

Abstract: P_29

Novel Therapeutic options for Hepatitis B, Hepatitis C or HIV

Anti-HIV activity of an African plant extract

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Background: Treatment of Human Immunodeficiency Virus (HIV) infection with antiretroviral therapy (ART) has remarkably reduced HIV/AIDS related mortality and morbidity of HIV-infected patients. ART is nevertheless associated with adverse effects, long-term toxicity, emergence of drug resistance and high costs limiting the availability of antiretroviral drugs in developing countries. Alternative therapeutic approaches are therefore still required. In this study we identified a strong anti-HIV activity in an African plant extract and characterized the mechanism of action.

Material and Methods: An African plant known to be used in decoction by HIV-infected-patients was collected in Kenya. Several Methods of extraction of hydrophilic and hydrophobic compounds from ground barks were tested. Protection and toxicity against HIV-1 were first screened by MTT on MT4 cells infected by the HIV-1 IIIB reference strain. Crude extracts were further tested on human Peripheral Blood Mononuclear Cells (PBMCs) by measurement of P24 concentrations in supernatant using ELISA. Protection on U373-CD4-CXCR4/CCR5 cells infected by viral X4, R5 or vesicular stomatitis virus (VSV) pseudotype particles as well as on U87-CD4-CXCR4/CCR5 cells infected by HIV-1 pNL4-3 plasmid were measured using luciferase activity. Toxicity was assessed by flow cytometry using the LIVE/DEAD Fixable Dead Cell Stain Kit. To decipher if the antiviral activity was due to direct interactions of the compounds with either the virus, the cell membrane surface or the viral life cycle, pre-incubation and post-incubation of extracts with
U373-CD4-CXCR4/CCR5 cells or with the virus were tested.

Results: The active extract was obtained by incubation of ground material in 90% ethanol. Following ethanol evaporation the residue was dissolved in water and subjected to phase partitioning with ethyl acetate. The aqueous phase was protective against HIV-1 on MT4 cells at non-toxic concentrations and was used for further analyses. The protective effect against HIV-1 was confirmed on PMBCs at non-toxic concentrations. The plant extract inhibited strongly HIV infection but not VSV infection in U373-CD4-CXCR4/CCR5 cells and U87-CD4-CXCR4/CCR5 cells independently of co-receptor usage (IC50 between 3 and 12 µg/ml). Selectivity index ranged from 172 for U373-CD4-CXCR4/CCR5 cells to 355 for U87-CD4-CXCR4/CCR5 cells, respectively. The crude extract inhibited HIV-1 infection when added only at the time of infection and not post-infection. The antiviral activity of the extract was maintained when the extract was pre-incubated with the virus but not with the cells.

Conclusions: The African plant extract showed a strong anti-HIV activity in several cell lines and PBMCs. The extract acts on HIV-1 entry into the cells rather than on viral life cycle. Further studies are required to purify and identify the active component(s) of the African plant.

No conflict of interest

Abstract: P_30

Novel Therapeutic options for Hepatitis B, Hepatitis C or HIV

Labyrinthopeptin A1, a novel lantibiotic peptide, is a dual HIV and HSV entry inhibitor

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Background: Lantibiotics are peptides, produced by bacteria, that contain the noncanonical amino acid lanthionine and many of them exhibit antibacterial activities. Labyrinthopeptin A1 (LabyA1; MW: 2073,7 Da) is a prototype peptide of a novel class of carbacyclic lantibiotics. Here, we extensively evaluated its broad-spectrum activity against HIV and HSV in vitro, studied its mechanism of action and evaluated potential microbicidal applications.

Materials and Methods: Multiple types of viral (HIV, HSV) replication assays were performed in various cell types. Flow cytometry, p24/p27 HIV Ag ELISA, Bio-Plex human cytokine assays, surface plasmon resonance (SPR)/fluorometric imaging plate reader (FLIPR) technology and bacterial growth assays were performed. Combined effects of LabyA1 with other anti-HIV and anti-HSV drugs were performed and analyzed by CalcuSyn software.

Results: LabyA1 exhibited a consistent and broad anti-HIV activity (EC50: 0.70 - 3.3 µM) and anti-HSV activity (EC50: 0.29 - 2.8 µM) in cell cultures. It is also equally active against various classes of HIV-1 drug resistant strains (e.g. entry inhibitors, integrase and reverse transcriptase inhibitors) and acyclovir resistant HSV strains (EC50: 0.31 – 2.1 µM). The well-known reference lantibiotic, called nisin, had no antiviral activity whatsoever. In addition, LabyA1 showed weak anti-HCV activity (EC50, 19 µM). LabyA1 also inhibited viral cell-cell transmission between persistently HIV-infected T cells and uninfected CD4+ T cells (EC50, 5.2 µM) and inhibited the transmission of HIV captured by DC-SIGN+ cells to uninfected CD4+ T cells (EC50, 4.2 µM). Time-of-drug addition studies revealed that LabyA1 acts as an entry inhibitor against HIV-1 and HSV-2, as its antiviral activity is completely diminished when added 2 h post-infection. Cellular and virus binding studies combined with SPR/FLIPR technology showed that LabyA1 interacted with the HIV-1 envelope protein gp120 of X4 and R5 viral strains (Kd: 7.8 – 12.3 µM), but not with the HIV cellular (co)receptors (CD4, CXCR4 and CCR5). In combination with various anti-HIV-1 and anti-HSV-2 drugs such as enfuvirtide (fusion
inhibitor), tenofovir (reverse transcriptase inhibitor), raltegravir (integrase inhibitor), saquinavir (protease inhibitor) and acyclovir (DNA polymerase inhibitor), LabyA1 demonstrated additive (whereby the CIs were between 0.9 -1.1) to synergistic (CIs are <0.9) effects with combination indices (CIs, at the 95%-level) varying for HIV-1 between 0.59 and 0.90 and for HSV-2 between 0.63 and 0.88. Compared to the mitogenic lectin phytohemagglutinin (PHA), the pre-treatment of PBMCs with LabyA1 had no effect on the expression of the cellular activation markers CD69 and CD25. It also never enhanced HIV replication and it did not induce any (inflammatory) cytokines/chemokines in PBMCs as evaluated by Bio-Plex multiplex assays. No toxicity was observed on vaginal epithelial cells in vitro (CC50, >50 µM). LabyA1, in sharp contrast to nisin, did not affect the growth of various vaginal Lactobacilli populations at concentrations up to 120 µM.

Conclusions: LabyA1 can be considered as a novel lead peptide as it has profound and consistent antiviral activity against HIV and HSV. Based on the lack of toxicity on the vaginal Lactobacillus strains and its synergistic/additive profile in combination with clinically approved anti(retro)viral drugs, it deserves further attention as a potential microbicidal candidate in the prevention of sexually transmitted diseases.

No conflict of interest

Abstract: P_31

Treatment Strategies for HIV infected Patients

Virologic and immunologic response of Maraviroc plus OBR after 24 weeks of treatment in Mexico

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Background: Maraviroc (MVC), the first approved CCR5 antagonist, has demonstrated 48-week safety and virologic efficacy in CCR5-tropic HIV-infected, treatment-experienced patients. Switching suppressive antiretroviral therapy (ART) to MVC-contained regimen, based upon genotypic tropism prediction from proviral DNA also improves tolerability. We reported 24-week effectiveness and safety data on real life situation of maraviroc plus optimized Background: regimen (OBR) in mexican population.

Material and Methods: We conducted an observational, prospective study. Patients were included if they had maraviroc as part of their antiretroviral regimen. Baseline demographic characteristics data were obtained. We evaluated baseline RNA HIV-1 viral load and CD4+ cells count. We assessed virologic and immunologic response after 24 weeks of treatment. Adverse events were also evaluated at week 4, 12 and 24.

Results: A total of 14 patients were included in our study. The median age of our subjects was 43 years old (IQR 33-51); 11 patients (78%) were men. OBR included DRV/RTV plus RAL in 50% of patients, TDF was used as part of OBR in half of patients. At the beginning of treatment, 5 subjects (35%) had CD4+ cells count < 200 cells/mm3. Eleven (78%) patients had viral tropisms for virologic failure, and 3 (22%) had proviral tropism to change a toxic regimen. Regarding patients with virologic failure, median of previous antiretroviral regimens were 4 (IQR 3-6). At 24 weeks of treatment, 8 patients (57%) had RNA HIV-1 viral load < 50 copies/mL; all patients had RNA HIV-1 viral load < 400 copies/mL. At 24 weeks of treatment 12 (86%) had CD4+ cells count > 200 cells/mL, Median CD4+ cells count at the beginning of the study were 272 (IQR 152-513) and at week 24 were 339 (IQR 224-668). During 24 weeks of treatment we only found 2 patients with minor adverse events associated to maraviroc. They didn't require change of regimen.

Conclusions: Maraviroc plus OBR is effective and well tolerated in HIV multidrug resistance patients and in those switching suppressive ART to MVC containing regimen based upon genotypic tropism prediction from proviral DNA improved tolerability.

No conflict of interest
Abstract: P_32

Treatment Strategies for HIV infected Patients

Virological and immunological response in the MITOX study – a 24 week analysis

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Background: NRTIs are well known to cause mitochondrial toxicity. The MITOX study was initiated to monitor safety and toxicity while changing efficient NRTI containing ART to NRTI-free regimen. This analysis is focussed on the safety data after 24 weeks.

Methods: A two-armed randomized prospective study of 80 HIV-infected men and post-menopausal women receiving two NRTI+PI/r were randomized either to continue their ART or to switch to MVC+PI/r regimen. Inclusion criteria were undetectable viral load for >6 months, no contraindication to maraviroc (including X4 tropism at screening from proviral DNA in unicate, FPR>15%), and the absence of genotypic drug resistance to the current PI regimen (historic). Virological failure was defined as any viral load >50 copies/ml.

Results: From 124 screened patients 40 for each arm were included. Six (MVC) and three (NRTI) patients were lost of follow-up, eight after screening and one after week 4, respectively. The mean baseline CD4 cell counts were 620 (MVC) and 662 (NRTI), and changed until week 24 to 607 and 661. In week 24, six (MVC) and two patients (NRTI) were viremic with >50 copies/ml, four of those (MVC) had >200 copies/ml. Tropism testing during failure was performed successful in five cases, one switch to X4 tropism could be observed. Maraviroc and PI drug levels were available for five patients and showed insufficient drug levels in three cases. In the MVC arm in two failure cases viral load dropped again <50 copies/ml despite no change in treatment (blips). Another case with unchanged treatment showed continuous low level viremia. The remaining three patients reached sufficient viral suppression by switching to NRTI+PI/r based regimen in two cases, while in the third case the PI was changed from ATV to DRV.

Of the two patients in the NRTI arm one showed despite multiple changes in treatment continuously low level viremia, the other reached undetectable viral load without treatment modification (blip).

Conclusions: The safety data analysis of this first prospective clinical study is a further demonstration of the possibility to switch to MVC-containing regimens based on proviral DNA tropism testing. In contrast to previous studies this study was performed as a prospective randomized clinical trial. The strategy to perform proviral tropism testing in unicate with a FPR cut-off of 15% proved to be safe, since in this study MVC failure is caused by factors like insufficient drug levels and incompliance, but very rarely due to X4 virus occurrence. Additionally, virologic failure could be handled easily by changing the PI/r or by switching back to a NRTI+PI/r based regimen. Thus we conclude MVC+PI/r regimens show excellent stability in combination with a very low risk to challenge consecutive ART.

Conflict of interest

financial relationship(s): Study sponsored by ViiV Healthcare Germany

Abstract: P_33

Treatment Strategies for HIV infected Patients

Switching from NRTI to Raltegravir due to NRTI toxicities in virologically suppressed HIV infected patients
Background: to describe the feasibility of switching NRTI backbone to raltegravir (RAL) in virologically suppressed HIV-1 infected patients.

Material and Methods: a database of a HIV reference centre in Florence was analysed, collecting demographic, behavioural, clinical and laboratory information. Patients were assessed at initiation of RAL therapy (baseline) and every 3-4 months thereafter up to a median of 31 months (IQR 20-40) of follow-up. Date of RAL interruption and reason of interruption were also recorded. Primary end-point was virological failure defined as two consecutive measures of HIV RNA >50 copies/mL. Survival analysis was used to assess probability of failure.

Results: Overall, 77 subjects, median age 51 years (24% female,) with HIV-RNA <20 copies/mL for ≥6 months were switched to RAL (400 mg bid): 25% were HCV and/or HBV co-infected, 91.8% B subtype , median CD4 nadir was 145 (IQR 52-247), 38% had AIDS diagnosis. The median time on ARVs was 16 years (IQR 9.9-18.1). According with HIV db interpretation algorithm, 46/60 (81.7%) had NRTI-related resistance mutations (median 3, IQR 1-6), 38/60 (63.3%) NNRTI-related (median 1, IQR 0-2) and 19/60 (31.7%) major PI-related mutations (median 0, IQR 0-2). Reason for change was NRTI toxicity: ipo-phosphoremia and creatinine increase (50.6%), mostly related to TDF; lipodystrophy (24.7 %); AZT-related toxicity (10.4%); CNS toxicity (1.3%), ABC-related toxicity (9.1%), FTC/3TC related toxicity (2.6%). CD4+ median monthly increase was 3.6 cells/mL (IQR -0.2-7.7). RAL was associated with PI (74%), NNRTI (28.6%) and NNRTI-PI (7.8%). 40% of patients switched to dual therapy, 51.9% had NRTI sparing regimes. Virological failure was observed in five patients. One patient developed RAL-resistance mutations: 156N and 163R. Probability of failure were 3.9% at one and two years and 6.5% at three years. Drug discontinuation without virological failure was observed in eight patients. Two patients died for cause unrelated to RAL and one patient was lost to follow-up. Virological failure was more frequently detected in patients harbouring higher number of RT and PI mutations (mean 7.4, ± 4, vs. 3.6, ± 3.2, per patient, p=0.01; mean 3.2, ± 3.6, vs. 0.76, ± 1.4, per patient p=0.001, respectively); and was related to the number of previous ARV regimens: mean 8.8, ±5.93 vs 4.96, ± 2.56 p=0.005 in failures vs non failures, respectively. Reversion of baseline ipo-phosphoremia after RAL switch was observed, mean 2.3 mg/dL , ± 0.21 at baseline vs 2.9 mg/dL, ± 0.54 six months later, p=0.001) and was the only metabolic improvement observed from baseline .

Conclusions: In our experience, switching to Raltegravir-containing regimen maintains virological success and reduce toxicity, particularly TDF-related ipo-posphoremia.

No conflict of interest

Abstract: P_34

Novel Therapeutic options for Hepatitis B, Hepatitis C or HIV

Treatment with boceprevir or telaprevir in a group of HIV/HCV co-infected patients: a new challenge

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Background: Co-infection with HCV is a leading cause of morbidity and mortality in HIV population. Two DAAs recently have been approved for the treatment of HCV in mono infection: telaprevir and boceprevir. Albeit to the date these drugs have not been approved for use in HIV/HCV patients, this population needs urgently other therapeutic options besides standard therapy. We describe our experience in some of these patients.

Material & Methods: Retrospective analyses of the patients admitted to our clinic that have
been started on telaprevir or boceprevir. Demographic, clinical and immunologic characteristics were obtained, as well as preliminary virologic response and adverse events. All the patients were also on standard therapy with ribavirin and peginterferon weight adjusted.

Results: A total of 20 patients have been analyzed: nine were on boceprevir and 11 on telaprevir. 14 of the patients were men, 19 were Caucasian, and their mean age was 41 years. Mode of transmission of HIV/HCV was MSM in 9 cases, IVDU in six and heterosexual sex in five cases. Median TCD4 cell was 666 and all had HIV undetectable viral load (19 were on HAART). 15 had been previously submitted to standard HCV therapy and 5 were naïve (four were submitted to boceprevir and one to telapevir). 15 patients had genotype 1a and five 1b; IL28B alleles: 11 were CT, six were TT and tree were CC; fibroscan revealed a mean Kpa of 9; and RNA -HCV median was 5.83log10. 15 patients achieved a greater than 1 log drop at week1 (Week5 in the boceprevir group). 13 patients that already reached week 12 , the 7 that reached 24 weeks, the 3 that reached 40 w and the 3 that already stopped all the treatment (72 weeks) remained suppressed. One patient on the boceprevir group has a virologic failure at week 12. On boceprevir group four patients had a hemoglobin drop below 10g, but only 1 needed EPO and transfusion support; thrombocytopenia was reported in two patients; there was also reported neutropenia in two patients. Commonly reported side effects were asthenia and disgeusia; 2 patients had oral ulcers. On telaprevir group two patients on efavirenz and telaprevir (1125mg/8h) were stopped at day two and at day 10 due to a rash who covered 80% of the body surface, not associated with DRESS. The skin biopsy performed revealed only a mild lymphocytic infiltrate.

Conclusions: In this small group of HIV patients, treatment with telaprevir and boceprevir was until now, associated with a good virologic response and had few adverse events already known. With the new DAAs for HCV achievement of viral suppression, adverse events and therapeutic interactions are even harder to overcome or predict in the HIV population.

No conflict of interest
genotype 2 and 3, the treatment protocol and having risk factors had significant effects on viral load of HCV patients (all P-Value<0.001). Of these two models, the estimators of zero inflated Poisson mixed model had the minimum standard errors.

**Conclusions:** The results showed that the zero inflated Poisson mixed model, has the best fit. This model proposes a compound Poisson subject specific random effect to account for excessive zeros. The proposed model can capture serial dependence, additional over-dispersion and excess zeros in the longitudinal count data. Since a wrong model would yield unreliable results; therefore, choosing the best and correct model for analyzing the data is highly important.

No conflict of interest

**Abstract: P_36**

**Treatment Strategies for co-infected Patients**

**Higher rate of sustained virologic response in women with HCV genotype 2 or 3 infection regardless HIV infection**

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**Background:** Higher rates of sustained virological response (SVR) are achieved in patients with easy to treat HCV genotype. Anyway, little is known about treatment outcome in co-infected women with HCV genotype 2-3 and HIV infection.

**Methods:** Variables associated with sustained viral response (SVR) in HCV infected women with genotype 2 or 3 who started PegIFN/RBV, followed in a single centre of Infectious Diseases in Brescia, Italy, have been retrospectively analysed. Chi-Square or Fisher’s Exact test and t-test for unpaired samples were used for statistical analysis. A p value <0.05 was considered to be significant. Multivariate logistic analysis has been adjusted for HIV infection, HCV-RNA plasma level, age, presence of cirrhosis and type of PegIFN used.

**Results:** A total of 464 patients were included in the analysis; the 52.8% (n= 245/464) was infected with HCV genotype 2 or 3. In this group of patients the 6.9% (n= 17/254) was co-infected with HIV, had a median age of 50 years (IQR 40-61) and weight was 63 Kg (IQR 55-72). The 62% was more than 45 years old, and the 9.5% had a diagnosis of cirrhosis. SVR has been achieved by the 70.2% of patients (n= 172/245). Multivariate logistic analysis showed the absence of significantly relation between SVR and the following variables: age > 45 [AOR 0.8 (0.4-1.5), p value: 0.5], HIV infection [AOR 2.8 (0.9-8.4), p value: 0.05], HCV-RNA > 500.000 IU/ml [AOR 0.6 (0.3-1.2), p value: 0.2] and type of PegIFN [AOR 1.3 (0.7-2.5), p value: 0.2]. Cirrhosis was the only variable related to a poor outcome [AOR 0.1 (0.06-0.4), p value: 0.0006].

**Conclusion:** Our data show that co-infection with HIV does not affect the achievement of SVR in women with easy to treat HCV genotypes. However, we need to emphasize the importance of an early treatment of HCV infection in this group of patients, particularly before the cirrhosis diagnosis.

No conflict of interest

**Abstract: P_37**

**Treatment Strategies for Hepatitis infected Patients**

**Aging and anti HCV treatment outcome in HCV infected women with and without HIV infection**

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Abstract: P_38

Treatment Strategies for Hepatitis infected Patients

HBV Case Report: sequencing results in a HBsAg negative patient

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Background: The spectrum of HBV infection ranges from asymptomatic infection to self-limited hepatitis, fulminant hepatitis and chronic HBV infection (CHB) depending on several factors, among which patient's age at the time of infection, viral characteristics and host factors. Indeed, the natural course of CHB is the final result of the interaction between virus, hepatocytes and host immune response. As HBV uses a reverse-transcriptase, mutant viral genomes emerge frequently. Selective pressures, both endogenous (host immune clearance) and exogenous (vaccines and antiviral drugs), readily generate these mutants, reportedly able to seriously affect the clinical course and the outcome of HBV replication.

Materials and Methods: A case of a 59-year-old male patient with a likely perinatal HBV infection is reported. The diagnosis was made at 22-year-old, when the Australia antigen was first searched and detected. The patient is being followed up at the Hepatology Unit of San Martino University Hospital in Genoa, Italy, since 1997. Virus sequence was obtained by Trugene® HBV Genotyping kit (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Viral load was evaluated by Artus® HBV-RG PCR (Qiagen) and HBsAg status by Monolisa Ultra (Biorad Laboratories S.r.l.), EIAgen (Adaltis S.p.A.), Axsym and Architect (Abbott S.r.l.).

Results: Until 2007 HBsAg, HBCAb and HBeAb were positive whilst HBsAb and HBeAg were negative, HBV-DNA levels below detection limits and transaminases always
normal. An “inactive HBV carrier state” with seroconversion from HBeAg to HBeAb described the condition of the CHB patient and no treatment was given. In June 2008 HBV serological tests revealed disappearance of the HBsAg and appearance of HBsAb; however, six months later HBsAg was again found positive, HBsAb negative and for the first time HBV-DNA detectable. Then, again, and until May 2011, HBsAg, HBsAb and HBV-DNA resulted negative. In May 2011, despite HBsAg and HBsAb were persistently negative, the virus was detected. In this occasion, virus sequencing was performed, revealing a D genotype and the presence of the mutations A181S in the pol-gene and P120P/A, M133M/L/V, D144D/G and W172C in the S-gene. Four different commercial kits were used to detected HBsAg, all confirming a negative result.

Conclusions: In addition to the already known escape mutants sP120P/A, sM133M/L/V and sD144D/G this treatment-naive patient presents the rtA181S mutation. The rtA181T was described in patients failing regimens with lamivudine and/or adefovir. This pol-gene mutation, due to the overlapping reading frames with surface gene, generates the W172C stop codon in the surface antigen. Ming-Wei Lai et al. described a case of a patient with hepatocellular carcinoma who was HBsAg negative, HBeAg and HBV-DNA positive. The concomitant presence of rtA181S and sW172C leads to impaired secretion of HBsAg and perhaps is capable of inducing cell transformation. Further studies are needed to confirm such hypothesis. In our case a mutation occurred in the same position but with the presence of serine instead of threonine. Open questions are: is HBsAg seronegativity due to the presence of multiple escape mutants that limit HBsAg detection or is it real and, if so, does the presence of the sW172C stop codon affect HBsAg secretion?

Abstract: P_39

Novel Diagnostic Technologies & Approaches

Next-generation sequencing technology in the clinical HIV laboratory: a more sensitive alternative to Sanger sequencing


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Background: Sanger based HIV sequencing is currently the method of choice for the identification and follow-up of HIV drug resistance. To assess the utility of bench-top next generation sequencing (NGS) platforms for the clinical HIV diagnostic laboratory, we have analyzed results obtained by Roche GS Junior, Illumina MiSeq and ABI’s IonTorrent, and compared the mutations load and the prediction of HIV resistance derived from GS Junior and Trugene (Sanger).

Methods: Plasma samples and/or peripheral blood mononuclear cells (PBMCs) were isolated from ten HIV carriers representing the major HIV subtypes in Israel. GS Junior HIV-1 reverse transcriptase (RT) and protease (PR) nucleic acid sequences were compared to MiSeq data by BWA mapping and GATK tool (one sample was run also on IonTorrent and compared). DeepChek®-HIV software, dedicated to Roche NGS analysis, was used to analyze the GS Junior and Trugene amino acids mutations Results: Mutations detected at > 1% threshold in GS Junior were included in the analysis.

Results: Correlation between the nucleic acid sequences obtained by the three platforms
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(one sample) and between GS Junior and Miseq sequences derived from ten samples, was rather strong (mean Spearman correlation=0.65). Comparison between the results of GS Junior and Trugene demonstrated that the latter identified less amino acid substitutions in PR and RT (153 and 268 substitutions by GS Junior, respectively, and only 104 and 153 substitutions by Trugene, respectively, in the ten samples). 95% of all substitutions detected by Trugene were also detected by GS Junior, while only 55% of the amino acids substitutions detected by GS Junior were also found by Trugene. Though mostly similar, some discrepancies between drug resistance mutations (DRMs) identified by these platforms were noted, mainly at low frequency in GS Junior. These included the RT M184V identified by Trugene (and by GS Junior at 0.25% frequency) and K65N and K103E identified only by GS Junior at frequency below 10%. In two cases, where PBMCs and plasma samples from the same HIV carrier were compared, similar molecular pattern was observed.

Conclusions: The higher number of mutations detected by GS Junior compared to Trugene (at < 20% frequency), suggest that deep sequencing may be more sensitive for DRM detection, though the clinical significance of our observations requires long term follow-up. Our study further suggests that PBMCs can be utilized as an alternative to plasma RNA samples, for prediction of HIV drug resistance. While DeepChek®-HIV is CE-IVD marked and compatible with clinical genotyping for routine use, bioinformatics tools have to be developed for the analysis of IonTorrent or MiSeq results, to enable efficient use in the clinical HIV laboratory.

No conflict of interest

Abstract: P_40

Novel Diagnostic Technologies & Approaches

The first HIV tropism determination kit development based on genotypic methodic for routine in vitro diagnostics in Russia

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Background: Since CCR5-antagonist becomes available in the Russian Federation, determination of the HIV tropism in appropriate patients is required. Genotypic methodic for HIV-1 tropism determination adapted for subtype A was recently developed in Central Research Institute for Epidemiology (CRIE), and validated in collaboration between CRIE and University of Cologne, Germany. Tropism testing is needed throughout Russian territory, but there are certain difficulties in samples delivery from distant regions to the central laboratory. So it was necessary to develop reproducible methodology for each regional laboratory. To do this the in vitro diagnostics (IVD) kit was developed, based on previously validated assay. It can be used by any laboratory equipped with capillary Sanger sequencer. The kit was certified according to Russian IVD certification requirements.

Materials and Methods: Limit of detection (LOD) for HIV-1 RNA has been estimated with NIBSC samples. LOD for HIV-1 DNA has been estimated with artificial DNA plasmids containing almost full HIV-1 genome. Comparison with comparator assay was performed using plasma and purified leucocytes samples which were collected from HIV-infected CCR5-antagonist naïve patients (n=149). For the samples with viral load over 500 copies RNA/ml analysis was done using viral RNA; for other samples analysis was done using proviral DNA. HIV-1 gp120 gene of a V3 domain of HIV-1 RNA was amplified by nested RT-PCR. DNA was amplified by a single step PCR. Both strands were sequenced for all samples studied using BigDye® Terminator Cycle sequencing Kit. Sequence contigs were assembled using

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DEONA version 1.0.1.8 Tropism module. The geno2pheno[coreceptor] genotypic tropism prediction system was used with clonal interpretations at false positive rate (FPR) cut-off in accordance with European guidelines on the clinical management of HIV-1 tropism testing.

**Results:** Limit of detection was 500 and 100 copies RNA/ml depending on the input of plasma volume 0.2 ml and 1 ml respectively. Limit of detection was 500 genome equivalents DNA/ml using the 0.25 ml of PBMC. During the comparison with a comparator assay 146 (98%) samples were successfully amplified. Comparison of results showed 100% concordance at 10% FPR cut-off level.

**Conclusions:** Until recently the AmpliSens HIV-Resist-Seq – diagnostic kit registered in Russia for IVD – have been used only for HIV drug resistance determination by analysis of viral protease, reverse transcriptase and integrase sequences. In April 2012 genotypic methodic for tropism determination was registered and included in that kit. Presently a genotypic tropism determination test based on HIV-1 \( gp120 \) V3 domain sequencing and following interpretation with geno2pheno[coreceptor] is available as IVD kit in Russia.

**Conflict of interest**
financial relationship(s): The work was funded by ViiV Healthcare Russia

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**Abstract: P_41**

**Novel Diagnostic Technologies & Approaches**

**HIV tropism as a suitable tool to predict immune response?**

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**Background:** HIV infection via the chemokine receptor CXCR4 (X4) correlates with faster disease progression, a more rapid decline in CD4 cells, and therefore earlier signs of AIDS related illnesses. We hypothesize that impaired CD4 cell response might correlate with the viral tropism considering that X4 tropism seems to associate with a poorer outcome of the disease and that in a substantial number of HIV infected individuals under suppressive ART CD4 cell recovery is often not adequate. The aim of this study was to substantiate such a link between HIV tropism and immunological outcome.

**Material & Methods:** This retrospective study included patients from the SHCS who were under ART for > than 3 years, with complete virus suppression and no virological failure. Patients were grouped according to their CD4 counts after 3 years on ART: either at >500 cells/µL (‘responder group’) or <500 cells/µL (‘plateau group’). Plasma samples were analyzed for viral tropism at the time of initiation of ART using geno2pheno[coreceptor] (FPR 5%).

**Results:** Ninety-five patients, 48 responders and 47 plateaus, were included in the study. For the respective CD4 range in this population, literature reports about 82% of patients to carry R5 tropic viruses (Brumme et al, 2005). Our data with 81.3% R5 (74/91) correlate well with this expectation. We found 7% (3/46) of patients in the responder group to carry X4-tropic virus, whereas the percentage of X4 in the plateau group was 31% (14/45) \([p = 0.0061]\). Average CD4 cell increase in responder group was 508 cells/µL vs. 225 cells/µL in plateau group \([p = <0.0001]\). Overall we found that 82.4% of all X4 tropic viruses in the study population could be assigned to the plateau group. Four samples were indeterminate by G2P. As a surprising fact viral kinetics and level of suppression were indistinguishable for both groups. Our multivariate analysis yielded only low baseline CD4 counts as predictors.

**Conclusions:** Unexpectedly more than 80% of patients with a X4 tropism at initiation of ART ended up with an impaired immune response, characterized by a plateauing CD4 recovery at below 500 cells/mL. Since tropism determination was performed on specimens years before the development of a CD4 plateau our data suggest the utility of early tropism testing. As a diagnostic tool tropism...
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Abstract: P_42

Novel Diagnostic Technologies & Approaches

Frequency of G516T single-nucleotide polymorphism in CYP2B6 among Slovenian HIV-1 patients and its association with efavirenz treatment switch

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Background: Efavirenz is often associated with adverse side effects in HIV treated patients, therefore many studies aimed to determine genetic markers influencing its metabolism. Recent publication (JID 2012; 206:1453-61) reported more than 3 fold increase in efavirenz plasma and hair concentrations among patients carrying 516TT genotype of CYP2B6 compared to patients with TG/GG genotype. In our pilot study we investigated whether such association exists also among Slovenian HIV-positive patients treated with efavirenz.

Material & Methods: 83 patients with a history of efavirenz treatment were included in the study: 23 patients were with a documented treatment switch following efavirenz exposure (study group) and 60 patients were without a recorded efavirenz discontinuation (control group). Real-time PCR for the determination of TT, GT and GG 516 genotype was used on genomic DNA isolated from blood samples obtained in previous studies. For statistical analysis Chi-square test or Fisher exact test were employed and P values of <0.05 were considered significant.

Results: Overall, the genotype TT was found in 4 (5%), GT in 35 (42%) and GG in 44 (53%) of 83 patients. Observed genotypic frequencies were in Hardy-Weinberg equilibrium. The polymorphism frequencies of TT, GT and GG genotypes were 0% (0/23), 61% (14/23) and 39% (9/23), respectively, among patients with reported therapy switch following efavirenz treatment and 7% (4/60), 35% (21/60) and 58% (35/60), respectively, among patients without a recorded efavirenz discontinuation. No significant difference was observed among the two groups, although a tendency of more frequent genotype GT appearance in switch vs. control group was observed (p=0.0595; odds ratio 2.889, 95% confidence interval: 1.072-7.784). The main limitation of our preliminary study is current lack of information about reason(s) for efavirenz discontinuation in the study group.

Conclusions: No significant difference in polymorphism CYP2B6 G516T genotype distribution among Slovenian HIV-positive patients, who discontinued efavirenz therapy, and patients without recorded efavirenz discontinuation was observed.

The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the project 'Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)' - grant agreement n° 223131.

No conflict of interest
Abstract: P_43

Novel Diagnostic Technologies & Approaches

A Cost effective 2 step approach for HLA-B*57:01 typing

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Introduction: Abacavir is a nucleoside reverse transcriptase inhibitor used as a part of combination antiretroviral therapy in HIV-1-infected patients. Initial reports indicated that abacavir use causes hypersensitivity in 5-8% of patients and this hypersensitivity was observed to be correlated with the presence of the HLA-B*57:01 allotype. Currently, abacavir is recommended as first line drug in several national and international treatment guidelines, but the presence of the HLA-B*57:01 allele should be tested before treatment. Although numerous genetic tests have been developed for HLA-B*57:01 screening, the turnaround time and the assay costs need to be improved to allow a wider and more efficient use of HLA-B*57:01 screening. Here the feasibility of a complementary two step approach for HLA-B*57:01 testing was evaluated. The first step consists of a flow cytometric analysis with a monoclonal antibody specific for HLA-B*57 and B*58 that excludes the presence of HLA-B*57:01 in 85-90% of the patients. The subsequent test is an improved genotypic sequence specific real-time PCR.

Material and Methods: For flow cytometry, blood samples of 135 patients with unknown HLA status were procured at Ghent University Hospital and enriched with 9 fresh samples of known HLA-B*57:01 positive patients as confirmed by the Laboratory for histocompatibility and immunogenetics of the Belgian Red Cross through sequence specific PCR and oligonucleotide hybridization. This is considered as the standard validated method. After assessment with flow cytometry all samples were sent blinded to the Red Cross for confirmation. Real-time PCR was performed on DNA isolated from 15 HLA-B*57:01 positive and 15 HLA-B*57:01 negative patients using sequence specific primers for HLA-B*57:01. The specificity of this PCR was increased by comparing an already established assay using sequence specific primers and SYBR green dye to a new assay with sequence specific fluorescently labeled hydrolysis probes instead of SYBR green.

Results: By flow cytometry 15.6% of the samples were assessed HLA-B*57 or B*58 positive. Three of these samples were confirmed HLA-B*57:01 positive by the Red Cross. This flow cytometric analysis reaches a sensitivity of 100%, a specificity of 88%, a positive predictive value (PPV) of 14% and negative predictive value (NPV) of 100% making it a useful pre-screening test. The genetic test by real-time PCR resulted in an excellent distinction between negative and positive patients using the probe based assay. The SYBR green assay provided a quantitative distinction requiring a more elaborate assay analysis, whereas the probe based assay provided a qualitative difference between HLA-B*57:01 positive and negative samples. The probe based assay results in a sensitivity, specificity, PPV and NPV of 100%.

Discussion: This study shows that the combination of a flow cytometric pre-screening can substantially decrease the number of required genetic tests for HLA-B*57:01 typing. This strategy not only allows a cost efficient screening, but also substantially decreases turnaround time of the HLA-B*57:01 testing for the majority of patients, since assay results can be available concurrently with the first CD4 count. In addition, the optimized real-time PCR method allows a better distinction, further simplifying assay evaluation.

Conflict of interest: financial relationship(s): The project was supported by ViV healthcare. ViV healthcare was not involved in the data analysis and interpretation of the results.
Abstract: P_44

Novel Diagnostic Technologies & Approaches

Touch down digital PCR for quantification of difficult amplicons in the HIV genome

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Background: Quantification of total HIV DNA by PCR is a promising tool in the context of HIV eradication and simplification strategies. With current technological developments digital PCR is now applicable at a large scale, providing a superior method for absolute quantification of nucleic acids compared to classical real-time PCR. In addition, the use of end point PCR in multiple reactions provides an increased flexibility in assay design without hampering quantification. In this study, a two-stage touch-down digital PCR was developed for a specific PCR assay that targets a highly conserved region of HIV. The probe of this assay has a low melting temperature and does not work with high annealing temperatures, yet low annealing temperatures may induce non-specific amplification. Consequently a PCR was developed with an initial round of cycles at stringent conditions, i.e. an annealing temperature of 58°C (optimal annealing for the primer pair) and a second PCR amplification at 50°C to allow probe binding and hydrolysis. An optimal accumulation of fluorescence was observed with a combination of 30 cycles at stringent conditions and 9 cycles at 50°C Ta. This set up, used to assess the two fold standard dilution curve provided a good linear correlation. In addition, this assay also performed well in the investigated patient derived PBMCs.

Material & Methods: Plasmids containing the NL4.3 HIV sequence, as well as infected Jurkat cells and patient derived PBMC DNA were used for PCR optimization. After isolation, genomic DNA was restricted using EcoRI digestion (Promega) and digital PCR was performed on the digest with the QX100™ Droplet Digital™ PCR (ddPCR) platform (Bio-Rad). PCR amplification reactions consisted of an initial denaturation at 95°C for 5 min, and

39 cycles of 15 sec. denaturation at 95°C and 1 min. annealing/elongation at the annealing temperature (Ta) using HIV specific primers and probes: (HIVpF, forward: GCCTCAATAAAGCTTGCCTTGA; HIVpR, reverse: GGGCGCCACTGCTAGAGAT; HIVppr, probe: 5’FAM-GTA[t/a/g]CTAGAGATCCCTCAGA). Initially a gradient PCR was performed for Ta (54-58°C). Subsequently, different PCR procedures with an initial number of reactions at high Ta (58°C) and a subsequent round at lower Ta (50°C) were assessed for maximal accumulation of fluorescent signal. Finally, a 2-fold standard curve of infected Jurkat gDNA as well as different gDNA isolates from PBMCs of 12 patients were assessed with the most optimal procedure.

Results: Gradient ddPCR revealed that amplification of plasmid DNA could only be detected at an annealing temperature lower than 54°C, but distinction between positive and negative replicates was sub optimal to allow accurate quantification. Subsequently, a two stage touchdown PCR was optimized that included a first PCR amplification at stringent conditions, i.e. an annealing temperature of 58°C (optimal annealing for the primer pair) and a second PCR amplification at 50°C to allow probe binding and hydrolysis. An optimal accumulation of fluorescence was observed with a combination of 30 cycles at stringent conditions and 9 cycles at 50°C Ta. This set up, used to assess the two fold standard dilution curve provided a good linear correlation. In addition, this assay also performed well in the investigated patient derived PBMCs.

Conclusions: This data shows that by using an end point PCR for quantification, a higher flexibility in assay set-up and optimization can be obtained in digital PCR. The combination of a more accurate sensitivity and a higher flexibility in assay optimization makes digital PCR a promising tool for future clinical HIV diagnostics.

No conflict of interest
Abstract: P_45

**Novel Diagnostic Technologies & Approaches**

**Application of the TRUGENE CLIP Sequencing Core Reagent for "in house" DNA sequencing on the TRUGENETM platform.**

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**Background: and Aim: CLIP DNA sequencing on the TRUGENETM platform is frequently used in routine laboratories for HIV genotypic resistance testing, HBV genotype & resistance mutations detection, and in some cases for HCV genotyping. Many laboratories also use this platform for 'in house' DNA sequencing. Here we have used the TRUGENE CLIP Sequencing Core Reagent (Siemens) to sequence the V3 loop of HIV for genotypic tropism determination, and compared this results to those previously obtained using the TS Primer Cycle Seq KIT, from General Electrics (GE).**

**Patients & Methods:** Samples from 25 patients with previous results using GE were re-sequenced, using the same nested PCR product, with the reagents from Siemens. 22 samples were proviral DNA specimens from suppressed patients (82% males; median age, 44 (IQR 42.5-48.5); median CD4, 630 (IQR 362.5-922.5); 18, 2%Tropism X4) and 3 were from viraemic patients (median age, 35 (IQR 31-48); median CD4, 89 (IQR 26-842); median Viral Load 24000 (IQR 4500-26113) and all with R5 Tropism). All samples were again re-tested in parallel using a modification of the Siemens protocol. Tropism was estimated using geno2pheno with a 10% FPR. A minimum quality (IUPAC mixtures in ≤8 codons in the V3 sequence) was required prior to geno2pheno interpretation. Pairwise score between sequences obtained with both Methods was estimated using multiple sequence alignment with Clustal Omega.

**Results:** Three samples that could not be sequenced using GE and 1 sample that did not meet QC criteria, were successfully sequenced with the Siemens kit. Overall, a 90% concordance (19/21) between both Methods was observed. Discordances were as follow: one sample, scored as R5 through GE amplification (FPR 20.2%) did not meet QC criteria after Siemens amplification; a second sample was scored as 3.7% FPR after GE sequencing and as 21.2% FPR after Siemens amplification, with a pairwise comparison score of 97. Pairwise score of concordant sequences, obtained through GE & Siemens, ranged from 89-100 (median 99.5; IQR 96.25-100). Parallel sequencing with the Siemens modified protocol resulted in the same Results:

**Conclusions:** The TRUGENE CLIP Sequencing Core Reagent kit is a valuable tool for the 'in house' sequencing of the V3 loop of HIV-1. A simple modification in the protocol allows making testing more cost-effective.

**No conflict of interest**

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Abstract: P_46

**Novel Diagnostic Technologies & Approaches**

**Use of DeepChek v1.1 and VisibleChek® for the analysis and integration of 454 GS Junior data from the RT and Protease of HIV-1.**

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**Background:** Analysis of 454 derived sequences from the RT and the PRO of HIV-1 has traditionally required a profound bioinformatic analysis. Here we present the
use of DeepChek v1.1 for RT & Pro sequence analysis, and VisibleChek for their integration with virological and clinical data.

**Patients & Methods:** Plasma samples from 46 HIV-1 infected patients were included in the study. Median age (IQR) was 36, 5 (32.75-44.25), median CD4 count (IQR) was 551 (211.75-777.50), and 93% were males. 95% were naïve patients and the rest were failing their current antiretroviral regimen. After extraction, reverse transcription and amplification, RT & Pro 454 sequences were obtained using a GS-Junior (Roche). Sequences were preprocessed in the GS Junior system; aligned fasta UDS sequences were uploaded into the DeepChek v1.1 module (Therapy Edge, ABL SA). Sanger sequences, obtained through Trugene HIV-1 genotypic kit, were uploaded in parallel. Stanford 6.2.0 version mutations scored as >=5 were considered for analysis. For resistance interpretation data the Spanish HIV Research Network (RIS) algorithm was chosen and interpretations were evaluated using Sanger information, and UDS data with different mutations thresholds (1%, 5%, 10% and 15%); any degree of resistance (resistant and intermediate resistant categories) was considered.

**Results:** Using VisibleChek for analysis, we were able to describe the detection of any mutation using Sanger sequencing in 29/46 patients, with a total number of 79 Stanford >=5 mutation, being K103N the most prevalent mutation (n=11). Using UDS, with a 1% cutoff, we found that 40/46 patients had at least one mutation, with a total number of 140 Stanford >=5 mutation. Using Sanger Resistance data, 3/46 (6.5%) patients showed any resistance to NRTIs, 14/46 (30%) to NNRTIs; no resistance to PIs was detected. Using UDS data for resistance interpretation resulted in an increase in the resistance to NRTIs, in comparison to Sanger sequencing data: 4/46 (8.6%) using 15% and 10% as thresholds, 5/46 (10.8%) using 5% as threshold, and 10/46 (21.7%) using 1% as threshold. UDS data also resulted in an increase in NNRTI resistance: 15/46 (8.6%) using 15%, 16/46 (8.6%) using 10% as threshold, 17/46 (10.8%) using 5% as threshold, and 19/46 (21.7%) using 1% as threshold. However, no differences in protease resistance interpretation between Sanger and UDS data using 15, 10, and 5%, and only one patient showed intermediate resistance to Atazanavir, with the data generated using 1% as threshold.

**Conclusions:** DeepChek and VisibleChek allow for an easy, reliable and rapid analysis of UDS data from HIV-1 reverse transcriptase and protease. Compared to Sanger data, UDS resulted in an increase of the number of resistance mutations, and the number of patients with any degree of resistance to NRTI and NNRTIs, while no increase in resistance to Protease Inhibitors was observed.

No conflict of interest

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**Abstract:**

**P_47**

**Novel Diagnostic Technologies & Approaches**

**Pre-existing minority HIV-1 variants in treatment naïve Ethiopian HIV patients detected by allele-specific real-time PCR**

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**Background:** Transmission of drug resistant HIV-1 is usually analysed by direct sequencing. However, mutations may disappear from the major population and the frequency may therefore be underestimated

**Material and Method:** Peripheral EDTA blood samples were collected from 92 drug naïve HIV-1 infected patients, attending various HIV clinics in Addis Ababa, Ethiopia, during 2008-
2009, before ART (stavudine or zidovudine/3TC/nevirapine) was initiated. Genotypic analysis in the reverse transcriptase was performed by direct sequencing. Allele-specific PCR was done for the Y181C and K103N mutations.

**Results:** No drug-resistance mutations were detected by standard sequencing. Neither was any K103N mutations identified by ASPCR. Y181C was detected in six individuals (6.5%), corresponding to a viral population of >0.25%. The frequency of mutants in the quasispecies ranged from 0.25 to 4.5%. There were no statistically significant differences in terms of viral load, CD4+ T cell counts, age, sex and BMI between patients with and without Y181C.

**Conclusions:** Our findings report the presence of the Y181C-mutation in minor quasispecies of a low proportion of treatment naïve HIV-1 patients in Ethiopia, but not in the major viral population. The data suggest that surveys with standard sequencing techniques may underestimate the presence of TDR in Ethiopia, although the use of NNRTI in first line regimen will be effective in the vast majority of patients.

*No conflict of interest*
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