Drug resistance genotyping profile and phylogenetic particularities of F clade in a group of antiretroviral naive Romanian patients

Ene L[1], Temereanca A[2], Radoi R[1], Mehta S[3], Ruta SM[2], Duiculescu D[1], Smith D[3]

[1]'Dr. Victor Babes' Hospital for Infectious and Tropical Diseases, AIDS Department, Bucharest , Romania ,

[2] "Stefan S. Nicolau" Institute of Virology and „Carol Davila" University of Medicine ,Bucharest , Romania

[3] University of California at San Diego Department of Medicine, La Jolla , California , USA

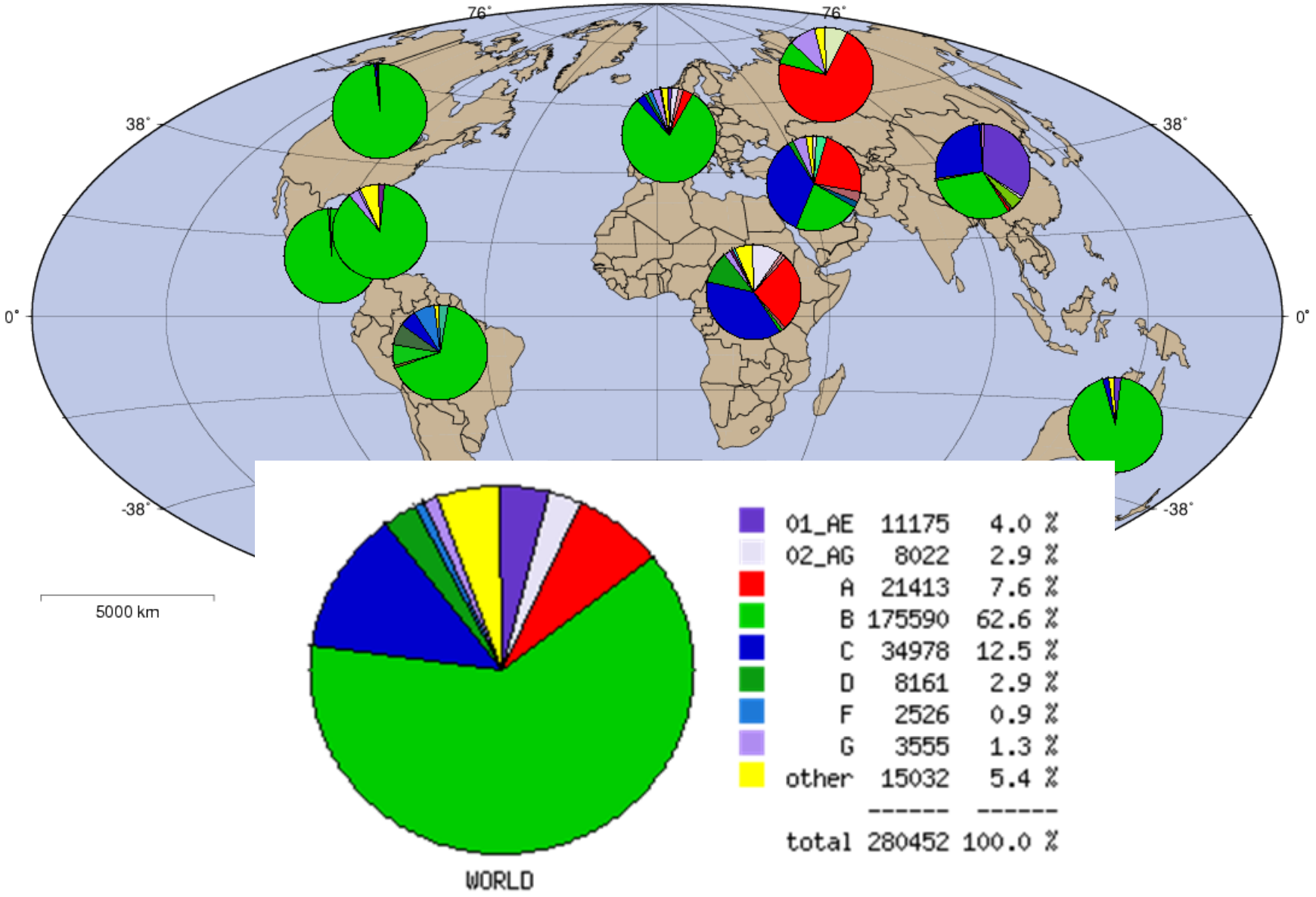
Background

"Victor Babes"Hospital cohort

- Adolescents – unique homogenous group
  - currently aged 20-21 years
  - having a similar genetic background
  - that were infected with HIV-1 subtype F by parenteral route in their first year of life (between 1989-1992)
  - some of the patients from this group survived with good immunological status and without treatment, other were diagnosed after 15-20 years of chronic HIV infection
  - most of the adolescents started sexual life and are prone to have unprotected sexual relations with HIV positive and negative partners[1] with major epidemiological implications: spread of HIV and possible superinfection with drug-resistant HIV strain
- Children with MTCT HIV-infection, born to mother infected with HIV by heterosexual route
- Adults with sexual route of transmission of HIV

[1]Johnson BT AIDS Behav. 2007 Sep;11(5):716-25

Clade diversity wordwide



Source: http://www.hiv.lanl.gov/

HIV sequence database

DATABASES SEARCH ALIGNMENTS TOOLS PUBLICATIONS GUIDES Search Site

Distribution of all HIV-1 sequences: ROMANIA

Please note that this map only includes sequences for which the sampling country is known.

Subtype distributions represent the frequency in the HIV Database and not the population

About this geography site.

Select organism: HIV-1

Select (if a country is selected, it supersedes region)

Region or

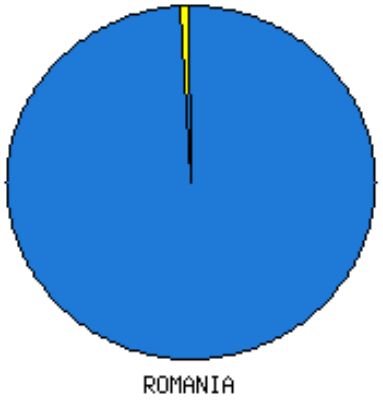
ROMANIA

Show all non_recombinant recombinant sequences

Table (html) of the compiled subtype distribution.

Click pie slice to retrieve sequences

or get allsequences:



F	660	99.2 %
other	5	0.8 %
total	665	100.0 %

Source: http://www.hiv.lanl.gov/

Impact of clade diversity HIV-1 virulence, antiretroviral drug sensitivity and drug resistance

- Clades may show differences in co-receptor usage and syncytia inducing capacity : absence of CXCR4 phenotype among clade C (Abebe, A.,1999, AIDS **13**, 1305–11, Zhang, L., 1996Nature **383**, 768)
- **clade F** shows some measure of resistance to the noncommercialized NRTI, the TIBO compound, while remaining sensitive to other NNRTIs, such as nevirapine and delaviridine (DLV), as well as NRTIs and PIs (Apetrei 1998, Journal of Virology **72**, 3534–38)
- Y181C and Y181I mutations render group O and HIV-2 resistant to all drugs within the entire NNRTI class, respectively (Quinones-Mateu, 1998 Journal of Virology 72, 9002–15.
- The baseline polymorphism at codon 106 in clade C viruses facilitated development of a novel V106M mutation, conferring efavirenz resistance (Loemba 2002Antimicrobial Agents and Chemotherapy **46**, 2087–94.)
- differential levels of vulnerability within the CNS – eg evidence of **faster progression to dementia among individuals in Africa infected with clade D** virus vs infected with clade A (Sacktor N Clin Infect Dis. 2009 Sep 1;49(5):780-6.)

JOURNAL OF VIROLOGY, May 1998, p. 3534–3538
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Human Immunodeficiency Virus Type 1 Subtype F Reverse Transcriptase Sequence and Drug Susceptibility

CRISTIAN APETREI,[1,2]* DIANE DESCAMPS,[1] GILLES COLLIN,[1] IBTISAM LOUSSERT-AJAKA,[1] FLORENCE DAMOND,[1] MIHAI DUCA,[2] FRANÇOIS SIMON,[1] AND FRANÇOISE BRUN-VÉZINET[1]

FIG. 2. Phylogenetic analysis comparing the RT regions of HIV-1 *pol* genes from different strains. Tree topology was inferred by the neighbor-joining method. The tree was based on an alignment of nucleotides from which columns containing gaps have been deleted (597 nucleotides). The tree was rooted with HIV-1 group O sequences. The numbers given at the branch points are the 50% threshold majority consensus values for 100 bootstrap replicates. Vertical distances are given for clarity. The cluster of sequences from nosocomially infected children (*) had already been observed by analyzing the *env* gene (1). Strain RO-BCI13 (**) was isolated from a vertically infected child. Strains RO-BCI17, RO-BCI18, RO-BCI19, RO-BCI20, and RO-BCI23 were isolated from HIV-1-infected adults.

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Polymorphisms and resistance mutations in the protease and reverse transcriptase genes of HIV-1 F subtype Romanian strains☆

Simona Paraschiv, Dan Otelea*, Magdalena Dinu, Daniela Maxim, Mihaela Tinischi

Main findings

- all the HIV strains isolated from adults and adolescents infected in childhood clustered together into a distinct group
- No primary resistance mutations were found,
- but every HIV strain analyzed was harboring at least two accessory mutations
- The M36I mutation in the protease gene (associated with resistance to ritonavir and nelfinavir in B subtype viruses) was found in all the Romanian strains.

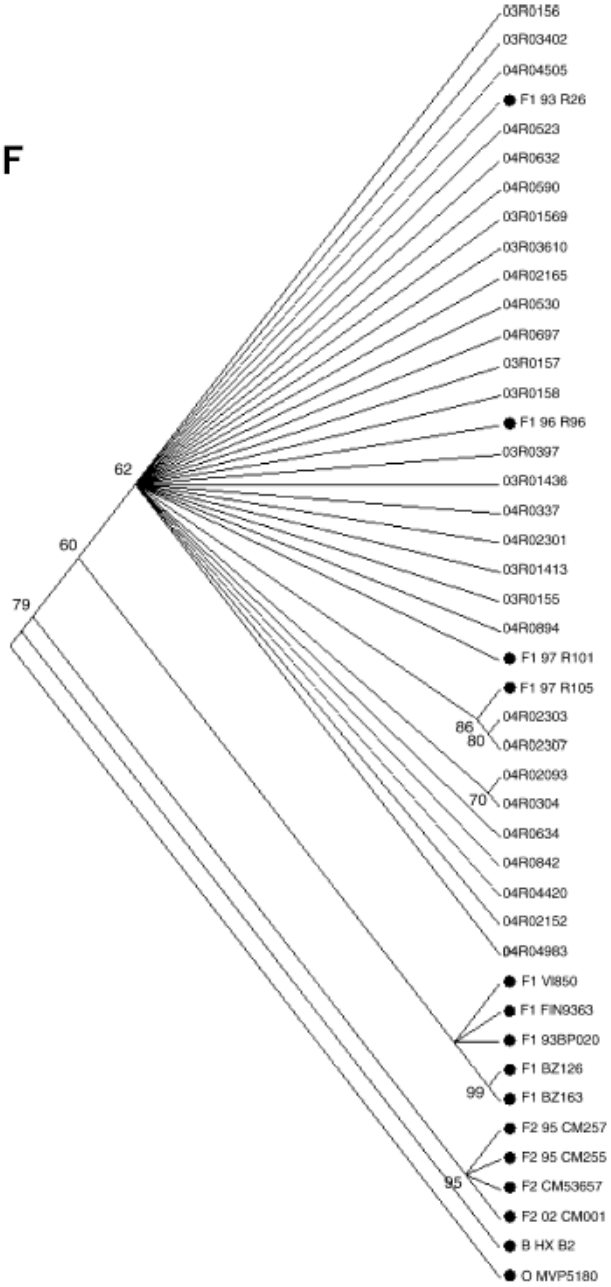

Figure 2 Phylogenetic analysis of PR and RT nucleotide sequences (1149 bp) from 29 Romanian strains and reference strains available in the Los Alamos HIV-1 database. The tree was generated as described in the Methods. The reference strains are presented in the same section and are distinctly marked (●). The numbers at the nodes indicate the percentage of the bootstrap value.

Retrovirology

Research

Close phylogenetic relationship I HIV-1 subtype F1 isolates

Monick L Guimarães[1], Ana Carolina I FC da Silva[2,3], Moises Francisco[3], Filo Ducelina Serrano[4], Mariza G Morgad

Address: [1]Laboratório de AIDS & Imunologia Molecular, Instituto Osv Molecular de Microorganismos, Instituto Oswaldo Cruz – FIOCRUZ, I Ministério da Saúde, Luanda, Angola and [4]Instituto Nacional de Luta

Email: Monick L Guimarães - monicklg@ioc.fiocruz.br; Ana Carolina Koko Otsuki - kotsuki@ioc.fiocruz.br; Rosa Ferreira FC da Silva - rosai Filomena Gomes da Silva - inst@ebonet.net; Ducelina Serrano - ducel Mariza G Morgado - mmorgado@ioc.fiocruz.br; Gonzalo Bello* - gbe

* Corresponding author

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Retrovirology 2009, 6:39 doi:10.1186/1742-4690-6-39

Figure 1

Majority-rule Bayesian consensus tree of HIV-1 subtype F1 *env-gp120* (310 bp) sequences. Posterior probabilities are shown for key nodes. The names of HIV-1 isolates include reference to subtype, country of isolation, and year of isolation. The color of each branch within the subtype F1 cluster represents the country (or geographic region) of origin of sequence corresponding to that branch, according to the legend in the figure. The asterisks point at the subtype F1 Angolan sequences described in the present work. Brackets indicate the different monophyletic clusters identified. The trees were rooted using subtype C reference sequences as outgroups. Horizontal branch lengths are drawn to scale with the bar at the bottom indicating 0.1 nucleotide substitutions per site.

Background -2

- HIV clade F1 is dominant in Romanian population[2]
  - shares phylogenetic similarities with Angolan sequences[3]
  - Romanian strains seem to be diferent from F1 Brasilian and F1 European strains (Belgium & Finland)[4]

[2] Apetrei C, AIDS Res Hum Retroviruses. 1997 Mar 1;13(4):363-5
[3]Guimarães ML, *Retrovirology 2009,* 6:39
[4]Paraschiv S , Int J Infect Dis. 2007 Mar;11(2):123-8

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PLoS one

Drug Resistance Mutations for Surveillance of Transmitted HIV-1 Drug-Resistance: 2009 Update

Diane E. Bennett[1], Ricardo J. Camacho[2], Dan Otelea[3], Daniel R. Kuritzkes[4], Hervé Fleury[5], Mark Kiuchi[6], Walid Heneine[7], Rami Kantor[8], Michael R. Jordan[9], Jonathan M. Schapiro[6], Anne-Mieke Vandamme[10], Paul Sandstrom[11], Charles A. B. Boucher[12,13], David van de Vijver[12], Soo-Yon Rhee[6], Tommy F. Liu[6], Deenan Pillay[14], Robert W. Shafer[6]*

1 World Health Organization, Geneva, Switzerland, **2** Molecular Biology Laboratory, Centro Hospitalar de Lisboa Ocidental, Lisbon, Portugal, **3** Molecular Diagnostics, "Prof. Dr. Matei Bals" Institute for Infectious Diseases, Bucharest, Romania, **4** Brigham and Women's Hospital Harvard Medical School, Boston, Massachusetts, United States of America, **5** Laboratoire de Virologie EA 2968, Université de Bordeaux, Bordeaux, France, **6** Division of Infectious Diseases, Stanford University, Stanford, California, United States of America, **7** Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America, **8** Division of Infectious Diseases, Brown University, Providence, Rhode Island, United States of America, **9** Tufts University School of Medicine, Boston, Massachusetts, United States of America, **10** Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium, **11** Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada, Ottowa, Ontario, Canada, **12** Department of Virology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands, **13** Department of Medical Microbiology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands, **14** Centre for Virology, Division of Infection and Immunity, University College London and Centre for Infections, Health Protection Agency, London, United Kingdom

Abstract

Programs that monitor local, national, and regional levels of transmitted HIV-1 drug resistance inform treatment guidelines and provide feedback on the success of HIV-1 treatment and prevention programs. To accurately compare transmitted drug resistance rates across geographic regions and times, the World Health Organization has recommended the adoption of a consensus genotypic definition of transmitted HIV-1 drug resistance. In January 2007, we outlined criteria for developing a list of mutations for drug-resistance surveillance and compiled a list of 80 RT and protease mutations meeting these criteria (surveillance drug resistance mutations; SDRMs). Since January 2007, several new drugs have been approved and several new drug-resistance mutations have been identified. In this paper, we follow the same procedures described previously to develop an updated list of SDRMs that are likely to be useful for ongoing and future studies of transmitted drug resistance. The updated SDRM list has 93 mutations including 34 NRTI-resistance mutations at 15 RT positions, 19 NNRTI-resistance mutations at 10 RT positions, and 40 PI-resistance mutations at 18 protease positions.

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* E-mail: rshafer@stanford.edu

Background -3

- No surveillance drug-resistance mutations (SRDMs) were identified so far in the Romanian group of children and adolescents who were never exposed to antiretroviral treatment[4]

- The relatively reduced number of available sequences for non-B clades make difficult the interpretation of low-level polymorphic mutations (that can occur in the absence of selective drug pressure) vs transmitted drug resistance (TDR)[5]

[4]Paraschiv S , Int J Infect Dis. 2007 Mar;11(2):123-8
[5]Shafer RW, Antiviral Therapy 13, suppl 2: 59-68

Objectives

- We aimed to
  - evaluate the prevalence of subtype F, and the transmitted drug resistance (TDR) mutations among antiretroviral treatment (ART) naïve patients from “Dr. Victor Babes” Hospital for Infectious and Tropical Diseases Bucharest (VBH)
  - assess the phylogenetic relationship of VBH strains with other HIV-F strains

Methods

- Drug resistance genotyping was performed using
  - an in-house assay and Sequencer DNA analysis software version 4.8. performed at University College, Division of Infection and Immunity, London
  - Comercially available TRUGENE GENOTYPING KITS, Bayer Diagnostics

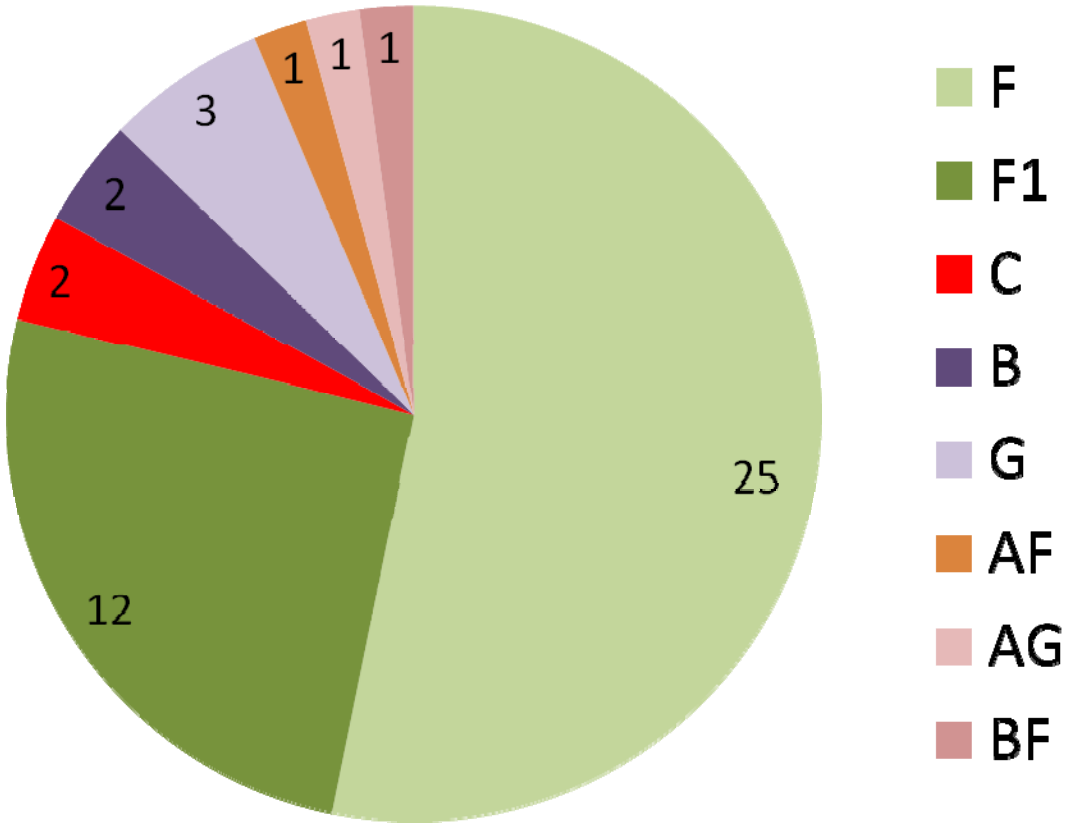
- Drug resistance interpretation was undertaken using the Stanford University HIVdb (http://hiv.db.stanford.edu/).
- Mutations associated with transmitted drug resistance (TDR) were identified using the WHO 2009 list of mutations for surveillance of TDR HIV strains http://hivdb.stanford.edu/pages/WHOResistanceList.html
- Study population: patients followed in “Victor Babes” Hospital without medical evidence of exposure to antiretroviral treatment
- Evaluation of CD4 count, HIV RNA (Cobas Amplicor Monitor 1.5 assay) at the moment of sample collection

Phylogenetic analysis

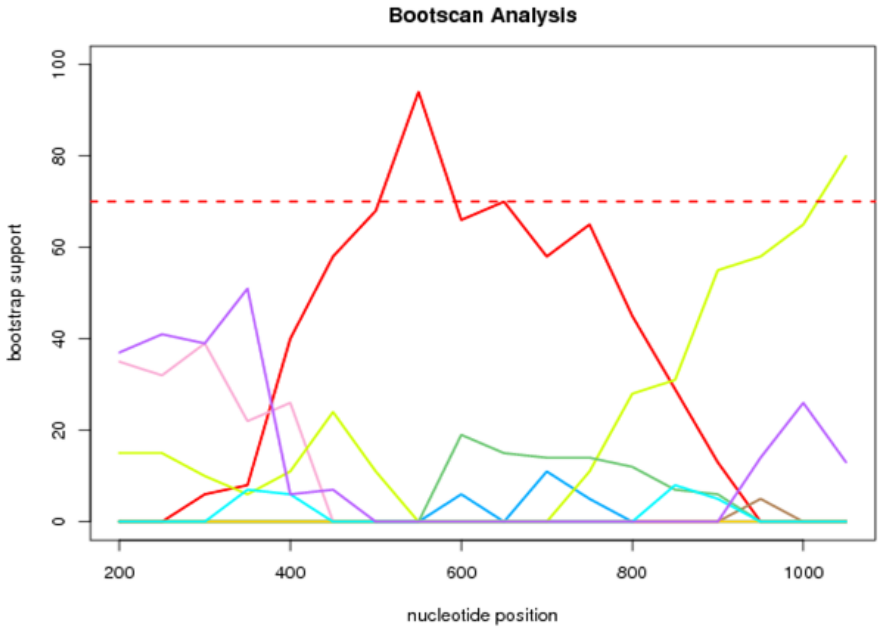
- All clade F, F1, F2 and recombinant HIV-1 pol sequences were downoladed from the HIV LANL database (http://www.hiv.lanl.gov/content/sequence).
- These sequences were aligned with the Romanian sequences using Clustal W and manually edited
- Neighbor-joining trees were then generated under the HKY model in Geneious program and re-sampled via bootstrapping 1,000 times

Results

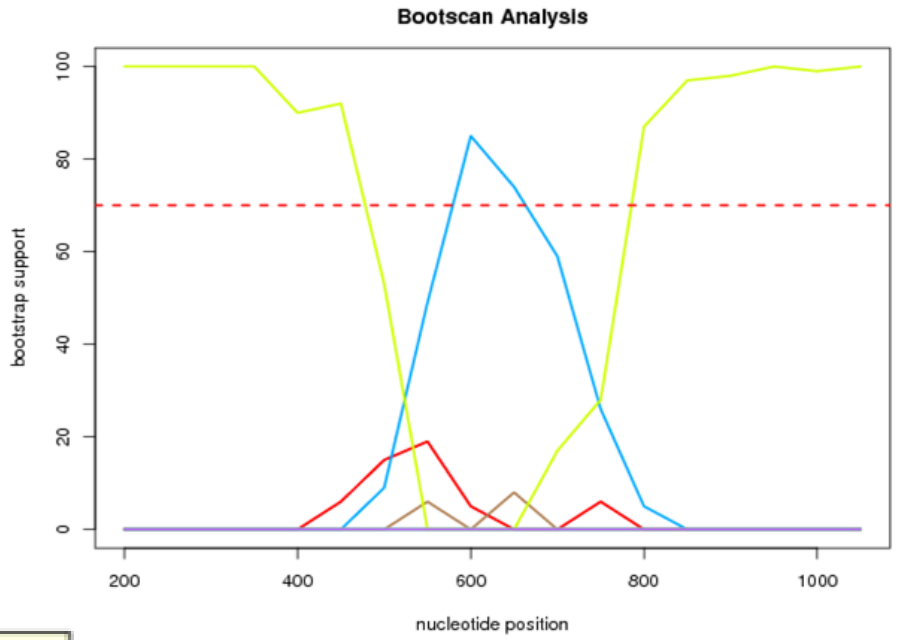
- 47 samples tested – 79 % clade F



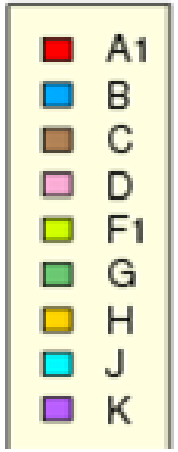
Recombinants? REGA



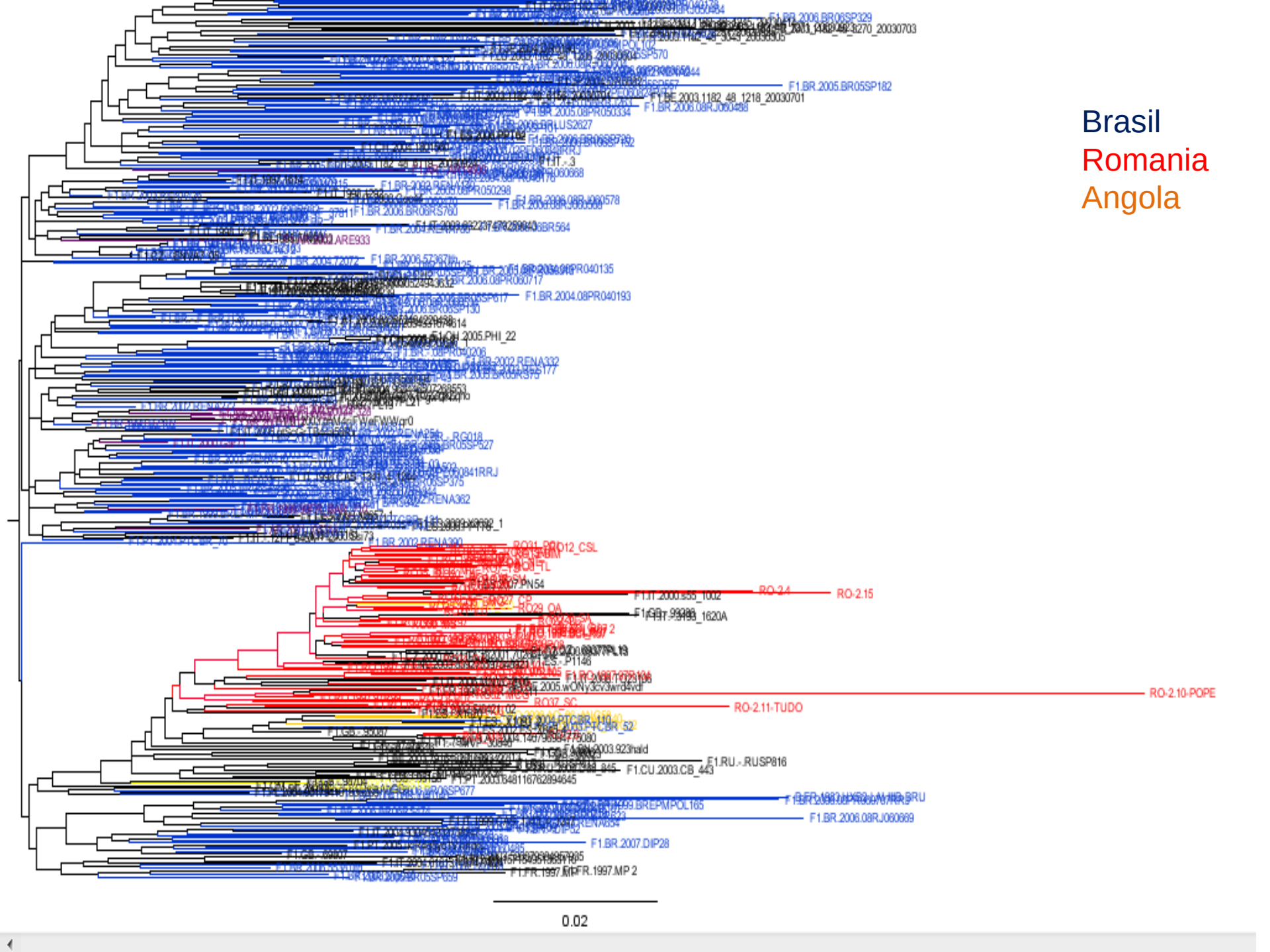
A/F

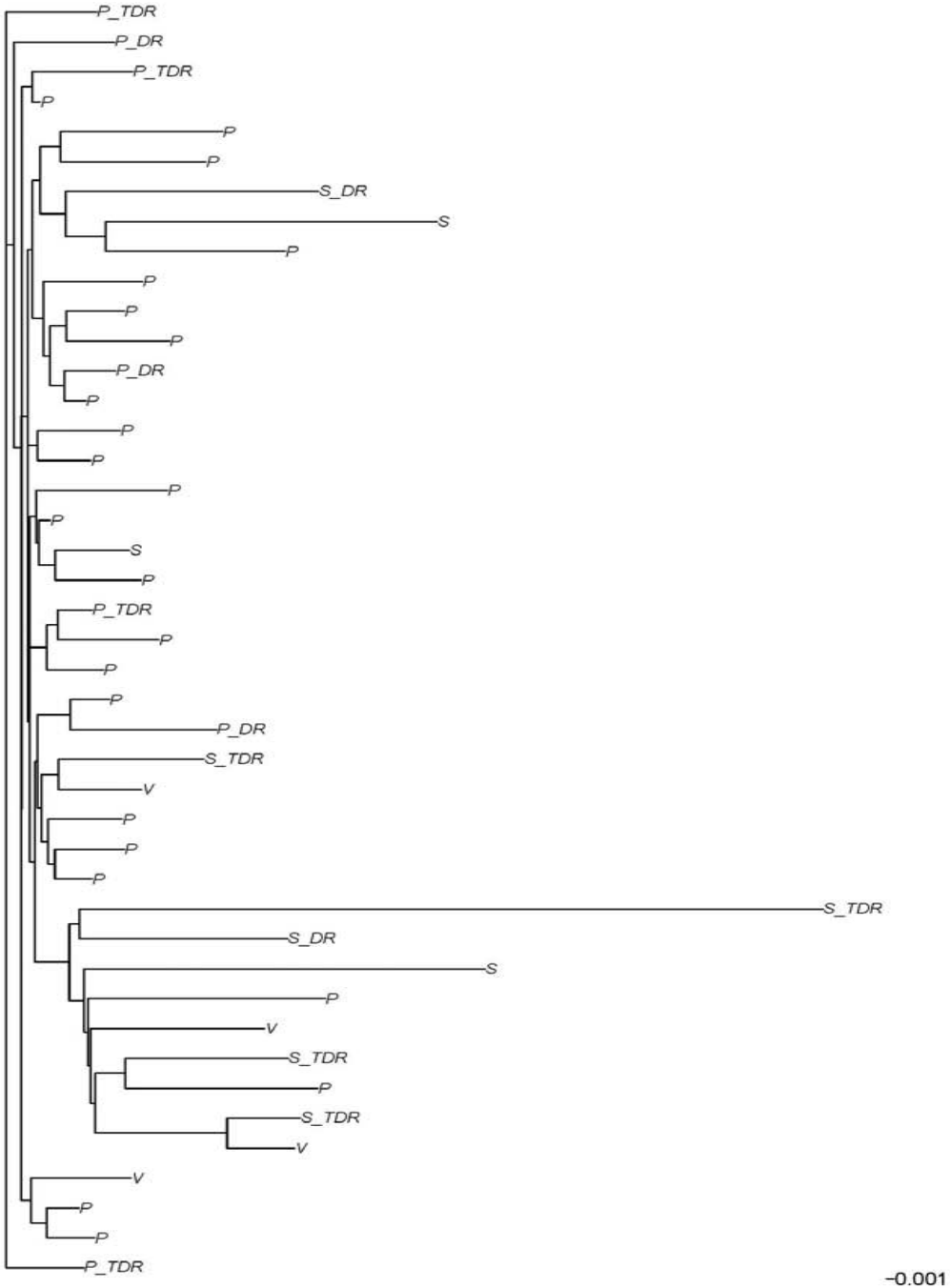


B/F



General characteristics of the naïve patients with clade F		
No pts		37
Female/male		17/20
HIV transmission route	Parenteral	24
	Vertical	4
	Sexual	10
Mean age at dg of HIV infection (years)		16.9 (0.1-33.5)
Mean age at sample colection (years)		20.8 (0.1-34.8)
Pts with AIDS diseases		10
CD4 count at evaluation lf/mmc(median, limits)		168 (range 2-2478)
HIV RNA log10 c/ml (median, limits)		5.1 (range 2,3-6.8)


Brasil
Romania
Angola
RO-2.4
RO-2.15
RO-2.10-POPE
RO-2.11-TUDO
F1.BR.2006.08RJ060669
F1.BR.2007.DIP28
F1.BR.2005.BR05SP182
F1.BR.2004.08PR040193
F1.CU.2003.CB_443
F1.RU.-.RUSP816
0.02


P_TDR
P_DR
P_TDR
P
P
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S_DR
S
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P
P
P
P_DR
P
P
P
P
S
P
P_TDR
P
P
P
P_DR
S_TDR
V
P
P
P
S_TDR
S_DR
S
P
V
S_TDR
P
S_TDR
V
V
P
P
P_TDR
−0.001

TDR

- 8/37 pts (21.6%) had any TDR*
  - 3 pts NNRTI 8.1%
  - 6 pts NRTI 16.2%
  - 1 pt IP 2.7%

* 1 patient had IP+RT TDR

2 pts had > 1 RT TDR

Mutations in the PR at key positions in the following amino-acids and positions

L10	I13	I15	G16	K20	E35	M36	N37	R41	K43	R57	D60	Q61	L63	I64	E65	H69	K70	I72	T74	V82	L89	Q92	I93
I1	V7	V29	E16	R19	D13	I37	DN2	K31	KR1	K34	E2	HRKNQ1	T35	IL1	D30	K1	R1	T25	S3	I1	LM2	K1	L6
V17					L23		D1		R1						ED1				SK1	**F1**	M24		D1
IV3							T1								N1								

Subscripts indicate the number of isolates in which the aminoacid was found

Mutations in the RT at key positions in the following amino-acids and positions									
M41	**D67**	T69	**K70**	**F77**	**K219**	K101	**K103**	E138*	V179*
L2	**G1**	ADNT1	**R1**	**L1**	**Q1**	Q1	**N3**	AE1, G2 D1,DV1	

Subscripts indicate the number of isolates in which the aminoacid was found
The bold red mutations are SRDM

*E138 A/G and V179D were associated with decreased susceptibility to Etravirine

Patients with SDRMs						
Pt s initials	Age at sampling	HIV transmission route	AIDS defining disease	Mutation	CD4 (lf/mmc)	HIV RNA (c/ml)
CV	16.5	parenteral	No	K219Q	36	50800
BM	17.9	parenteral	HIVE	K103N	45	193000
DIF	18.3	parenteral	HIVE	K103N	504	119000
BMG	20.3	parenteral	No	T69F		
IN	34.8	Sexual	TB	M41L	182	
MM	36	Sexual	No	K70R	281	
PA	32	Sexual	No	M41L, D67G, F77L, Q151R,		
TP	32	Sexual	No	M41V, T215N, K219Q	192	164000

Children with MTCT HIV-infection had no major or minor resistance mutations. Their mothers were ART-naive and only one child received MTCT prophylaxis.

Discussions

- We found major RT resistance mutations among 8 of 37 (26.1%) ART-naïve, patients
- 4 of 8 pts with TDR were adolescents with parenterally aquired HIV-infection →as initial infection with TDR is doubtful, occurrence of resistance mutations to NNRTI might be explained either by superinfection with another HIV strain by sexual route or due to undisclosed use of ART
- Paucity of subtype F sequence data in currently interpretation algorithms means that the effect of polymorphisms affecting the response to Etravirine is uncertain in this population

Discussions

- Phylogenetic analyse showed the existence of an distinct cluster of F1 clade in the Romanian group , by comparison with other F strains
- There seams to be no difference inside the romanian strains between the 3 age groups
  - the adolescents with parenteraly –acquired HIV infection
  - children with vertical HIV infection born to mothers who acquired HIV-infection by heterosexual route
  - the adults with heterosexual tranmission of HIV

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- The patients who participated at the study