COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

HIV molecular epidemiology and new treatment strategies in Russia and Eastern Europe: results of the research in CARE

Anastasia Antonova, Gamaleya Institute

Francesco Saladini, University of Siena

Anders Sönnerborg, Karolinska Institutet – Karolinska University Hospital

www.careresearch.eu

The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673

COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

UNIVERSITÀ DI SIENA 1240

CARE - HIV-1 VIROLOGY RESEARCH

Francesco Saladini, PhD
Department of Medical Biotechnologies
University of Siena, Italy

www.careresearch.eu

The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673

CARE WP4 background information (HIV-1 virology)

- Integrase strand inhibitors (INSTIs) are the mainstay of antiretroviral therapy (ART)
  - First-generation raltegravir (RAL) and elvitegravir (EVG)
  - Second-generation dolutegravir (DTG) and bictegravir (BIC), more potent and less affected by drug resistance
  - Cabotegravir (CAB), second-generation and long-acting formulation
- WHO recommends replacement of efavirenz (EFV) with DTG in first-line therapy, **DTG roll-out** has thus been started in Russia
- The HIV-1 epidemic in Russia is dominated by the **sub-subtype A6**
- There are **no data on the susceptibility of A6 HIV-1 to DTG**, however there has been a signal of **A6 being associated with failure to respond to CAB** based therapy in recent trials (Cutrell et al., AIDS 2021)

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

CARE WP4 planned activities (HIV-1 virology)

Analysis of the mechanisms of INSTI drug resistance in the highly prevalent HIV-1 sub-subtype A6, including the role of natural polymorphisms and off-target mechanisms based on mutations outside the integrase gene

- Evaluation of the in vitro susceptibility and the genetic barrier to resistance to INSTIs in A6 isolates

- Creation of a molecular clone with near full-length A6 genome for further analysis on drug resistance

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

In vitro susceptibility to INSTIs in A6 isolates

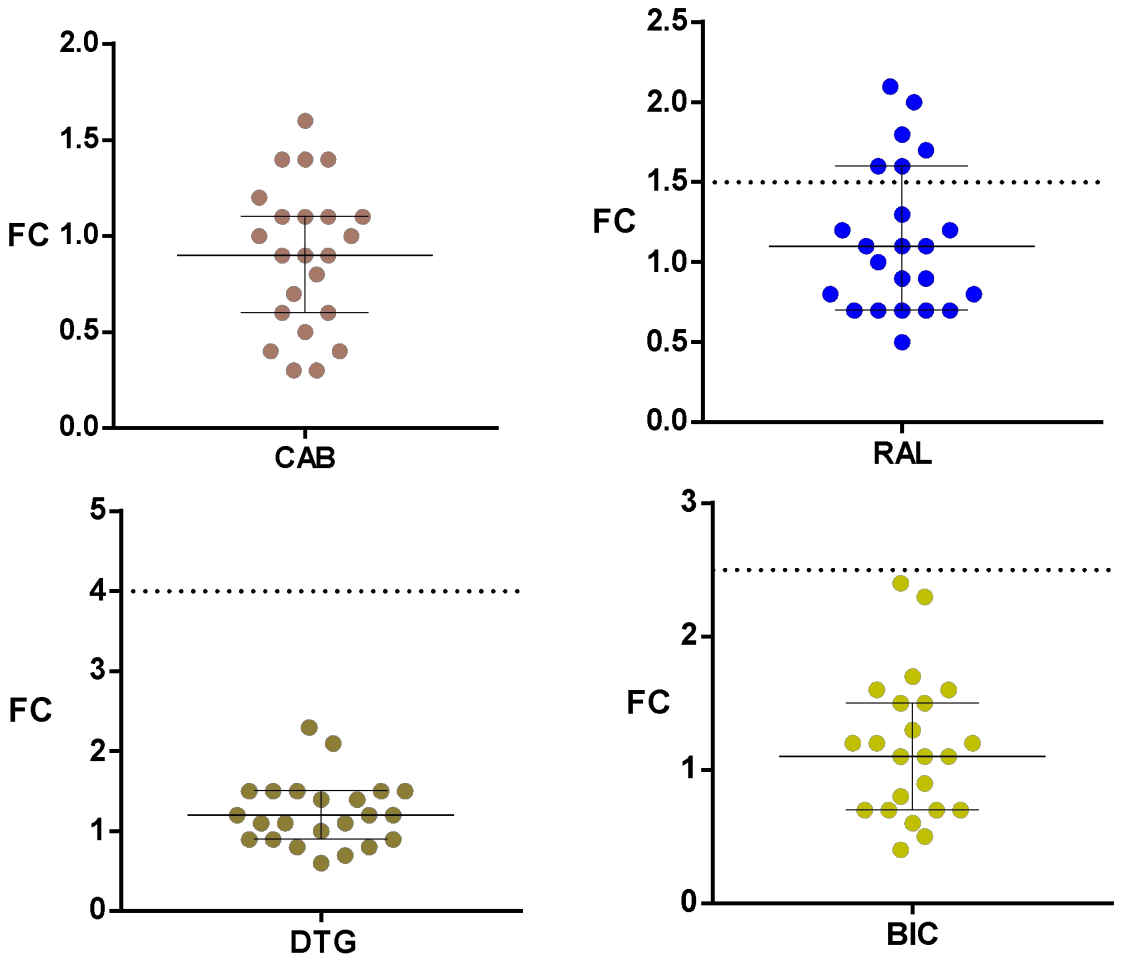
- We created **23 NL4-3 based recombinant viruses** harboring clinically derived **sub-subtype A6 integrase** coding region from samples collected at the HIV and Hepatitis Monitoring Laboratory (HHML) of Siena (n. 8), the Institute of Virology of Cologne (n. 2) and the Gamaleya Center of Moscow (n. 13)

- None of the 23 A6 sequences harbored major INSTI resistance associated mutations
- 22/23 (96%) sequences of samples had **L74I** variant
  - Consensus aminoacid in sub-subtype A6 and weakly selected under INSTI therapy
  - Signature mutation in the recent CAB treatment failures

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

In vitro susceptibility to INSTIs in A6 isolates

- In vitro susceptibility to raltegravir (RAL), dolutegravir (DTG), bictegravir (BIC) and cabotegravir (CAB) determined as fold-change (FC) with respect to the wild-type NL4-3 strain
- All samples fully susceptible to **DTG** and **BIC**
- Susceptibility to **CAB** comparable to that of NL4-3 virus
- 6/23 viruses with minimally decreased susceptibility to **RAL**



FC cut-offs (dotted line): RAL 1.5 – biological; DTG lower 4, upper 13 – clinical; BIC lower 2.5, upper 10 – clinical, estimated; CAB not available)

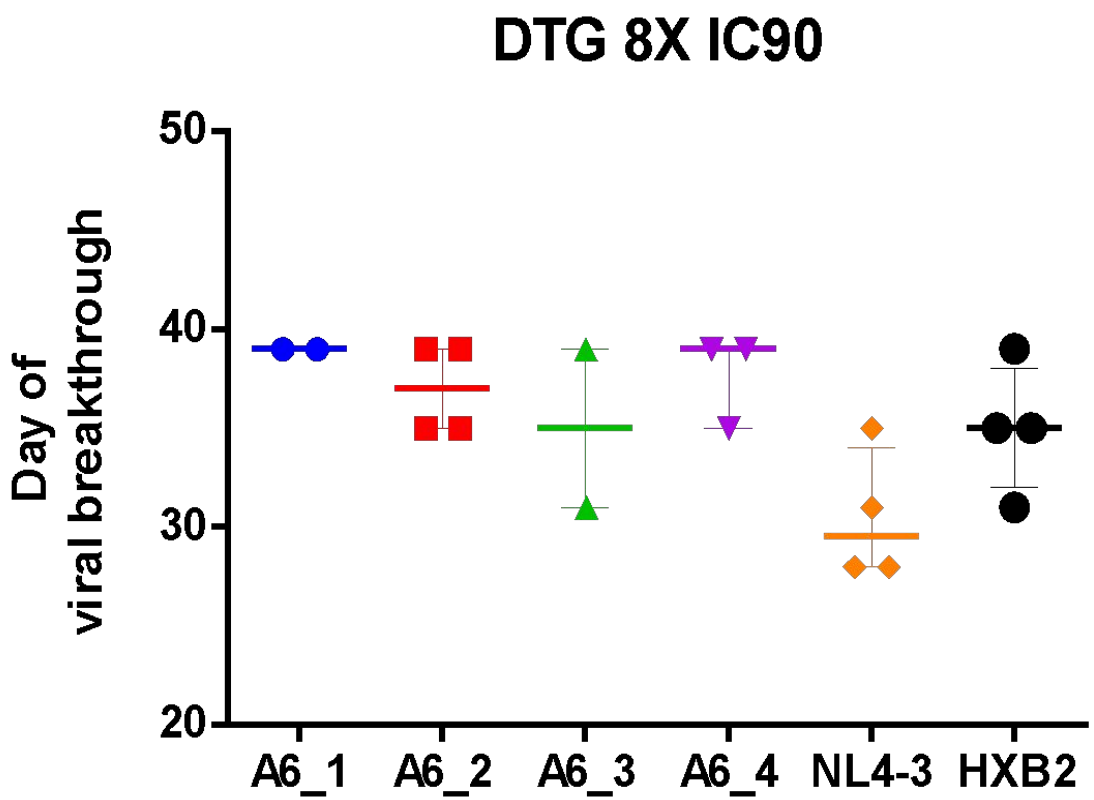
This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

In vitro genetic barrier to resistance to INSTIs in A6 isolates

- MT-2 cells infected with A6 recombinant viruses (A6_1 to A6_4) or reference NL4-3 and HXB2 viruses and exposed to DTG at 8X IC_{90} value as calculated with NL4-3 virus
- Viral breakthrough monitored every 48/72 hours to detect virus-induced cytopathic effect (syncytia)

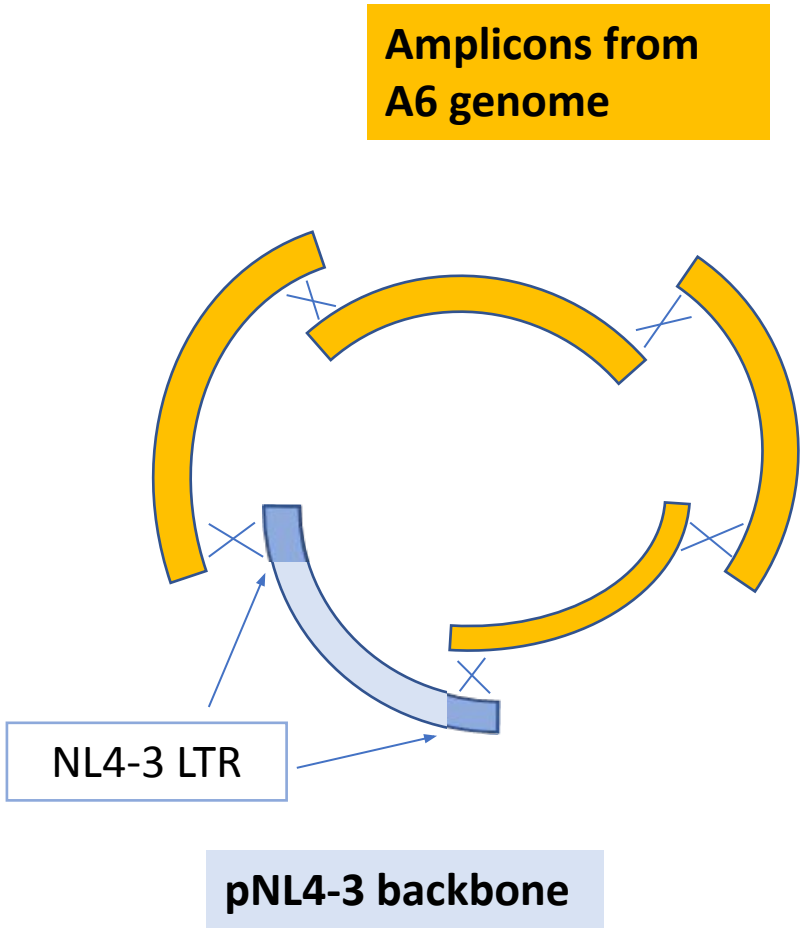
- No significant differences observed between A6 recombinant viruses and NL4-3/HXB2 strains
- No emergent mutations detected at viral breakthrough
- Further experiments ongoing with higher drug doses (16X and 32X IC_{90})



This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

Creation of a molecular clone with near full-length A6 genome for further analysis on drug resistance



- Amplification of four overlapping PCR fragments from viral RNA spanning from the whole GAG to ENV coding regions
- Assembly with the NL4-3 vector including the LTR region through homologous recombination carried out by Gibson assembly method
- Identification of replication competent clones after transfection in 293 cells and propagation in CD4+ T-cell lines
- Final assembly still ongoing

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

CARE WP4 (HIV-1 virology) – Summary

- A6 integrase appears to be fully susceptible to INSTIs, except for few viruses showing minimal reduction in RAL susceptibility

- Virus breakthrough in *in vitro* selection experiments also does not appear to differ substantially between A6 and the reference subtype B
  - Further experiments in progress, emergent resistance at higher drug dosing still to be evaluated

- The creation of a full-length A6 molecular clone is in progress
  - Valuable to better investigate resistance not only to INSTIs but also to other drugs as well as to define the synergistic effect of drug combinations

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

ФНИЦ эпидемиологии и микробиологии имени Н.Ф. Гамалеи

CARE - HIV-1 VIROLOGY RESEARCH IN RUSSIA

Anastasia Antonova, Junior researcher

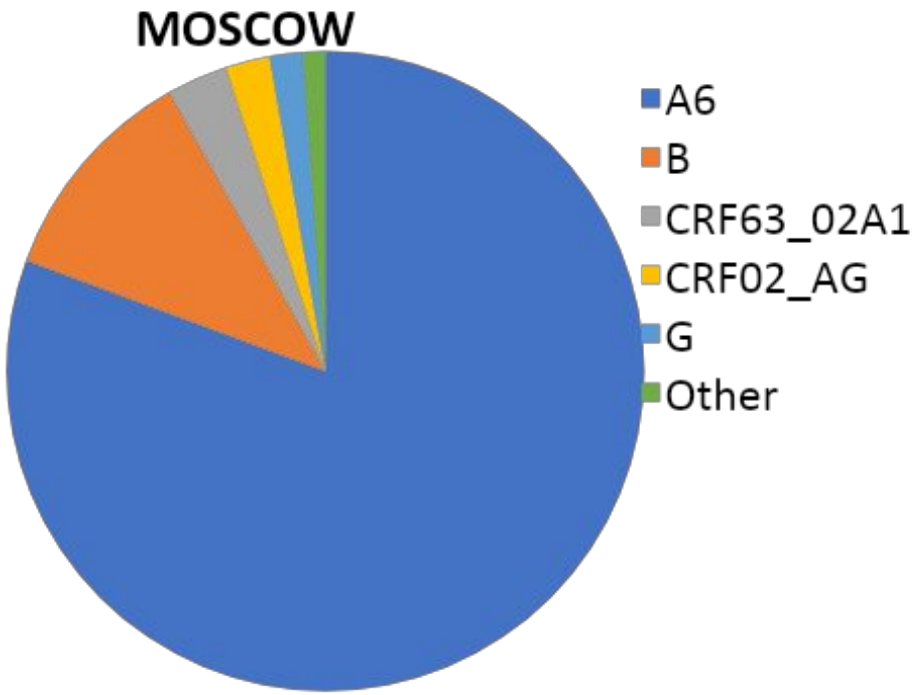
Gamaleya National research center of epidemiology and microbiology

www.careresearch.eu

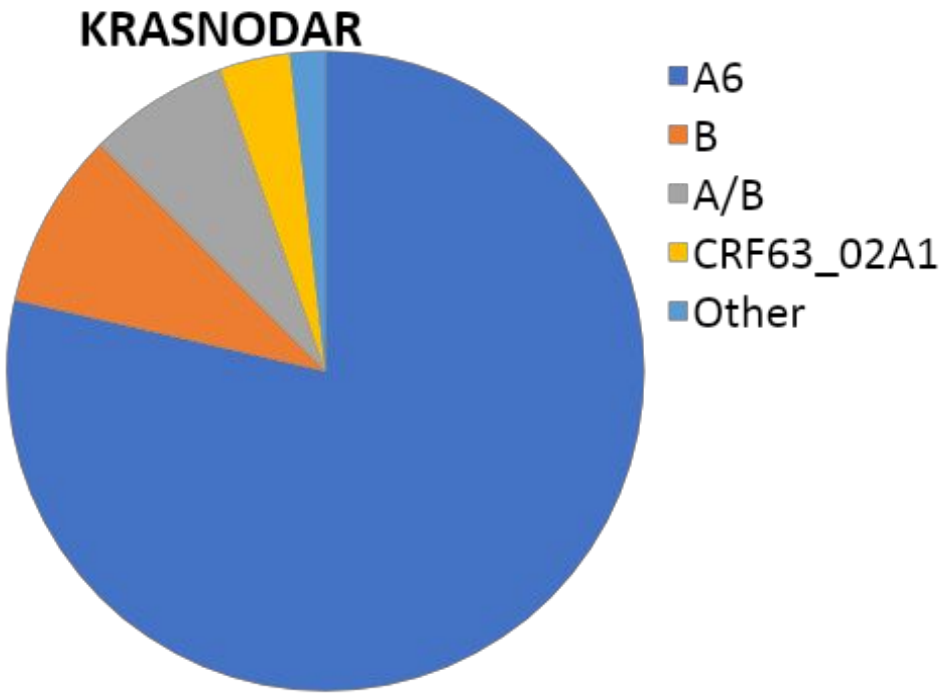
The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673

HIV molecular epidemiology and new treatment strategies in Russia



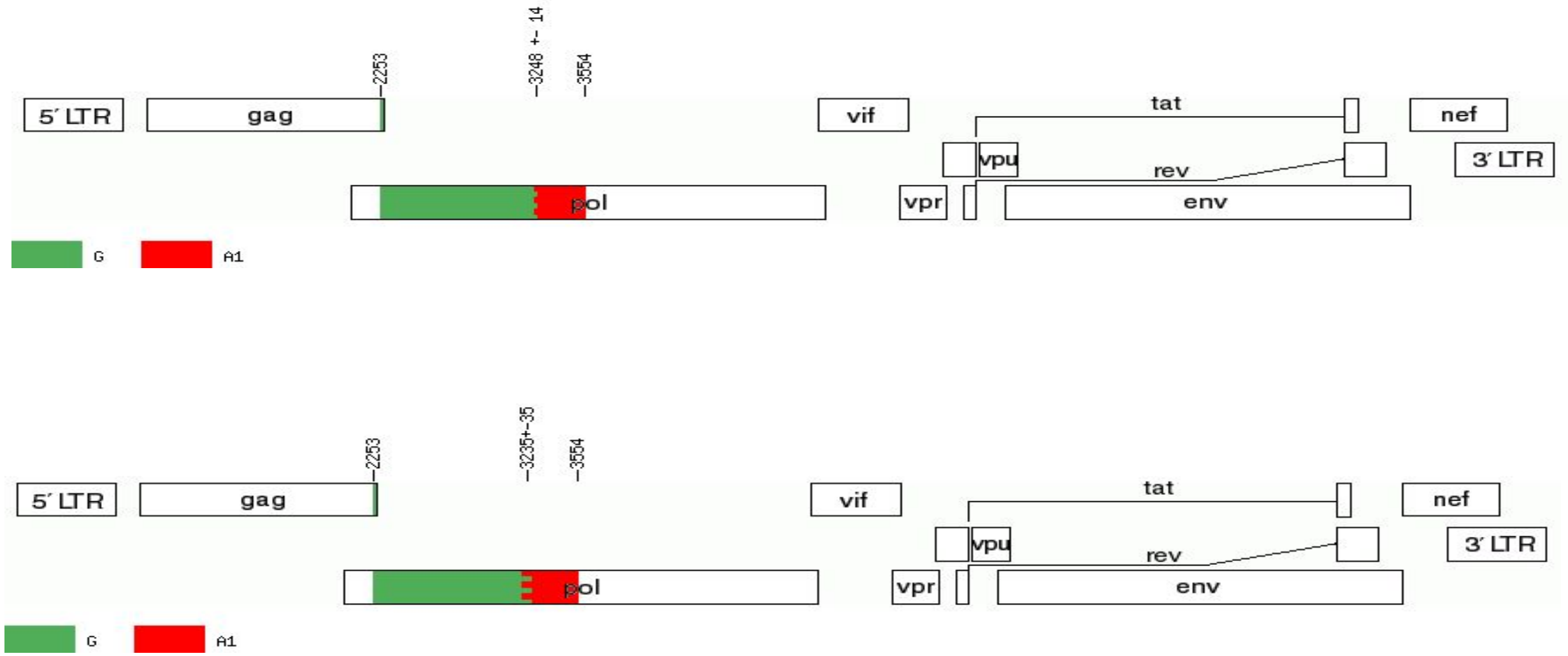
n = 356



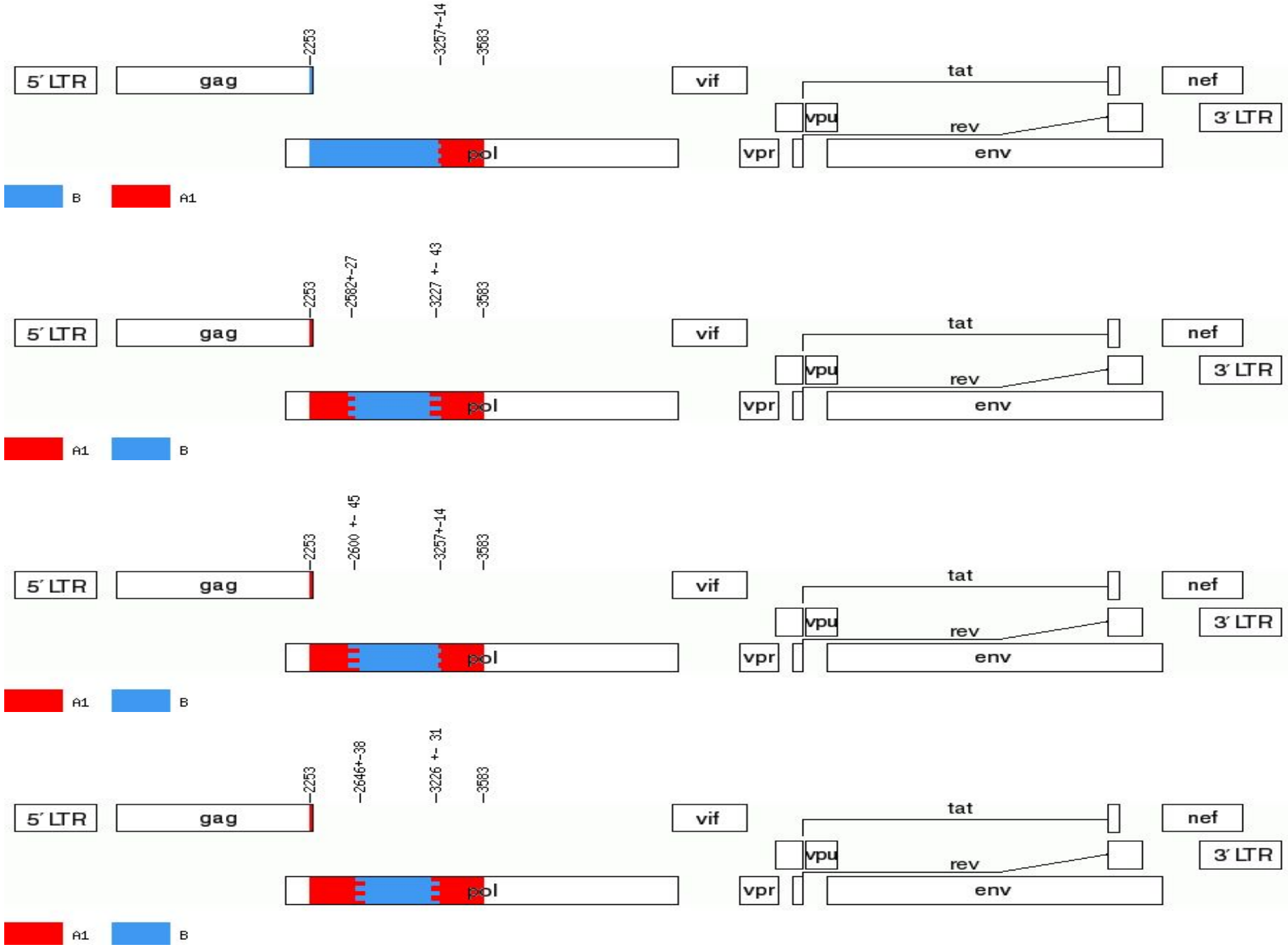
n = 56

The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673



The genome map of recombinants from MSK



The genome nap of recombinants from KRD

Pretreatment Drug Resistance (PDR)

MSK

Proportion of sequences with SDRMs

Resistance Category	No. Analyzed	No. Containing SDRM	%
Sequences with any SDRM	356	11	3.1%
PR Sequences with any PI SDRM	356	2	0.6%
RT Sequences with any NRTI SDRM	351	2	0.6%
RT Sequences with any NNRTI SDRM	351	9	2.6%
RT Sequences with any NRTI + any NNRTI SDRM	351	1	0.3%
PRRT Sequences with any NRTI + any NNRTI + any PI SDRM	351	0	0.0%

KRD

Proportion of sequences with SDRMs

Resistance Category	No. Analyzed	No. Containing SDRM	%
Sequences with any SDRM	56	7	12.5%
PR Sequences with any PI SDRM	53	0	0.0%
RT Sequences with any NRTI SDRM	56	4	7.1%
RT Sequences with any NNRTI SDRM	56	6	10.7%
RT Sequences with any NRTI + any NNRTI SDRM	56	3	5.4%
PRRT Sequences with any NRTI + any NNRTI + any PI SDRM	53	0	0.0%

Structure of PDR

Sequences with SDRMs

Sequence Header	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
442373	None	K103N, Y181C	None
442382	None	K103N	None
488710	None	K101E	None
486866	None	K103N	L24I
486921	K65R, Y115F, M184V	Y181C, G190S	None
494609	None	None	M46I
494610	None	K103N	None
504122	None	K103N	None
499797	None	Y188L	None
499777	None	V106M	None
508614	T69D	None	None

Sequence Header	NRTI SDRMs	NNRTI SDRMs	PI SDRMs
KRD0002	None	K103N	None
KRD0008	D67N, K70E, M184I	K101E, Y181C	None
KRD0009	None	K103S	None
KRD0021	K65R, M184V	K101E, Y188HL	None
KRD0030	None	K103N	None
KRD0056	M41L	None	None
KRD0059	K65R, Y115F, M184V	G190S	None

Three recommended options for the preferred first-line ART regimen for adults in the Russian Federation:

EFV + 3TC (or FTC) + TDF

K103N, Y181C M184V/I K65R

DTG + 3TC (or FTC) + TDF

M184V/I K65R

VM-1500 + 3TC (or FTC) + TDF

M184V/I K65R

The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673

COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

FUTURE PERSPECTIVES, VIROLOGY

Anders Sönnerborg, MD PhD
Divisions of Infectious Diseases and Clinical Microbiology
Karolinska Institutet / Karolinska University Hospital

The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

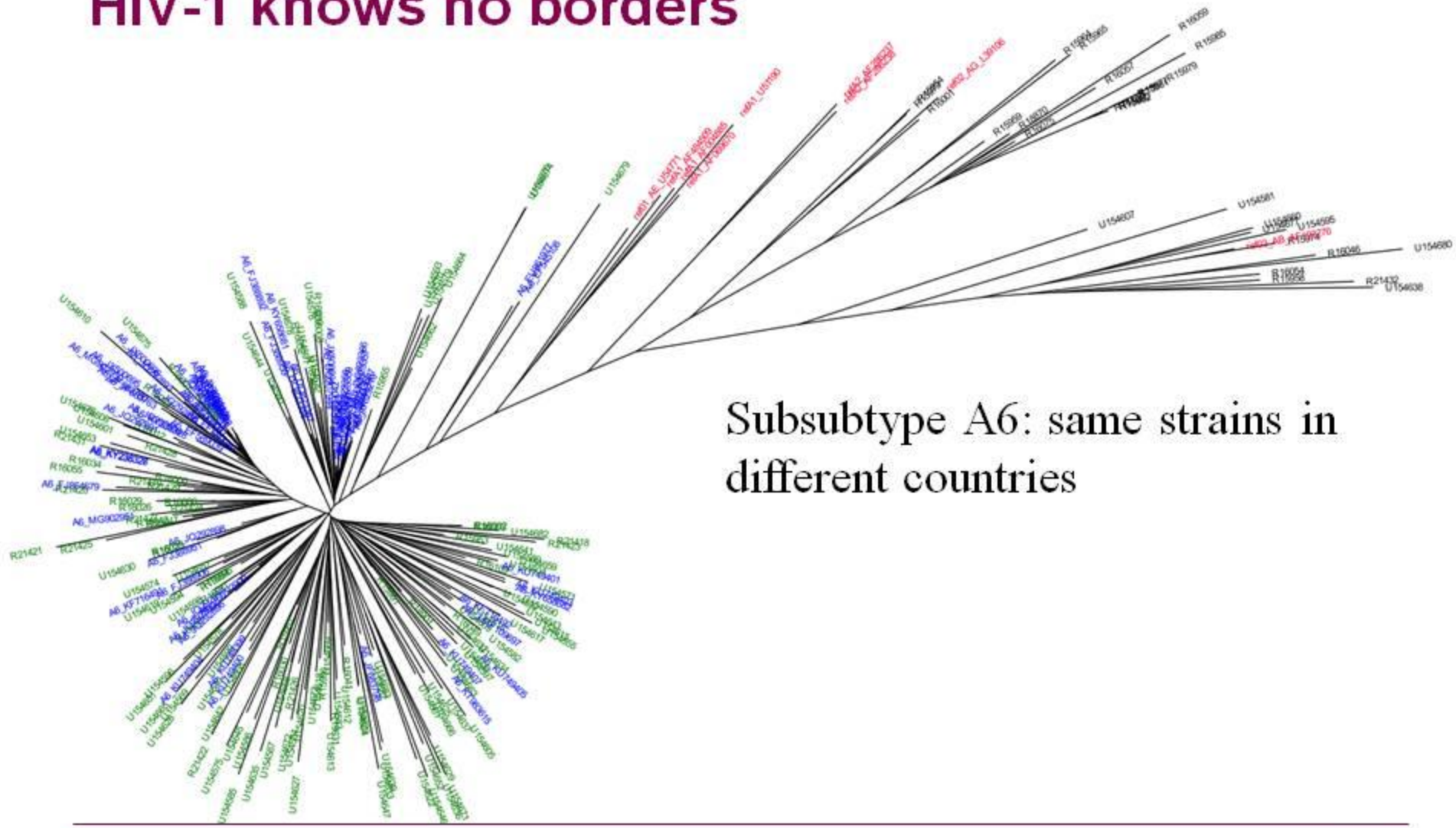
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673

Clinical/translational HIV-1 virology research over the borders, WP4

- Clinical HIV-1 cohorts/databases
- Sharing technical knowledge
- Molecular epidemiology through sharing viral sequences
- Basic and clinically oriented virology
- Educational knowledge transfer

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

HIV-1 knows no borders



Subsubtype A6: same strains in different countries

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

Future perspectives - ongoing

- Analyse pooled clinical and viral sequence data from all countries
  - → molecular epidemiology
  - → pretreatment drug resistance
  - → any drug resistance in failing patients

- Finalizing experimental virology
  - → Final insights in the geno- and phenotypes of the A6 strains

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

Karolinska Institutet

Future perspectives – continuation of the collaborations

- A common infrastructure has been established
- Logistics for exchange of knowledge and technical skills are in place
- Insights in partners knowledge and skills have been obtained
- A platform for continuation of surveillance of HIV-1 molecular epidemiology and drug resistance is established
- When new HIV-1 variants (or other viruses) emerge the collaboration will facilitate a rapid adaptation

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

COMMON ACTION AGAINST HIV/TB/HCV ACROSS THE REGIONS OF EUROPE

Karolinska Institutet

Thanks for your interest

This presentation is based upon work from CARE Consortium, that has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 825673 and the Ministry of Science and Higher Education of the Russian Federation, federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

CARE Consortium

Denmark

REGION HOVEDSTADEN/CHIP

Georgia

INFECTIOUS DISEASES, AIDS AND CLINICAL IMMUNOLOGY RESEARCH CENTER

Germany

EBERHARD KARLS UNIVERSITAET TUEBINGEN

FORSCHUNGSZENTRUM BORSTEL

KLINIKUM DER UNIVERSITÄT ZU KÖLN

Italy

EURESIST NETWORK

UNIVERSITA' DEGLI STUDI DI SIENA

Lithuania

VIESOJI ISTAIGA VILNIAUS UNIVERSITETO LIGONINE SANTAROS KLINIKOS

Moldova

INSTITUTIA MEDICO-SANITARA PUBLICA - INSTITUTUL DE FTIZIOPNEUMOLOGIE "CHIRIL DRAGANIUC"

Russian Federation

FEDERAL RESEARCH CENTRE FOR EPIDEMIOLOGY AND MICROBIOLOGY NAMED AFTER THE HONORARY ACADEMICIAN N.F. GAMALEYA OF THE MINISTRY OF HEALTH

FEDERAL STATE INSTITUTION SAINT PETERSBURG RESEARCH INSTITUTE OF PHTHISIOPULMONOLOGY OF THE FEDERAL AGENCY FOR HIGH TECHNOLOGY MEDICAL AID

Sweden

KAROLINSKA INSTITUTET

United Kingdom

IMPERIAL COLLEGE OF SCIENCE TECHNOLOGY AND MEDICINE

Ukraine

STATE INSTITUTION PUBLIC HEALTH CENTER OF THE MINISTRY OF HEALTH OF UKRAINE

The project has received funding from the Ministry of Science and Higher Education of the Russian Federation, Federal target program 2014-2020, unique project identifier RFMEFI61019X0020.

This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 825673