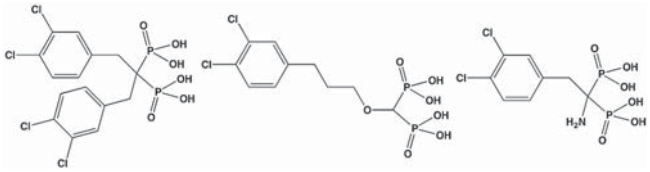
infection still remains a significant health threat. The key steps of the replication cycle of HIV-1 are the viral DNA synthesis and its integration into the cell genome. One of the current problems associated with HIV therapy is the emergence of drug-resistant virus variants. The resistance emergence is often associated with phosphorolytic excision of the 3'-drug-terminator. To date lots of inhibitors of different viral enzymatic activities are developed, however, a promising approach for development of novel and effective anti-HIV drugs is the design of multitarget compounds suppressing simultaneously several of these activities.

Herein, we synthesized 40 methylenebisphosphonates (BPs), five of which simultaneously inhibited phosphorolytic activity of native and drug-resistant forms of HIV-1 reverse transcriptase (RT), RT catalyzed elongation and RNAse H activity and, moreover, two integrase activities. We assessed structural elements required for simultaneous inhibition of these reactions. BPs should be constructed of three pharmacophores: the methylenebisphosphonate backbone, the aromatic halogenated pharmacophore linked to the backbone through the inert aliphatic linker, and the



Mg^{2+}-coordinating group. The activity of BPs was also affected by the nature of the second substituent at the bridging carbon. The most active BPs inhibiting activities of the main HIV-1 enzymes are demonstrated below.

Interaction between HIV-1 integrase and the host protein Ku70: a promising approach to antiretroviral therapy

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The Ku protein is a heterodimer composed of two subunits: Ku70 and Ku80. The main function of this heterodimer is the binding of DNA termini produced by double-strand DNA brakes during the first steps of the non-homologous end joining repair process, that makes Ku an essential complex for cellular survival during genotoxic stress. Besides its role in NHEJ, Ku can also take part in transcription regulation, telomere maintenance, protein turn-over, cytoplasmic DNA-sensing and some other processes. Recently, it has been reported that Ku participates in the HIV-1 replication. Ku favors different stages of the HIV-1 life cycle, such as the formation of 2-LTR circles, integration and transcription of the integrated viral DNA. Viral replication is diminished in cells depleted of either component of Ku and this effect is more pronounced during the early stages of viral replication. However, an exact mechanism by which Ku affects the replication of HIV-1 remains to be evaluated. It has been proposed, that the binding of Ku70 subunit to HIV-1 integrase (IN) protects the later from proteasomal degradation. Considering this, the inhibition of the complex formation between IN and Ku70 might affect viral replication. The drug design would be greatly facilitated if a detailed structure of the IN/Ku70 complex were present. Unfortunately, the exact structure of HIV-1 IN is not yet known, and only single domains of IN can be effectively crystallized.

We have shown that a stable complex can be formed between recombinant Ku70 and IN with a Kd ~ 70 nM. A series of deletion mutants were created both for Ku70 and IN that helped us localize the binding sites within both proteins. N-His6-tagged HIV-1 IN separate domains (N-terminal (1–50 aa), catalytic (51–220 aa) and C-terminal (220–270 aa)) were expressed in *E. coli* as well as several truncated IN variants containing amino acids 1–160, 1–220, 51–160 and 51–280. A full-size Ku70 with a GST-tag on its N-termini together with a number of truncated variants (Ku70(1–250), Ku70(250–609)) were purified also from *E. coli*. Using the GST-pull down technique we have gained data suggesting that the binding of Ku70 with HIV-1 IN relies at least on two sites in the proteins structure. Specifically, the Ku70(1–205) domain makes contacts with an α-helix located in the (160–230) IN region. This observation is further supported by inserting point mutations in various positions in this α-helix. Ku70(250–609) can also bind to IN but the site of this interaction localizes closer to the N-terminus of the protein around the region of IN(130–160). The data obtained from experiments on recombinant purified proteins were corroborated by expressing C-terminal HA-tagged full-length IN and its various deletion mutants in HEK 293T cells with or without a WT Ku70–3FLAG and truncated variants. The coexpression with Ku70 stabilized IN in the cells, while the IN expression in cell that were knocked down of Ku70 was greatly reduced. Based on the data collected in our experiments we intend to construct an optimized computer model of the complex between HIV-1 IN and Ku70 that will be further used for molecular docking of potential inhibitors of their interactions.

Diversity of HIV-1 recombinant forms in Russia and the former USSR countries

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Background: The massive HIV-1 epidemic in Russia and the former USSR started in the mid-1990 years. In the

early years of the epidemic when the leading risk factor was intravenous drug use, the population of HIV genetic variants was characterized by a high degree of homogeneity, with subtype was A1 being the most prevalent. In the late 90-es the recombinant form CRF03_AB was first registered in the Kaliningrad region; later on it was found as rare cases in all fUSSR countries, including Russia, and caused the outbreak in the city of Cherepovets in 2006. In addition, CRF02_AG recombinant, presumably originating from Cameroon, where this variant is quite widespread, was found in 2005 in Uzbekistan.

The purpose of this study was to conduct an analysis of prevalence and nature of HIV-1 recombinant forms in Russia and fUSSR countries at the present time.

Methods: From 2008 to 2015 years, 1347 sequences of HIV-1 *pol* gene from patients from Russia, Kazakhstan, Kyrgyzstan and Armenia were analyzed in the laboratory. The sequences were obtained using ViroSeq HIV-genotyping system, as well as *in house* method. Genotyping and phylogenetic analysis were performed using the COMET HIV-1 / 2v.0.5, MEGA 6.06 and PhyML program. There were 141 recombinant forms found among the samples analyzed. In addition, 50 pol gene sequences of HIV-1 recombinants from GenBank (Russia, Ukraine, Belarus) were included into this study.

Results: Three groups of circulating recombinant forms were found among the HIV-1 *pol* gene sequences analyzed — CRF02_AG (102/191, 53,4%), CRF03_AB (37/191, 19,4%) and CRF63_02A1 (42/191, 22%) — the double recombinant generated by viruses belonging to subtype A1 and CRF02_AG, as well as 10 unique recombinant forms of the same origin. All CRF02_AG sequence from Russia and FSU without exception clustered with the variant of Uzbekistan.

The frequency of recombinant forms of HIV-1 differed in different countries: we found 45,6% of them in Kyrgyzstan, 34,6% in Kazakhstan, 2,9% in Armenia and 4,5% in Russia.

Conclusion: Currently, the widespread of HIV-1 recombinant forms can be traced in all fUSSR countries and Russia, thereby increasing its diversity, with the appearance of unique recombinant forms. In general, it may be associated with the increased activity of migration, and with the active co-circulation of different HIV-1 genetic variants in the population.

Comparative analysis of AFSU HIV-1 variants circulating in IDUs and heterosexua populations

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Introduction: The large-scale epidemic of HIV-infection in Russia started when AFSU (IDU-A) subtype A1 HIV-1 variant was introduced into the population of injecting drug users (IDUs) in 90-s, and was characterized with the rapid distribution in this risk group together with the very low (1–2%) of the genetic differences between the viruses circulating in the population. Since the beginning of the 2000s the output of the virus outside the risk group specified was noted, with the gradual spread of AFSU variant through the heterosexual contacts whose share reached 37% among HIV-infected persons in 2015.

The aim of the present study was to compare the rate and nature of AFSU divergence over time in the main risk groups using the well-studied example of HIV-infection epidemic in the Perm region.

Materials and methods: Blood samples from 142 naive HIV-infected patients from Perm who were infected between 1996 and 2011 were collected, of which 91 belonged to the IDUs risk group and 51 were heterosexuals. The fragments of *pol* gene (PR-RT, 954 bp) and *env* (C2-V4, 498 bp) was obtained by the «nested» PCR, followed by sequencing. Phylodynamic analysis was carried out using Beast software package v 1.8.2 (http://beast. bio.ed.ac.uk). Reconstruction of the most recent common ancestor (tMRCA) and divergence estimation were performed using MEGA 6.0 program (http://megasoftware.net/). The search for the codons positive with regard to selection was carried out using DataMonkey (http://datamonkey.org); glycosylation sites were searched using the programs N-GlycoSite (http://hiv.lanl.gov) and NetPhos 2.0 (http://cbs.dtu.dk).

Results: The average genetic distance among all AFSU samples studied (142) and their tMRCA increased with time ($r=0,71$; $p<0,001$) and from 1996 to 2011 rose from 1,75 to 3,02% and from 2,98 to 6,29% in *pol* and *env* genes, respectively. The average rate (±SE) of the *pol* gene fragment evolution in A_{FSU} variants circulating in 2003–2011 among IDUs (n=57) and heterosexuals (n=51) was $1,83\pm0,13$ ($\times10^{-3}$) and $2,78\pm0,10$ ($\times10^{-3}$) substitutions per site per year, respectively. The similar index for the *env* gene fragment was $2,73\pm0,17$ ($\times10^{-3}$) and $6,18\pm0,14$ ($\times10^{-3}$) substitutions per site per year for IDUs and heterosexuals, respectively. Positive selection at the level of 6 codons was detected in the *pol* gene, with three differing positions depending on the risk group. There were 21 such codons detected within env gene, with the differences between the risk groups in 14 positions. The profile and frequency of 17 glycosylation sites in *env* gene showed no differences between the virus variants circulating in different risk groups.

Conclusions: Analysis of the genome of A_{FSU} HIV-1 viruses circulating in the region shows the unequal rate and nature of the diversity both in different viral genes and between different risk groups.