nine protein kinase that mediates sustained activity of FOF_1 ATP synthase, and the latter enzyme complex to which oligomycin A is a known antagonist. Regardless of its cytotoxic potency oligomycin A is inconvenient for clinical use due to general toxicity. In collaboration with Gause Institute of New Antibiotics, Moscow we have synthesized 25 oligomycin A derivatives differentially active against human tumor cell lines. To further screen this series we developed a test system in actinobacterial strain *Streptomyces fradiae* ATCC 19 609 that is exceptionally sensitive to this compound. This bacterial test system is valid for screening of antitumor derivatives since the amino acid sequences of oligomycin A binding sites in the C-subunit of FOF_1 ATP synthase of this strain and *H. sapiens* are close. The β-subunit can be phosphorylated by an upstream kinase, therefore, its inhibition should be therapeutically relevant.

The proposal implies rational design of oligomycin A derivatives and beta subunit antagonists of FOF_1 ATP synthase on Hodgkin's lymphoma cells and patients' samples collected in Moscow. The synthesis and test systems are original. Finally, we will elucidate the mechanisms of lymphoma cell death induced by the leading derivative in combination with C-subunit inhibitor.

Replication of HIV-1 with accessory gene deletions in cell-coculture and cell-free modes of transmission

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HIV-1 restriction factors are cellular proteins that inhibit viral replication at post-entry stage of virus life cycle. The dozens of cellular factors with antiviral activities have been described. However, only six main protein families, i.e. APOBEC, TRIM, Tetherin, SAMHD, and MX2, specifically restrict the replication of HIV. The mechanisms of restriction are specific for each factor, and affect different stages of viral replication that overall makes cells highly resistant to virus. Nevertheless, HIV has evolved its own proteins Vif, Vpr, Vpu, Vpx, Nef, which efficiently counteract restriction factors and abolish their protective effects.

The role of restriction factors in HIV-1 replication has been studied extensively upon infection with cell free virus. However, an HIV-1 restriction during cell-to-cell mode of virus transmission, which dominates at the early stages of infection in vivo, is not studied comprehensively.

In this work, we have constructed HIV-1 packaging plasmids with deletions in Vif, Vpu, Vpr or Nef gene, and determined the levels of defective virus replication at the different settings including various types of producer and target cells, and modes of transmission. To quantify the levels of cell-to-cell infection, the improved replication dependent vectors were used (Shunaeva A et al. J Virol. 2015 Oct 15;89 (20):10591–601). The effect of accessory gene deletion on HIV-1 infectivity was dependent on cell type and mode of transmission. Particularly, the differences between wild type and mutated HIV-1 replication levels were significant in lymphoid cells and neglectable in HEK 293T cells. This suggests that lymphoid cells express a wide gamma of HIV-1 restriction factors in the response to infection. When comparing cell-free infection with cell-coclulture infection, the defect in the expression of one of accessory proteins resulted in ten fold and more decrease in infectivity with cell-free virus derived from Jurkat T cells. In contrast, the levels of replication of defective virus in coculture of lymphoid cells were reduced less than 1,5–2 times in comparison to replication of wild type virus, indicating that cell-to-cell transmission overcomes the restriction imposed by deletion of HIV-1 accessory genes. Interestingly, the replication of Vpu-defective HIV-1 was even 3–5 fold more efficient in cell cocultures than infectivity of wild type virus, whilst under cell-free mode of infection the deletion of Vpu decreased the HIV infectivity. Overall, our data suggest that hijacking cell-to-cell mode of transmission can be an additional mechanism that HIV-1 uses to counteract cellular restriction.

Immunotherapy against drug resistance as a therapy compliment increasing the duration of the effective use of art

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Introduction: Clinical trials demonstrated validity of therapeutic HIV vaccines for reducing viral load and improving patient well-being towards «functional cure» (Noto & Trautmann, 2013; Wahren & Liu, 2014). HIV immunotherapy becomes actual in view of the latest findings of an effective broad T-cell response clearing HIV-1 from the latent reservoirs (Deng et al, 2015; Rawlings SA et al, 2015). Strong immune response against viral antigens responsible for drug resistance can create a bottle-neck to viral evolution forbidding or hindering the development of drug resistance. In HIV-1, such response can complement highly active antiretroviral treatment, and thus prolong the time for its effective application. By controlling the emergence of resistant HIV strains, a combination of ART and immune therapy would delay and may ideally prevent the emergence of HIV/AIDS associated co-morbidities such as cancer or co-infections.

Aims: Develop a complex approach for immunotherapy of HIV/AIDS including generation of synthetic antigens,

methods for in vitro, ex vivo and in vivo testing of immunogens, with the emphasis on HIV enzymes: reverse transcriptase/RT, protease/PR, and integrase/IN.

Materials & methods: The consensus humanized HIV enzyme genes, wild-type and with primary resistance mutations were created. Respective genes were designed, synthesized (Evrogen) and cloned into plasmids for DNA-immunization. Eukaryotic expression was confirmed by PAGE of the plasmid-transfected cells with subsequent Western blotting. Inactivation of enzymes was carried by site-mutagenesis yielding prototype DNA-immunogens. Gene immunogenicity was tested in BALB/c mice, injected with respective plasmids with subsequent electroporation employing flat or multi-needle electrodes (BEX). Immune responses were assessed by IFN-g/IL-2 Flourospot, and multiparametric FACS after stimulation of murine lymphocytes with HIV-derived peptides. Two *in vivo* challenge systems were developed, one based on co-delivery of HIV-1 and reporter genes with follow-up of reporter expression using bioluminescent imaging (Spectrum CT), and the other utilizing challenge with tumorigenic reporter-labelled murine cell lines expressing HIV antigens.

Results: Panel of HIV-1 enzyme genes were synthetised and their expression was confirmed by PAGE with Western blotting. Delivery protocols for plasmid DNA were optimized to support strong in vivo immunogen expression required for a potent immune response. Classical immunological tests demonstrated PR gene to be a potent Th1 immune; IN — a moderate mixed Th1/Th2-; and RT, a potent Th2 immunogens. Prime with their genes was sufficient to reduce the expression of the reporter gene in vivo, when the latter was introduced in a boost mixed with any of the given HIV genes. The latter phenomenon, dubbed «antigen challenge», turned to be useful for testing lytic capacity of HIV specific cellular response in rodents. In absence of straight-forward models to test the protective capacity of anti-HIV responses in small animals we suggested also a cancer-cell based model. We set such model of HIV challenge where mice received syngenic tumor cells (here of murine adenocarcinoma 4T1) expressing HIV proteins. In model experiments, expression of a foreign (reporter) protein prevented 4T1 cells from establishing tumours in immunocompetent mice pre-immunized with the reporter gene. Murine adenocarcinoma cell lines expressing nine variants of HIV proteins were already obtained, and shown to form tumors in immunocompetent mice. Models are currently used to assess of immune response against HIV enzymes can protect immunized mice against a challenge with HIV enzyme expressing 4T1 cells.

Conclusions: Promising immunogens inducing an immune response against HIV enzymes responsible for drug resistance in HIV-1 infection were obtained, and shown to be highly immunogenic in mice. In vivo challenge systems were established to assess the protective capacity of the immune response, which are of use in other immune therapy applications.

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Psychosocial aspects of comorbidity of HIV infection and dependence on psychoactive substances in pregnant women

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Russian Federation since the beginning of the XXI century is one of the first places in the world for the registration of people with HIV/AIDS and is one of the most involved in the epidemic process of European regions. Over 80% are in active reproductive age, which led to an increase in the number of pregnancies and births, including on the background of drug addiction.

The most pronounced emotional factor in a woman's life that causes change in its mental state, attitude to life, self-esteem is pregnancy. The diagnosis of HIV infection is lifelong and requires mental adaptation of personality to the new conditions of life. Psychic disadaptation in HIV-infected patients reduces the patient's motivation to treatment, which is especially important when carrying out chemo prevention of HIV transmission from mother to child during pregnancy. Related addiction may have a significant impact on the psychoemotional status of pregnant woman. Objective: to examine the psychosocial aspects of comorbidity of HIV infection and dependence on psychoactive substances during pregnancy.

Materials and methods. On the basis of specialized obstetric Department (Department № 16) City infectious diseases hospital № 30. S.P.Botkin surveyed 160 HIV-infected women in the third trimester of pregnancy: 72 women with heroin dependence (study group) and 88 women without addiction (comparison group). Age of women ranged from 17 to 34 years (mean age of 22,6±2,2 years). Used clinical-anamnestic and experimental-psychological methods, including a set of psychological techniques: the method «life style Index», test Kheima, the scale of anxiety Spielberg. Statistical analysis was performed using the statistical software package. A comparison of statistical samples and their parameters was performed using t-test and χ^2. Correlation analysis was conducted with calculation of correlation coefficients Spearman and Pearson. The critical confidence level of null statistic hypothesis is of 0,05.

The results of the study and their discussion.