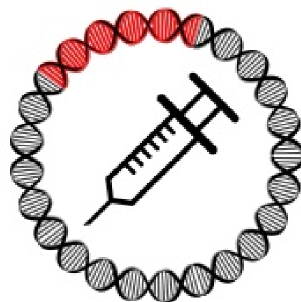
ABSTRACT BOOK

INTERNATIONAL CONFERENCE

"TOOLKITS FOR DNA VACCINE DESIGN, AN UPDATE"

Moscow, November 17-21, 2016



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Thursday November 17, 2016

SESSION I:
DNA AND OTHER VACCINE MODALITIES

Joseph Joan & Saubi N	Pre-clinical development of recombinant BCG based HIV vaccine. Lessons learned	key-note
Shneider Alexander	Can a DNA cancer vaccine turn out to be an anti-inflammatory gene therapy?	key-note
Sominskaya Irina	Protein against DNA, a review of vaccines against hepatitis B virus	key-note
Žusinaite Eva	Progress in Zika virus vaccine development	oral

PRE-CLINICAL DEVELOPMENT OF RECOMBINANT BCG BASED HIV VACCINE. LESSONS LEARNED.

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Our group previously proposed to develop a heterologous recombinant BCG prime-recombinant modified vaccinia virus Ankara (MVA) boost dual pediatric vaccine platform against transmission of breast milk HIV-1 and *Mycobacterium tuberculosis* (*Mtb*). There is strong evidence in favour of a role for HIV-1 specific T-cell responses in the control of HIV-1 replication. One promising approach for T-cell induction is *Mycobacterium bovis* BCG as a bacterial live recombinant vaccine vehicle. Initially, we engineered BCG.HIVA^{222} vectored by a lysine auxotroph of BCG and expressing HIVA immunogen, designed by Prof. Tomáš Hanke from University of Oxford. HIVA immunogen is derived from consensus Gag protein of HIV-1 clade A, prevalent in Central and Eastern Africa, and a string of CD8^{+} T-cell epitopes. BCG.HIVA^{222} carrying an episomal plasmid expressing HIVA was shown to be stable and to induce durable, oligo functional HIV-1-specific CD4^{+} and CD8^{+} T-cell responses in BALB/c mice. Furthermore, when the BCG.HIVA^{222} vaccine was used in a prime-boost regimen with heterologous vectors, HIV-1-specific responses provided protection against surrogate virus challenge expressing HIVA, and was also as efficient in protecting against aerosol challenge with *Mtb* as the BCG 1173 P2 vaccine Pasteur strain. The BCG.HIVA^{222} vaccine candidate was vectored by a lysine auxotroph of BCG Pasteur strain that carried an *E. coli*-mycobacterial shuttle plasmid pJH222.HIVA with a lysine A complementing gene and a weak promoter to drive HIVA gene expression. This design increases the plasmid stability *in vivo* and prevents heterologous gene expression disruption by genetic rearrangement. We compared modified Danish (AERAS-401) and Pasteur lysine auxotroph (222) strains of BCG expressing the immunogen HIVA for their potency to prime HIV-1-specific responses in adult BALB/c mice and examined four heterologous boosting HIVA vaccines for their immunogenic synergy. We also evaluated the influence of BALB/c mice age and immunization routes on induction of HIV-1 and *Mtb*-specific immune responses. Administration of BCG.HIVA^{222} to newborn mice was safe and primed HIV-1-specific immune responses boosted by subsequent MVA.HIVA administration. Recently, we have engineered a new BCG.HIVA^{2auxo} vaccine strain harbouring an antibiotic-free plasmid selection system and maintenance. The BCG.HIVA^{2auxo} vaccine in combination with modified vaccinia virus Ankara (MVA).HIVA was safe and induced HIV-1 and *Mycobacterium tuberculosis*-specific interferon-γ-producing T-cell responses in adult BALB/c mice.

CAN A DNA CANCER VACCINE TURN OUT TO BE AN ANTI-INFLAMMATORY GENE THERAPY?

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P62/ SQSTM1 is a multi-domain protein playing a key role in autophagy and intracellular signaling. It is highly expressed in tumors, but not in normal tissues. It forms multi-protein complexes in cancer cells which do not exist in non-malignant cells. It is vital for survival of the cancer cell and not vital for normal cells. Finally, if a cancer cell would lose p62 protein due to selective pressure applied by a vaccine, a cancer cell would not be able to form a tumor.

We have created a DNA vaccine based on p62 and demonstrated in laboratory cancer models that it works against a broad spectrum of cancer models (lung cancer, melanoma, sarcoma, breast cancer). The vaccine has demonstrated its effect both against primary tumors as well as preventing the metastatic process. As a next step, we applied our p62 DNA vaccine against spontaneous cancers in dogs and cats. The vaccine treatment led to tumor shrinkage and prevented metastatic relapses. It also induced tumor encapsulation. It became possible to surgically remove tumors that were otherwise inoperable. The full scope of preclinical toxicology studies on p62 has revealed no toxicity.

We have conducted phase I/II clinical trials in 27 patients. All patients have exhausted all available standards of care and were at the disease progression stage when enrolled into the trial. The vaccine has demonstrated high safety profile. Also, we observed stop of disease progression in 5 out of seven patients with ovarian cancer, 4 out of 8 with breast cancer and some cases with other cancer types. A serendipitous observation was that 10 out of 10 patients treated with chemotherapy after they have progressed on p62 vaccine have responded to the chemotherapy although they have failed chemotherapy in the past. This effect cannot be explained simply by antigen-encoding DNA vaccine model.

Detailed analysis of cytokine profiles during p62 vaccine treatment has demonstrated that our vaccine possesses strong effect against chronic inflammation. It reduced levels of pro-inflammatory cytokines and increased anti-inflammatory ones. Based on this observation, we have hypothesized that by administering p62-encoding vector one would be able to mitigate diseases that have chronic inflammation as a significant component of their pathogenesis. We have proven this hypothesis by demonstrating effects of p62 DNA vaccine in animal models of osteoporosis, psoriasis, metabolic syndrome, inflammation-associated CNS disorders.

It is not possible to attribute p62-SQSTM1 vaccine to either a vaccine or an immunotherapy/gene therapy realms because it obviously possesses both properties.

PROTEIN AGAINST DNA, A REVIEW OF VACCINES AGAINST HEPATITIS B VIRUS

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Aim: To overview recent developments in the field of creation of new generation of vaccines against hepatitis B virus, and discuss advantages and disadvantages of different vaccine formulations.

Background: HBV prophylactic vaccination is the most important prevention measure. Hepatitis B vaccine provides protection against infection with HBV by producing immunity or antibodies to the surface protein or outer coat of the virus.

Topics overviewed: In my presentation I describe history of hepatitis B vaccines: the first HBV vaccines to be used in humans involved injection of empty 22-nm subviral particles purified from the plasma of chronic carriers; the second generation of HBV vaccines consisted of similar subviral particles, which are produced by recombinant DNA technology as recombinant proteins in stably transfected eukaryotic cell lines; the third-generation of HBV vaccines contained one (Pre-S2) or two (Pre-S1 and Pre-S2) additional HBV envelope proteins including DNA vaccines. Recent achievements in development of preventive and therapeutic vaccines will be overviewed.

Conclusion: Through vaccination, a drastic reduction in the mortality and morbidity of chronic HBV, including hepatocellular carcinoma, has occurred in recent years. The challenge for the development of an efficient vaccine with relatively low side effects is still in progress.

PROGRESS IN ZIKV VACCINE DEVELOPMENT

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Background. Zika virus (ZIKV) was isolated 70 years ago in the Zika forest of Uganda. This is an arthropod virus belonging to the *Flaviviridae* family and causing relatively mild (if at all) illness, therefore, attracting little attention until recently. During the last few years ZIKV rapidly spread to South-East Asia, Polynesia, Caribbean region and Brazil causing large outbreaks. The outbreaks have been associated with increased incidence of microcephaly in newborns and severe neurological syndromes leading WHO to declare ZIKV a Global Emergency.

Aim. To give an overview on current ZIKV vaccine development.

Topics overviewed. Despite the fact that successful vaccines against other flaviviruses exist (Dengue virus, Yellow fever virus, Japanese encephalitis virus), development of vaccine against ZIKV meets a number of challenges. First, little is known about ZIKV biology and immune response. Second, vaccine should protect pregnant women that undoubtedly raise concerns about its safety and possible side effects.
The primary immunological goal of Zika vaccine is achievement of strong neutralizing immune response against the Envelope protein resulting in sterilizing immunity. Multiple ZIKV vaccine candidates are now in development at different stages. Many of them are based on the existing vaccine platforms, which are known to be effective for other viruses: live recombinant vaccines based on viral vectors (MVA, adenovirus, measles, lentivirus), genetic DNA/RNA-based vaccines, chimera with other flaviviruses (e.g. Yellow fever virus), individual ZIKV protein subunit vaccines, inactivated Zika virus etc. Some of the most promising ones are planned to enter phase I clinical trials at the end of 2016.
Current advances in Zika vaccine development will be highlighted.

SESSION II:
DESIGN AND PRECLINICAL TESTING OF DNA VACCINES

Dovbenko Anastasija	Comparison of immunogenicity of HCV core and its alternative reading frame protein in mice	poster
Isaguliants Maria	HIV enzymes as prototype immunotherapeuticals against drug resistance: preclinical trials in mice	oral
Latanova Anastasia	Multicomponent DNA vaccine based on HIV-1 clade A enzymes induces cellular response of diverse profile in mice	oral
Lukashev Alexander	Viral RNA nucleotide content is affected by innate immune system recognition – implications for vector design	oral
Pankova Ekaterina	Single immunization with codon-optimised gene of consensus rabies virus glycoprotein is able to elicit neutralizing antibodies in mice	oral
Starodubova Elizaveta	Comparative immunogenicity of HIV-1 reverse transcriptase of clades A and B in mice	poster
Starodubova Elizaveta	Optimization of design and delivery of HIV reverse transcriptase based prototype DNA vaccines in a mouse model	oral

COMPARISON OF IMMUNOGENICITY OF HCV CORE AND ITS ALTERNATIVE READING FRAME PROTEIN IN MICE

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Background. Hepatitis C virus (HCV) persists in up to 85% of infected individuals as a chronic infection characterized by liver infiltration of inflammatory cells that can lead to fibrosis, cirrhosis and hepatocellular carcinoma. There is no vaccine against HCV and available therapy is expensive and related with different side effects. HCV core protein represents an attractive target for an HCV vaccine. Besides the core protein, the 5` terminus of HCV genome encodes core+1/ARF protein. ARFP participates in HCV morphology or replication, it can be important in gene regulation and also it can affect immune response mechanisms.

Aim. The main aim was the study of immunogenicity of plasmids expressing proteins encoded by the 5` terminus of HCV RNA in DNA immunization.

Methods. Plasmids carrying fragments encoding HCV core and its alternative reading frame protein were obtained. Eukaryotic expression of HCV core and ARFP variants were tested in HEK 293 cells, and expression level was defined by Western blotting with polyclonal rabbit anti-HCV or anti-ARFP antibodies. Mice were immunized by two injections with 40 μg plasmids or empty vector intradermally with four days interval; injections were followed by electroporation (BEX, Japan). Cellular immune responses were analysed by IFN-γ/IL2 Fluorospot after stimulation with proteins and antigen-derived peptides. Specific antibodies were assessed by ELISA.

Results. Immunization with plasmids carrying wild type, mutated and synthetic codon-optimized variants of HCV core or ARFP induced the specific immune response. Plasmids were introduced into mice to assess their immunogenicity and delineate the extent of HCV core and ARFP immune competition. Immunization with natural and mutated HCV core genes with prohibited frame-shift provide the same level of specific cellular and antibody responses. Prohibition of ARFP synthesis as well as reading frame shifting, however do not show any protein expression in HEK 293 cells. Efficacy of ARFP expression by the natural ribosome frameshift mechanism was low and obviously insufficient to induce a specific immune response in DNA immunization.

Conclusions. ARF protein apparantly do not accumulate in sufficient quantity in cells to be detected by Western blot. Anti-ARFP immune response is not competing with that against HCV core, and cannot explain the low immunogenicity of the latter in DNA immunization performed with the virus-derived genes.

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HIV ENZYME COMBINATION AS PROTOTYPE IMMUNOTHERAPEUTICAL AGAINST DRUG RESISTANCE: PRECLINICAL TRIAL IN MICE

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Background The success of antiretroviral therapy turns HIV-1 infection into a chronic but controllable disease. However, drug resistance (DR) evolves which may render the therapy ineffective. Primary infections with drug resistance HIV-1 strains occur in up-to 5% of cases making virus insusceptible to some of the treatment regimens. Ultimately, multiple DR may develop, where all therapy options would fail leading to AIDS and death. This motivates the development of immunotherapies which would prevent or thwart DR development. We assume that an immune response against primary DR mutations on the background of HAART would cause a bottle-neck hindering resistance development. We describe a set of such immunotherapeutical DNA vaccine candidates based on the enzymes of HIV-1 clade A FSU-A strain causing epidemics in the territory of former Soviet Union.

Methods HIV-1 FSU-A sequences derived from untreated patients resident in the former Soviet Union were collected from Gene Bank and Los Alamos databases, and aligned using MUSCLE engine. Consensus amino acid sequences of protease (PR), integrase (IN) and reverse transcriptase (RT) were created. Respective humanized genes (Evrogen) were cloned into pVax1. Genes were subjected to site-mutagenesis to introduce enzyme inactivating mutations, and primary mutations conferring resistance to drugs in use in the Russian Federation. Gene expression in eukaryotic cells was confirmed by Western blotting. Plasmids were delivered in BALB/C mice intradermally with subsequent electroporation (BEX Ltd) as one or two injections spaced 4 weeks. Immune response was assessed at the end-point by antibody ELISA, IFN-g/IL-2 Fluorospot (Mabtech), and multiparametric FACS assessing IFN-g, IL-2 and TNF-a expression in CD4+ and CD8+ T cells. In vitro splenocyte stimulations were done with recombinant proteins and peptides representing immunodominant epitopes of RT, PR and IN (Synpeptide). In a separate experiment, booster dose of HIV genes was delivered with pVax expressing luciferase (Luc), and mice were imaged for bioluminescence (BLI; Spectrum CT) on days 1, 3, 6, 9, 15 and 21 post injection.

Results Nine plasmids encoding inactivated enzymes: protease (PRi), PRi with 2 and 3 primary DR mutations to ritonavir; reverse transcriptase (RTi), RTi with 2 mutations of resistant to NRTI, or 3 mutations of resistance to NNRT, integrase (INi), two INs with different patterns of mutations to raltegravir, and one to elvitegravir, were designed. All encoded proteins were well expressed in HeLa, HEK293 cells and NIH3T3 cells. When injected into BALB/c mice, all plasmids induced strong IFN-g, and RT and IN, also an IL-2 response. PR gene variants performed as Th1-immunogens responding mainly by CD8+ T-cells producing INF-g/TNF-a; RTs genes, as Th2, inducing IFN-g/IL-2 by CD4+ T-cells, and specific IgGs in titer over 10(5). IN genes were mixed Th1/Th2 immunogens generating IFN-g/IL-2/TNF-a response by both CD4+ and CD8+ T-cells. To assess lytic responses, booster dose of HIV genes was introduced mixed with a plasmid encoding Luc reporter. Bioluminescence was compared to that in mice receiving empty vector, or HIV and Luc genes as a mix already in prime. In mice primed with HIV/Luc genes, BLI dropped by day 9, and drastically diminished by day 15, falling >100 times from the initial levels or levels observed in vector/Luc-injected mice. In mice primed with HIV- and boosted with HIV+Luc DNA, >100-fold diminishment of BLI signal was registered already by day 3. PR, IN and RT gene variants were selected capable of the most efficient reduction of BLI signal. These PR, IN and RT genes were then tested together, when introduced in mice as a mix or as three separate injections. Immune response to RT and IN was not influenced by mixing. On contrary, mixing strongly enhanced both IFN-g and IL-2 response of CD4+ and CD8+ T cells to HIV protease.

Conclusions Our data demonstrate the compatibility of DNA immunogens encoding HIV enzymes, and advantages of administering them as plasmid mixtures. A system was designed allowing to evaluate the effector capacity of HIV-specific immune response in a mouse dubbed "antigen challenge". A

combination of the most immunogenic variants of consensus protease, integrase and reverse transcriptase genes of HIV-1 FSU-A strain will now be tested in the non-human primate safety/immunogenicity trial.

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MULTICOMPONENT DNA VACCINE BASED ON HIV-1 CLADE A ENZYMES INDUCES CELLULAR RESPONSE OF DIVERSE PROFILE IN MICE

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Background Currently existing therapy against HIV-1 is represented by inhibitors of three HIV enzymes: reverse transcriptase/RT, integrase/IN and protease/PR vital for the viral life cycle. DNA-immunization with their genes, also for drug resistant/DR forms, could support ART by preventing the development of drug resistant HIV. Earlier, we evaluated immunogenicity of single DNA-immunogens encoding RT, IN and PR with or without DR mutations. Here, in continuation of this study, we investigated the immunogenicity of DNA encoding the consensus RT, IN and PR of HIV-1 clade A FSU-A strain, delivered as a mixture or as separate shots. Mixture of consensus genes is proposed as a prototype therapeutic DNA vaccine against HIV-1 FSU-A strain which is genetically stable and highly prevalent in a large territory within the former Soviet Union borders.

Materials and Methods DNA encoding the consensus RT, IN and PR of HIV-1 clade A were designed based on the multiple alignment of 40 to 400 amino acids sequences of HIV enzymes from the untreated HIV-1 patients (Los Alamos HIV-1 database). Genes for humanized inactivated RT/IN/PR (Evrogen) were cloned into pVax1. Gene expression in HeLa cells was confirmed by Western blotting. BALB/c mice (n=5/group) were intradermally injected with RT, IN and PR-encoding plasmids, or pVax1 and electroporated (BEX Ltd., Japan). RT, IN and PR DNA-immunogens were given at three sites, as a mixture/RTPTIN-m or separately as individual immunogens/RTPTIN-s. Immune response was assessed three weeks later by indirect ELISA, IFN-g/IL-2 Fluorospot (Mabtech) and multiparametric FACS. Statistical analysis was performed by Kruskal-Wallis, Mann-Whitney U and F-tests.

Results Synthetic RT, IN, and PR genes were highly expressed in eukaryotic cells. Mice immunized with RTPTIN-m and RTPTIN-s responded by strong RT-, IN-, and PR-specific IFN-g production (>500 sfu/mln cells). Cellular responses to RT and IN in mice receiving gene mixtures and separate injections were similar, but PR-specific cellular response was enhanced by gene mixing up-to 10-fold reaching 3000 for IFN-g and 1000 for IL-2 spot-forming cells/mln. Dual IFN-g/IL-2 production followed the same trend. Gene mixing enhanced RT-specific response manifested by CD8+ IFN-g+ cells, and PR-specific responses showed by 0.2%-1.2% CD8+cells positive for IFN-g, IFN-gamma/Il-2, IFN-g/TNF-a, IFN-g/Il-2/TNF-a. Meanwhile, in both separate and mixture immunizations IN-specific response was manifested by 0.1-0.3% CD4+cells positive for the same set of cytokines. Specific antibodies were detected only against RT. Anti-RT antibody titers reached 3x10(5) for IgG, 3x10(5) for IgG1 and 2x10(4) for IgG2a indicating a predominant Th2 response, whatever injection mode. Anti-RT IgA titers of 2x10(3) were detected only in mice receiving RTPTIN-s.

Conclusions A prototype multicomponent DNA-vaccine based on the enzymes of HIV-1 of clade A showed high immunogenicity in a mouse model. Administration of genes in a mixture induced strong cellular response against all three components. This data concludes the studies of immunogenicity of DNA encoding HIV enzymes in a mouse model, and opens up a route for further tests of RTPRIN-multigene in primates.

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VIRAL RNA NUCLEOTIDE CONTENT IS AFFECTED BY INNATE IMMUNE SYSTEM RECOGNITION – IMPLICATIONS FOR VECTOR DESIGN

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Background: Codon optimization became an integral part of vector design and development of nucleic acid-based therapeutics. The straightforward approach involves modification of codon usage to match that of the host in order to maximize translation efficiency. One of the main drivers of uneven codon use in mammals is methylation of cytosine in the context of CpG dinucleotide and subsequent transition of C into T. As a result, codons that carry CpG dinucleotide are underrepresented in mammals. This feature of host DNA also allows recognition of non-self DNA with high CpG content by TLR9. Recognition of non-self RNA is less understood.

Aim: Highlight the factors that affect viral RNA composition and may influence artificial RNA recognition in a cell.

Topics overviewed: It was well known that viruses avoid CpG dinucleotide; however it was unclear if this was because of preference for non-CpG codons that are also abundant in the host, or for other reasons. Our analysis of codon and dinucleotide biases in mammalian RNA viruses implies that the pressure against the CpG dinucleotide in one of the main drivers of codon usage in RNA viruses. Pressure to avoid CpG at a codon junction (codon positions 3-1) is almost as high as within a codon (codon positions 2-3). Other experimental studies showed that increasing the CpG content in viral RNA can be used to attenuate viruses and results in activation of innate immunity, although the exact sensor for CpG dinucleotide in RNA remains unknown. Another dinucleotide that is almost universally avoided in RNA viruses is UpA, hypothetically because it is the target of the antiviral RNAse L. Pressure against these dinucleotides is usually uniform within a virus genus, but may differ dramatically between closely related genera, implying that viruses can develop mechanisms to counter sensors or effectors aimed at these dinucleotides.

Conclusions: While a standard codon optimization would collaterally optimize the dinucleotide content at codon positions 2-3, dinucleotide content at codon junctions may be overlooked when designing artificial RNA. It can significantly affect function of RNA and may be used to decrease or increase immune recognition of artificial RNA.

SINGLE IMMUNIZATION WITH CODON-OPTIMISED GENE OF CONSENSUS RABIES VIRUS GLYCOPROTEIN IS ABLE TO ELICIT NEUTRALIZING ANTIBODIES IN MICE

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Background Rabies has a long history, and it has known for more than 4000 years. The history of the fights against this virus began with the work of Louis Pasteur. Nowadays, several types of anti-rabies vaccines are used worldwide. Inactivated rabies vaccines are most commonly used and are recommended by World Health Organization (WHO). Despite the wild spread of these vaccines, they have some delivering restrictions and can cause side effects. Rabies causes approximately 55 000 human deaths annually and a lot of animal deaths that is why the task of creating new generation vaccines is still actual. DNA-vaccines are the perspective candidates because they have some principal advantages.

Materials and Methods Data about 127 amino acids sequences from PDB of rabies viruses registered in Russia were used for developing consensus sequence of rabies glycoprotein. These sequences were aligned with BioEdit tools, and consensus sequence was created. This new sequence was integrated in pVax1 plasmid vector that is recommended for DNA-vaccine use. Expression of the glycoprotein was confirmed in BHK-21 cell culture. For selection the optimal condition of animal immunization, BALB/c mice were injected by the plasmid with the consensus sequence 50, 100 and 300 ug intramuscular and 20, 40, 100 ug subcutaneously. Mouse sera were collected and were pulled 21 days after injection. Sera were analyzed for presence of anti-rabies glycoprotein antibodies by ELISA, viral glycoprotein produced and purified from bacteria was used as antigen. Fluorescent Antibody Virus Neutralization test (FAVN) was used for detection anti-rabies virus neutralizing antibodies (VNA) in mouse sera. For this test, mice were injected by 100 ug of plasmid with the consensus sequence intramuscular. 21 days after injection, sera were collected and pulled. Besides sera, BHK21 cell line and CVS-11 virus strain were used for FAVN test, and inactivated rabies vaccine was used as control.

Results In our research plasmid coding consensus sequence of rabies glycoprotein, was created based on virus isolates from Russian Federation. This plasmid was used for immunization of BALB/c mice. ELISA has showed presence of specific anti-rabies glycoprotein antibodies in sera of mice in response for immunization. Optimal conditions for mice immunization were found – 100 ug of plasmid intramuscular. At the next step, generation of virus neutralizing antibodies after consensus glycoprotein gene injection was estimated by FAVN test. Titer of anti-rabies VNA after immunization with plasmid encoding consensus glycoprotein was 1,95 ME/ml and after inactivated rabies vaccine immunization was 0,95 ME/ml (threshold by WHO is 0,5 ME/ml). Immunization with viral gene of glycoprotein of Vnukovo-32 strain didn't elicit any VNA. Thus, created plasmid has anti-rabies activity higher than plasmid caring viral gene of glycoprotein and even than the control inactivated virus vaccine.

Conclusions All things considered, in this survey creating of new DNA-vaccine antigen based on consensus sequence of rabies glycoprotein was shown, optimal conditions for mice immunization were selected, and immune activity of new construction was proved by the ELISA and FAVN tests.

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IMMUNIZATION WITH GENES OF REVERSE TRANSCRIPTASES OF HIV-1 CLADE A AND B INDUCE STRONGE IMMUNE RESPONSE IN MICE

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Background: Recent progress in molecular biology techniques brings the DNA vaccines from a mere concept into a viable option of treating and preventing major health burdens. Consensus optimized genes were developed and applied to combat high variability of human pathogen. Here, we utilized such codon-optimized consensus genes to induce potent immune response against reverse transcriptase (RT) of HIV-1. Inactivated consensus reverse transcriptase (RT) of HIV-1 clade A strain FSU-A prevalent in the territory of the former Soviet Union was compared in immunogenicity to RT of HXB2 strain.

Materials & Methods: Protein consensus RT of HIV-1 FSU-A was designed based on multiple alignment of HIV RT FSU_A sequences from untreated HIV-1 patients using MUSCLE engine. Genes of the consensus FSU-A and inactivated RT of HXB2 were synthesized by Evrogen (Moscow, Russia) and cloned into pVax1 vector (Invitrogen) to receive pVaxRT_Ain and pVaxRToptin, respectively. Plasmids were delivered in BALB/C mice by ID injection followed by electroporation (BEX, Japan). DNA was co-injected with plasmid encoding firefly luciferase (Luc). *In vivo* bioluminescence imaging of reporter expression was performed on days 1, 3, 9, 15 and 21 post immunization (Spectrum CT, Perkin Elmer). Immune responses were assessed *in vitro* in isolated splenocytes stimulated with a panel of RT-derived antigens using IFN /IL-2 Fluorospot (Mabtech), and antibody production by ELISA.

Results: Expression of luciferase served as *in vivo* reporter reflecting the rate of initial gene expression and immune-mediated clearance of antigen-expressing cells. Mice DNA-immunized with HXB2 RT and consensus FSU-A exhibited similar levels of bioluminescence until day 9, and at the end of the study. Mice receiving pVaxRToptin nearly cleared the reporter expression by day 15, and pVaxRT_Ain, by day 21. Mice receiving either gene showed a significant number of IFN , IL-2, and IFN /IL-2 co- secreting cells. Immunodominant epitope was localized at aa 145-168 of FSU-A RT capable of inducing IFN- production in >$2x10^3$ SFC/mln splenocytes. Stimulation with clade B RT resulted in high specific response only in the HXB2-RT immunized animals, whereas the consensus RT of FSU_A was equally well recognized by the splenocytes of pVaxRT_Ain and pVaxRToptinn immunized animals. Both immunizations induced anti-RT IgG in titer $8x10^4$ and systemic anti-RT IgA in titer $5x10^3$.

Conclusions: This is the first demonstration of a high immunogenicity of consensus HIV RT of clade A, and the first comparison of immunogenic performance of DNA encoding RT of HXB2 and the consensus RT from HIV clade A. The latter was immunogenic, as was revealed by immune assays and complete extermination of the RT_A/reporter expressing cells. Current efforts are targeted towards designing and characterizing immunogenicity in mice of the variants of clade A RT with primary mutations of resistance to NRTI and NNRTI, in preparation for the trials in non-human primates.

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OPTIMIZATION OF DESIGN AND DELIVERY OF HIV REVERSE TRANSCRIPTASE BASED PROTOTYPE DNA VACCINES IN A MOUSE MODEL

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Background Potency of therapeutic HIV vaccines in reducing viral load in patient was demonstrated in several resent vaccine trials. HIV immunotherapy becomes actual in view of the latest findings of an effective broad T-cell response clearing HIV-1 from the latent reservoirs. We focused our study on approaches to induce strong immune response against viral antigen responsible for drug resistance – the reverse transcriptase. Such response can complement the antiretroviral treatment.

Materials and Methods Codon-optimized genes of HXB2 HIV-1 clade B RT and RT with mutations of drug resistance were designed and synthetized (Evrogen). DNA delivery was tested in BALB/c mice. Different regimes of electroporation was performed (BTX) and (DermaVax/Cellectis). In vivo monitoring approach using a plasmid encoding firefly luciferase (Luc DNA) was applied. On days 1, 3, 9, 15 and 21 post delivery of mixture of RT- and Luc-encoding plasmids into mice, they were monitored for Luc expression by in vivo bioluminescent 2D imaging (Lumina, Perkin Elmer) evaluating total photon flux from the injection area. After day 21, mice were sacrificed and IFN-g/IL-2 response was assessed by Fluorospot (Mabtech).

Results Synthetic codon-optimized genes of HXB2 HIV-1 clade B RT and RT with mutations of drug resistance driven increased expression in cell culture. Immunogenicity of genes was accessed in BALB/c DNA immunized mice. Regimens of EP were compared and were found the best to increase the immune response. Double prime scheme of immunization was tested and shown light effect on immunogenicity. Prime-boost regimen of DNA immunization gave prominent enhancement of immune activation. Also model of HIV challenge was tested, as the capacity of immunized mice to clear the expressing cells in vivo. For this, mice were primed with HIV genes, and boosted with HIV-1 genes mixed with luciferase reporter gene and them monitored for reporter expression. Screening of immune response of wt- and dr-RT gene immunized mice revealed difference in ability to elicit immune response to region of drug-resistance.

Conclusions Immunogenic potency of HIV RT as a DNA vaccine can be greatly increased by codon-optimization of gene, optimization of delivery by EP and prime-boost regimen. General efficacy of wt and dr RT variant are quite close, but repertoire of recognized epitopes differs. HIV RT has a potential as DNA vaccine antigen, however more detailed screening of immune recognition of regions of drug- resistance is needed.

Acknowledgements Supported by Russian Science Foundation grant 15_15_30039

Friday November 18, 2016

SESSION III:
DNA DELIVERY AND ADJUVANTS

Dzmitruk Volha	Dendrimer complexes as a platfrom for gene delivery	key-note
Gordeychuk Ilya	Visualization and quantification of protein expression after gene electroporation in vivo	oral
Shneider Alexander	Adjuvants for DNA vaccines: from ad hock vaccine design to technology platforms	oral

DENDRIMER COMPLEXES AS A PLATFORM FOR GENE DELIVERY

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Background. Gene therapy is one of the most effective ways to treat major infectious diseases, cancer, and genetic disorders. It is based on several viral and non-viral systems for nucleic acid delivery, including new supramolecular systems, complexes and composites. Among non-viral vectors dendrimers are very attractive due to their globular shape with a topological structure formed by monomeric subunit branches on all sides emerging from the central nucleus.

Aims. Aim of presentation is to draw attention to the latest applications of dendrimers in medicine as well as in nucleic acids delivery.

Topics overviewed. This presentation briefly discusses and summarizes recent advances in dendrimers both as biomedical complexes and as effective gene carriers *in vitro* and *in vivo*. In this regard, dendrimers are non-immunogenic and have the highest efficiency of transfection among other non-viral systems. The toxicity of dendrimers both *in vitro* and *in vivo* is an important question that has been addressed on many occasions. Several non-toxic and efficient multifunctional dendrimer-based conjugates for gene delivery, along with modifications to improve transfection efficiency whilst decreasing cytotoxicity, are discussed.

Conclusions: Thus, dendrimers are promising candidates for gene delivery, but further studies are required before using them in human gene therapy.

Acknowledgements. This work has been supported by grant No. 692293 VACTRAIN within EU 'Horizon 2020'; by a Marie Curie International Research Staff Exchange Scheme Fellowship within the 7th European Community Framework Programme, project No. PIRSES-GA-2012-316730 NANOGENE; by grants B15MS-001, B15RM-060, M15CO-041 from the Belarusian Republican Foundation for Fundamental Research.

VISUALIZATION AND QUANTIFICATION OF PROTEIN EXPRESSION AFTER GENE ELECTROPORATION IN VIVO

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Background. Near infra-red (650-900 nm) fluorescent proteins, engineered from the PAS-GAF domains of the *Rp*BphP2 bacterial phytochrome of the photosynthetic bacterium *Rhodopseudomonas palustris* have been widely applied to cancer studies[1]. They enabled visualization of small tumors at early stages, monitoring tumor growth and tracking metastases. They allow monitoring of the cells independently of their viability, oxygen or ATP supply, and without a substrate, since fluorescence by these reporters is independent of these factors, on contrary to the light emission by luciferase. This invites the use of near-infrared proteins for optimization of gene delivery in DNA immunization and gene therapy. Previous studies motivate the choice for these studies of two members of this series, iRFP670, and iRFP720[1].

Aims. To test possibility of using piRFP670 and piRFP720 genes to optimize the procedure of plasmid delivery into mice by intradermal injection followed by electroporation, and evaluate the efficacy of in vivo electrotransfection in terms of protein production.

Materials and Methods. Plasmids encoding iRFP670 and iRFP720 (Addgene)[1]. Plasmids were produced in E. coli and purified using endotoxin free plasmid purification kits (Qiagen). In vivo plasmid delivery was performed in 8-week female BALB/C mice (Taconic). Mice were injected intradermally with 10 to 30 µg of plasmid DNA in max 30 µl PBS delivered with 30G insulin syringes Omnican 50 (B Braun). Injections were followed by electroporation done using CUY21Edit II with the fork-plate electrode (both BEX Co Ltd). Reporter expression was quantified using in vitro and in vivo calibration curves built in the range of 0,05 to 2 µg of fluorescent proteins. In vivo fluorescence was recorded on Spectrum CT (Perkin Elmer) on days 1, 3, 5 and 9 after plasmid administration. Fluorescence signals of iRFP670 (excitation 645 nm, emission 670 nm) and iRF720 (702 nm and 720 nm, respectively) were quantified after unmixing from the baseline fluorescence.

Results. Regimen for electroporation (EP) providing the highest rate of gene transfer into the skin was optimized using mouse and human skin explants (see abstract by J Jansons & S Petkov, "Toolkits for DNA vaccine design, an update" Moscow, November 17-20, 2016). The regimen was applied for *in vivo* EP transfection. Efficacy of *in vivo* EP was proportional to the electric current and depended on the adherence to skin resistance in the range of 40-80 Ohm/mm^2. Protein expression was detected within 18 h after *in vivo* transfection into mice of 20 µg of piRFP670. *In vivo* fluorescence increased with time and reached maximum within a week after the *in vivo* transfection. After *in vivo* transfection of 20 µg of piRFP720, reporter expression was first detected only four days post DNA inoculation. Quantification of protein expression using calibration curves demonstrated that the level of iRFP670 synthesis at the injection site reached 200 ng by day 2, and increased to over 800 ng per site, while iRFP720 was synthesized at a several-fold lower level. Increase of plasmid dose from 20 to 40 µg caused a proportional increase in the levels of expression of both fluorescent proteins.

Conclusions. Both near-infrared fluorescent proteins are easily detectable in mice after *in vivo* transfection by EP. The high quantum yield characteristic to iRFP670 provides for its easy detection already at the early stages when it present in low quantities, which makes it preferable for reporting the efficacy of plasmid delivery. Experiments demonstrated that *in vivo* transfection of 20 µg of plasmid DNA can ensure the production of close to 1 µg of protein per injection site which in case of multiple injections/EP into a vaccinee, is comparable to the amounts provided by protein immunization.

Acknowledgements. Supported by Russian Science Fund 15-15-30039. Mobility was supported by TP 09272_2013 and PI 19806_2016 of the Swedish Institute, and training and method acquisition, by VACTRAIN #692293.

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ADJUVANTS FOR DNA VACCINES: FROM AD HOCK VACCINE DESIGN TO TECHNOLOGY PLATFORMS

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Nothing saved as many lives as vaccines. However, can we afford to spend years designing each new vaccine? A threat of bioterrorism dictates that we should be able to respond to a new pathogen as quickly as it may spread. Another example, advances in deep sequencing allow identifying particular mutations associated a tumor in a specific patient. Designing personalized vaccines based on the particular mutated proteins, neoantigens, may save many lives, but in order to be effective these vaccines have to be developed expeditiously.

DNA vaccines constitute a novel vaccine class, which holds the best promise to overcome the major limitations of currently available vaccines. However, despite their great promise, DNA vaccines have had limited success to date in humans due to the vaccine's insufficient immunogenicity and protective potency. Thus, it is relatively easy to propose a DNA vaccine antigen, which would work to protect mice against a particular pathogen or a tumor containing a set of neoantigens. However, in order to save humans we need to create a "Ford assembly plant" for rational and expeditious vaccine design. It has to be a set of reliable, safe and, ideally, pre-approved technologies which would turn an antigen into a highly efficient vaccine.

Multiple technologies are introduced today to enhance immunogenicity with DNA vaccines. Perhaps the most popular approach to enhance efficiency of DNA vaccination is electroporation. Delivery via electric charge may not only increase the plasmid uptake but also stimulate innate immunity necessary, which stimulates immune response to the vaccine. Alternative methods of delivering DNA vaccines (e.g intradermal or subcutaneous) are also widely tested today. Another approach is incorporating IL-12 and other immunestimulating molecules into vaccination regimens. This method has demonstrated its promise for melanoma treatment. Another approach is to suplement vaccine plasmid DNA with delivery system like liposomes or polymer particles. The latest version of this approach also includes incorporating into the delivery vehicles protein molecules specifically targeting an organ or a cell type. Another approach is to combine DNA vaccination with checkpoint inhibitors. The list could be continued.

It is widely known that vaccination with peptides and proteins, which are relatively small, does not lead to a strong immune response. In order for the peptides to elicit protective immunity they have to be supplemented with an adjuvant assembling small antigens into bigger particles. However, currently available adjuvant could be utilized to assemble intracellular peptides, which therefore makes them inapplicable to enhancing DNA vaccines. We have invented a new class of adjuvants, intra-molecular adjuvants, specifically designed to enhance the immunogenicity (both, T and B) of proteins encoded by DNA vaccines. Intra-molecular adjuvants are protein domains fused to an antigen, which cause its selective self-aggregation within the cell. The first intra-molecular adjuvant we have reported was poly-glutamine (polyQ).

Proteasome degradation is involved antigen presentation. We have demonstrated that combining genes encoding two forms of a protein, proteasome-resistant and proteasome-degradable, make a DNA vaccine induce stronger immune response.

SESSION IV:
VACCINE PRODUCTION AND CLINICAL TRIALS

Blinkova Larisa P	Detection and resuscitation of viable but nonculturable bacteria in vaccines and other biomedical	short oral
Karpenko Larisa	Vaccines against HIV-1 and other infectious and somatic diseases, developed in State Research Center of Virology and Biotechnology "Vector"	oral
Kozlov Andrei	The Results of Phase II Clinical Trial of HIV Therapeutic Optimized DNA Vaccine: The Possible Reach of Viral Reservoirs	key-note
Tarasov V.	The development of research universities as a new business model for production of innovative vaccines	oral

DETECTION AND RESUSCITATION OF VIABLE BUT NONCULTURABLE BACTERIA IN VACCINES AND OTHER BIOMEDICAL PREPARATIONS

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Background Problem of viable but nonculturable `(VBNC) cells and their resuscitation into active state is very important for industrial biomedical preparations. VBNC cells are appeared under stress and have not ability to form colonies. However, dormant nonculturable cells can return to the active proliferation with help different factors (sera, vitamin K etc.). Due to possible presence of VBNC cells in live bacterial vaccines and probiotics or contamination of viral vaccines and other biomedical preparations it is necessary to apply adequate control procedures. The aim of our research was the detection VBNC cells and testing some factors for the resuscitation of active division.

Materials & Methods We used light microscopy to count bacterial cells and assessed CFU/ml values on nutrient agar. Luminescence microscope was used to determine portions of viable and dead cells after staining with Live/Dead® Baclight™ double staining kit. Numbers of VBNC cells were determined by comparison of total cell counts and portions of viable cells [Blinkova L.P. et al 2014, Pakhomov Yu.D. et al, 2016]. Statistical analysis was conducted using Fisher-Student t-criteria for p value $\leq$0.05.Detection of VBNC bacteria was conducted for probiotic preparations *E. coli*, *Bifidobacterium*, *Lactobacillus*. As resuscitation factors of VBNC of different bacteria for example *Salmonella enterica Typhimurium* we used normal saline, blood substitute, inulin, vitamin PP etc.

Results Our analysis of native commercial lyophilized probiotics (Russia) showed that from 4% to more than 99% cells were nonculturable in assessed samples. Numbers varied for different bacterial preparations, their storage period and other factors. The normal saline, blood substitute were effective for reversion VBNC cells of probiotics to active state.
In experiment with *Salmonella enterica Typhimurium* of the resuscitation, which was VBNC for 8 months after exposure to different concentration of inulin or vitamin PP in nutrient broth we observed decrease in number of VBNC bacteria to 7 – 37% compared to 80-90% in controls. Exposure to vitamin PP acid was effective only in concentration 0.01%.

Conclusion Since VBNC cells were detected in bacterial probiotics subjected to lyophilization stress such cells can be identified in bacterial vaccines and can lead to underestimation of their viability, when only the CFU/ml is measured. Contamination of different biopreparations with VBNC microbial cells, which can return to active state is also possible and thus it is a hazard for bioproduction without appropriate control technique.

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VACCINES AGAINST HIV-1 AND OTHER INFECTIOUS AND SOMATIC DISEASES, DEVELOPED IN STATE RESEARCH CENTER OF VIROLOGY AND BIOTECHNOLOGY VECTOR

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Background. Since 2000 Vector State Research Center of Virology and Biotechnology has been designing DNA-vaccines against a number of viral pathogens including HIV, influenza, hepatitis and several highly dangerous virus infection, and also DNA-vaccines against oncological diseases. The main concept of our approach includes the design of synthetic polyepitope immunogens using wide range of protective T-epitopes of main virus antigens that can induce responses of cytotoxic (CD8+ CTL) constructed by reference to specific features of processing and presentation of T-cell antigens. This approach enables in theory to cope with virus antigenic variability, focuses immune responses on protective determinants and enables to exclude from the vaccine compound adverse regions of viral proteins.

Aim. The subject of presentation is the brief description of the strategies for designing artificial polyepitope T-cell immunogens and evaluation of their efficiency during clinical trials (as exemplified by vaccine against HIV-1 CombiHIVvac).

Topics overviewed. In the report will be discuss the questions of construction and rational design of polyepitope T-cell immunogens, including: features of organization of polyepitope immunogens, which can generate high-level CD8+ and CD4+ T-lymphocyte responses; the strategies to optimize efficient processing, presentation and immunogenicity of polyepitope constructs; original software to design polyepitope immunogens.

Using polyepitopes approach we have constructed T-cell HIV-1 immunogen TCI providing the basis for DNA-vaccine pcDNA-TCI. This DNA-vaccine has been included into CombiHIVvac composition. CombiHIVvac comprises micelle-like particles with TBI polyepitope protein and DNA-vaccine pcDNA-TCI. TBI protein has been developed for inducing humoral immune response; it comprises Env and Gag proteins. TCI immunogen comprises over 80 optimally selected overlapping determinants (both CD8+ CLT and CD4+ Th) from viral proteins Env, Gag, Pol and Nef. Selected antigenic determinants are conservative among A, B and C HIV-1 subtypes.

Resume of the progress ed achieved. Conducted pre-clinical and Phase I clinical studies demonstrated that CombiHIVvac is safe and well tolerated. Neither single nor double intramuscular injection of vaccine had persistent effects on the integral physiologycal, hematological or biochemical parameters in the individuals. No pathological signs were observed at the site of the vaccine injection. CombiHIVvac induces both humoral and cell HIV-specific immune response. Double administration is more efficient and provides a *more durable immune* response.

THE RESULTS OF PHASE II CLINICAL TRIAL OF HIV THERAPEUTIC OPTIMIZED DNA VACCINE: THE POSSIBLE REACH OF VIRAL RESERVOIRS

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Background. The human immunodeficiency virus (HIV) vaccine is urgently needed to curtail the global AIDS epidemic. We present the preliminary results of the first Russian Phase II clinical trial of therapeutic DNA vaccine against HIV-1 infection («DNA-4»). «DNA-4» is DNA vaccine containing 4 plasmid DNA encoding *nef*, *gag*, *rt* and *env* genes of HIV-1 subtype A. «DNA-4» induces mostly cellular immune responses as shown by antigen specific cytokines expression, T cell proliferation and cytotoxity

Aim. The study was conducted to estimate the safety and tolerability of the candidate vaccine in HIV-infected patients on HAART. We also investigated the dynamics of viral load (including blips ≥ 50 copies/ml) and $CD4^+$ T cells.

Topic overviewed. This was a multicenter, randomized, double-blind, placebo-controlled Phase II clinical trial. Trial participants were randomized in three groups: 0.5 mg of “DNA-4” (17 patients), 0.25 mg “DNA-4” (17 patients) and placebo group (20 parients). All patients were immunized 4 times at days 0, 7,11,15 intramuscularly without electroporation followed by 24 weeks follow-up period.
“DNA-4” was well tolerated. There were no deaths or severe adverse effects detected during the study. Adverse affects were more pronounced at lower DNA concentrations. Neutropenia and leukopenia were detected in four vaccinated patients. Local reactions were detected in vaccinated groups and placebo group with the same frequency.

We found that 3 trial participants (in groups 0.25 mg and 0.5 mg) had significant increase in blips amplitude (18000 copies/ml and 2800 copies/ml in group 0.25 mg; 709 copies/ml in group 0.5 mg) while the frequency of blips was similar in vaccinated and placebo patients. This might be caused by destruction of latent viral reservoirs as a consequence of induction of cell immunity and expression of TNFα in vaccinated patients as shown by previous studies.

Conclusions. DNA-vaccine against HIV-1 “DNA-4” is safe and well tolerated by HIV infected patients on HAART. The data suggest the possible influence of “DNA-4” on viral reservoirs. This hypothesis requires further investigation and analysis all available data and clinical samples.

THE DEVELOPMENT OF RESEARCH UNIVERSITIES AS A NEW BUSINESS MODEL FOR PRODUCTION OF INNOVATIVE VACCINES

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According to the forecast of some analysts, vaccine market should increase in the next decade: the growth rate in natural and monetary units could exceed the rate of growth of market of traditional chemotherapeutic drugs. Blockbusters Gardasil and Prevnar will bring over $ 1 billion a year for its owners. Dissemination of intellectual, financial and human resources between all participants of market is a big problem for Universities, Science and Business to develop and implement to a market new generations of vaccine.

The aim of abstract is to show advantages of public-private partnership between the pharmaceutical industry and State pharmaceutical and medical Research Universities to combine limited resources to implement new developments to the vaccine market.

One of the task of Universities is producing of Human Capital. In according to Fisher G., Human Capital (HC) is a measure in the person's ability to generate income. HC includes innate abilities and talents, as well as education and acquired skills. Human capital is a base to the Galor-Weil model. Technology could complement skill and increases the returns to investments in education. In the technological venture business, the concentration of highly qualified specialists leads to the higher efficiency of each of them and also it can cause more significant synergies in scientific research. That is why it is necessary to concentrate HC not only at the Universities. We should combine efforts with industrial science, private companies. It could give potent innovation growth in development of vaccines in according with Galor-Weil model. Claster model in development of vaccine business is used this theory also. Human capital is an important part of micro and macroeconomics. The main purpose the macroeconomics is economic growth. The factors include economic growth include the growth of education, natural resources, improving health, improving the organization of labor, scientific and technological progress fixed assets. So cluster can bring together the most important macroeconomic factors for the improvement of health by combining education, fixed assets, scientific structures, which dissevered at the present time.

Studies of professor Robert Zemsky, Lisa Lynch and Peter Cappelli have shown, that after increasing of level of education of specialists by 10 %, the overall productivity increased by 8.6%. In comparison the investment in the fixed assets increase productivity by 3.4%. In other words, the marginal return on investment in human capital is almost three times the return on investment in equipment. Our analysis have shown that immunobiological industry is waiting from system of Education modern specialists, which have skills and competences in gene design, development of drug delivery systems for DNA vaccines etc. Western Civilization achieved leadership in the World, because Research Universities offered advanced technologies and well educated specialist and scientists, which meet requirements of the national and global industry. The industry in its turn invested in education and science. Western transcontinental companies Sanofi, Pfizer and others take 65% of marketplace of vaccine in Russia in monetary units.

It is necessary to change system of the pharmaceutical and medical education from traditional educational institutes to the Research Universities , which could link manufacture, science and education to the one claster to build an effective business model for the development of modern vaccines by using innovative approaches.

SESSION V:
NOVEL TREATMENTS AND TECHNIQUES

Guryeva Svetlana	Spidroin-based bioartificial matrix with therapeutic agents for regenerative medicine	oral
Kalis Martins	Determination of parameters defining accuracy of widefield FRET measurements of molecular interactions	oral
Michlewska Sylwia & Maksim Ionov	Ruthenium dendrimers as anticancer delivery agents	oral
Osmakov Dmitry	Low molecular weight compounds inhibiting the acid-sensing ion channels	poster
Zamyatnin Jr. Andrey	Targeting at gluten intolerance culprit: development of enzymatic tools for effective gluten detoxification	oral
Zernii Evgeni	Antioxidant premedication as a new approach to prevention of perioperative corneal abrasions	poster

SPIDROIN-BASED BIOARTIFICIAL MATRIX WITH THERAPEUTIC AGENTS FOR REGENERATIVE MEDICINE

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Background. Delivery of bioactive molecules and drugs in slow, sustained, controlled release formats is required for many applications. Other desirable properties for such delivery system are biodegradability and biocompatibility. Advantageous features of spider silk, such as processing flexibility, biodegradability and adjustment of drug release profile, present considerable utility for a number of human therapeutic interventions [1]. Spider silk proteins are referred to as spidroins. Previous studies have identified that dragline silk is composed of two spidroins, major ampullate spidroin 1 (MaSp1) and major ampullate spidroin 2 (MaSp2). In the last decade, remarkable progress has been made in understanding silk genetics, structures and biophysics that allows producing recombinant silk proteins. Silk solutions can be formulated into a variety of biomaterial formats, including films, 3D porous scaffolds, hydrogels, micro- and nano-spheres, and coatings [2].

Aims In this research, we were interested in studying spidroin-based microcapsules as a possible delivery carrier for small molecules and proteins.

Materials & Methods : In this study, we prepared microcapsules using recombinant spidroin via a process of emulsifying of dialyzed protein suspension in toluene. The mechanically and chemically stable microcapsules were transferred into aqueous solution via centrifugation into an aqueous sub-layer. The chemicals were added to the protein solution prior to emulsification. In vitro chemicals release studies included the experiment with HEK cell line and primary fibroblasts using xCELLigence RTCA DP instrument. This instrument enables real-time monitoring of cell proliferation. Spidroin microcapsules with chemicals were added to cells into special plates for xCELLigence instrument. The cell index curves were analyzed to identify the influence of chemicals released from microcapsules on cells. The effects of a toxic agent (dimethyl sulfoxide [DMSO]), growth factors, and small molecules with potential improving effect were compared.

Results & Conclusions. In this study, we demonstrated that spidroin-based microcapsules were capable of encapsulation of chemicals whilst allowing small molecules to diffuse freely across the membrane. This study proved the opportunity of using spidroin-based microcapsules for aims of regenerative medicine. Spidroin-based microcapsules can be used as delivery carriers for small molecules and proteins, and such microcapsules can be the part of artificial matrices to promote regeneration via inhibiting inflammatory and fibrosis processes. Nevertheless, this approach will be explored in future works.

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DETERMINATION OF PARAMETERS DEFINING ACCURACY OF WIDEFIELD FRET MEASUREMENTS OF MOLECULAR INTERACTIONS

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Background. Fluorescent Resonance Energy Transfer (FRET) method for studying interaction of molecules situated at a distance 10 nm or less was first described over 50 years ago. It has a wide application in modern science in biomedical research and drug discovery when studying protein interaction and conformation change in immune and nonimmune cells. FRET is rather complicated method requiring precise identification of the correct setup for the experiments, measurements and data interpretation. We used CRAC channel as a model to approbate our FRET system on. Store-operated calcium channels are major pathway for calcium signaling and serve many functions in cells and tissues – gene expression, secretion and immune response. STIM proteins are located in ER, being Ca^{2+} sensors, and, after activation by ER Ca^{2+} store depletion, form ion channel with Orai proteins. These ion channels have an important role in human immune cells (T cells, mast cells, NK cells, B cells). Our aim was to identify the most optimal conditions for studying fluorophore-labelled protein interaction using wide-field dual-detector FRET system and to characterize the parameters affecting data accuracy.

Materials & Methods: HEK 293 cell line was cultured on poly-L-lysine-treated microscope coverslips and used for transfection with plasmid vectors using lipofectamine. Plasmids (STIM1 with CFP fluorescent protein and Orai1 with YFP) were used for studies of ion channel formation as detected by FRET. Exposure time and approach of data analysis varied through experiments in order to optimize FRET data quality. We used Kruskal-Wallis multiple comparison test for FRET data analysis and Pearson correlation to analyze dependence of FRET on CFP/YFP ratio.

Results. Different technical approaches were used for FRET data quality assessment. Too intensive exposure of the fluorophores during the preparation of cells for the FRET measurement resulted in fluorophore bleaching and low FRET efficiency values. Use of neutral density (ND4 combined with ND8) optical filters improved emmited fluorescence intensity and respective FRET efficiency values. Our data analysis confirmed that FRET pixel frequencies must conform to Gaussian distribution. We found correlation between FRET efficiency values with CFP/YFP ratio. Moreover FRET was considerably increased for the bad quality FRET measurements not conforming to Gaussian distribution. However, we did not found that CFP/YFP ratio would affect FRET quality.

Conclusions. 1. Optimal conditions for FRET experiments using a wide-field fluorescence microscope require use of ND filters during the preparation of cells for the FRET measurement in order to avoid photobleaching. 2. CFP/YFP ratio of the readouts and FRET signal quality must be evaluated for correct interpretation of the results.

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RUTHENIUM DENRIMERS AS ANTICANCER DRUG DELIVERY AGENTS

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Ruthenium belongs to the family of metals widely used in anticancer therapy (platinum, arsenic, antimony, bismuth, gold, vanadium, iron, rhodium, titanium, gallium). This presentation deals with a new kind of ruthenium terminated carbosilane dendrimers (CRD), consisting of carbosilane dendrons functionalized with N- , NH_2-donor monodentate and N,N-chelating ruthenium complexes. The biophysical characterization, hemolytic activity and the cytotoxicity towards cancer and normal cell lines, of CRDs have been determined. Obtained data indicate that: i) generation 0 metallodendrimers are the most effective drugs, being non-toxic for normal cells but induced significant cytotoxicity in cancer cells; ii) an increase of generation leads both increased cytotoxicity of CRDs and the levelling of the difference in their action on normal and cancer cells; iii) coordination modes of ruthenium in a dendrimers scaffold not correlate with their cytotoxicity. Moreover, the ability of CRDs to form the stable complexes with nucleic acids can allow them to deliver different types of genes to target cells. Thus new synthesized ruthenium terminated carbosilane dendrimers can be considered as new tool in therapies of reducing cancer cells proliferation.

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LOW MOLECULAR WEIGHT COMPOUNDS INHIBITING THE ACID-SENSING ION CHANNELS

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Acid-sensing ion channels (ASICs) are trimeric membrane proteins belonging to the family of degenerin/epithelial Na+-channels. ASIC3 channel, which is one of the types of these channels, is widely expressed in neurons of the peripheral nervous system and plays an important role in the perception of painful stimuli in various physiological and pathological processes. The usage of natural low molecular weight ligands is one of the most effective research methods of functioning of these channels not only in fundamental terms but also in practice, as allows the development of new drugs with target action. Medicinal plants are known for a long time as a rich source of various biologically active compounds - ligands of certain receptors or enzymes. From the plant Thymus armeniacus, we isolated low molecular weight compound that inhibits the ASIC3 channels functioning. It is a brand new member of lignans (named sevanol) that in micromolar concentrations inhibits the ASIC1a and ASIC3 channels and has, as a consequence, anti-inflammatory and analgesic effect in vivo tests on mice. Structure-functional analysis showed the influence of certain groups of sevanol on the activity to the channel. The structural information was used to design and original synthesis of sevanol structural analogs. The structural analogs of sevanol produce the same effect in vitro and in vivo but display better stability and pharmacological properties. Thus we performed rational design of ASICs antagonist based on the natural compound. Synthesized analogs could be useful as anti-inflammatory compounds as well as neuroprotective substances in ischemic conditions.

TARGETING AT GLUTEN INTOLERANCE CULPRIT: DEVELOPMENT OF ENZYMATIC TOOLS FOR EFFECTIVE GLUTEN DETOXIFICATION

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The term gluten intolerance may refer to three types of human disorders: autoimmune celiac disease (CD), allergy to wheat and non-celiac gluten sensitivity (NCGS). Gluten is a mixture of prolamin proteins present mostly in wheat, but also in barley, rye and oat. Gluten proteins are highly resistant to hydrolysis mediated by proteases of human gastrointestinal tract. It results in emergence of pathogenic peptides, which cause CD and allergy in genetically predisposed people. There is a hierarchy of peptide toxicity and peptide recognition by T cells. If nowadays gluten-free diet (GFD) is applied broadly for CD treatment, such a diet may be unsuitable for NCGS treatment because in this case gluten not always arises as the major or exclusive culprit of gastrointestinal disorder. Furthermore, it is some physicians' opinion that GFD can be deficient in fiber as well as in other vitamins and minerals. In this regard development of alternative medicinal approaches to GFD for effective gluten intolerance treatment is extremely important.

Since human digestive systems are incapable of degrading the entire gluten proteins, other organisms can be used as a source of proteases exhibiting additional activities helping an organism in complete gluten degradation, and plants are perspective candidates for this source. In this study recombinant wheat cysteine protease Triticain- was shown to possess glutenase activity at acidic (or close to neutral) pH levels at the temperature of human body. Further analysis showed that the products of Triticain- -catalyzed gluten hydrolysis revealed multiple cleavage sites within the sequences of gliadin toxic peptides, in particular, in the major toxic 33-mer α-gliadin-derived peptide initiating inflammatory responses to gluten in CD patients. Triticain- was found to be relatively stable in the conditions simulating stomach environment. We conclude that Triticain- can be exploited as a basic compound for development of pharmaceuticals for oral administration aimed at release of the active enzyme into the gastric lumen for CD treatment.

ANTIOXIDANT PREMEDICATION AS A NEW APPROACH TO PREVENTION OF PERIOPERATIVE CORNEAL ABRASIONS

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Background Perioperative corneal abrasion (CO) is an ophthalmological complication commonly found in patients undergoing general anesthesia. Etiology of perioperative CO was hypothesized to involve changes in quantity and quality of the precorneal tear film. To develop a paradigm for prevention of the anesthesia-induced CO, its time-response characteristics were evaluated together with associated alterations in biomechanical and biochemical properties of the tear film. Considering the obtained data, a new approach to prevention of perioperative CO based on antioxidant premedication was tested.

Materials & Methods The corneal abrasions were induced in experimental animals (rabbits) by their exposure to general anesthesia for 1-6 h with or without antioxidant premedication. The development of CO was clinically monitored by means of fluorescein test and verified by histopathological examination of the corneas. Secretion and stability of the tear film was evaluated using standardized Schirmer's test and BUT test, respectively. Tear proteins content was measured by BCA assay, the proteins were separated using SDS-PAGE and identified by mass-spectroscopy. Tear antioxidant activity was validated using the hemoglobin-hydrogen peroxide-luminol chemiluminescence assay. The content of antioxidant enzymes and inflammatory cytokines in tear samples was evaluated using specific colorimetric kits.

Results Fluorescein stained point lesions in cornea were occasionally detected 1 hour post injection of anesthetic whereas six-hour anesthesia resulted in corneal injury in all experimental animals. The lesions were histologically diagnosed as corneal abrasions restricted to the corneal epithelium and involved cell deaths in its mid- and deep layers. The corneal damage was associated with dramatically reduced tear secretion and stability of the tear film. Analysis of the tear proteome pointed to depression of lacrimal glands function, and suggested serotransferrin, serum albumin and annexin A1 as potential tear markers of the complication. The tear film alterations included fast drop of total antioxidant activity and activity of antioxidant defense enzymes, and delayed increase in proinflammatory cytokines. Premedication using antioxidants considerably restored biochemical properties of the tear film and recovered clinical and morphological sings of the disease.

Conclusions Our data suggests antioxidant premedication as prospective approach for prevention of perioperative CO. In addition our data should be considered in any ophthalmological study employing anesthetized animals as results of such a study might be affected by general anesthesia.

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Saturday November 19, 2016

SESSION VI: MOLECULAR MECHANISMS BEHIND DNA VACCINATION

Belikov Sergey	Regulation of transcription, exploiting advances in genome biology to understand and program immune response	key-note
Jansons Juris	Delivery and immunogenic performance of naked DNA vaccines vizualized by in vivo imaging of a novel near-infrared fluorescent reporter	oral
Östlund Farrant Anna-Karin	The packaging of chromosomes, epigenome is a regulator of nuclear processes	key-note
Rhee Joon Haeng	Adjuvants: 21st century toolkit for vaccines and immunotherapeutics, with a special emphasis on mucosal routes	key-note

REGULATION OF TRANSCRIPTION, EXPLOITING ADVANCES IN GENOME BIOLOGY TO UNDERSTAND AND PROGRAM IMMUNE RESPONSE

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Aim: to overview recent developments in the field of regulation of transcription, and discuss advances in the genome biology in connection to immunology.

Background: Human genome contains all information needed to build and maintain the organism. On top of this, epigenomic regulation which includes DNA methylation and histone modifications can alter the structure of chromatin and, thus, change to the function of the genome to achieve developmental regulation, tissue specific gene regulation, and response to environmental stimuli including immune response to infections and vaccines.

Topics overviewed: Immunology is one of the fields of biology that benefits most from the huge progress in genome biology made during the last ten years. In my presentation I describe recent technological advances in the next generation sequencing (NGS) methods such as mapping of DNA methylation, histone modifications, transcriptional factor binding, chromosome interactions as well as genetic variations). Examples will be given of how these methods can be used for a detailed characterization of the immune cells. Molecular mechanisms which can be exploited to enhance the immunogenicity of DNA vaccines will be discussed. The biology of immune system is characterized by tremendous genetic and environmental inter-individual alterations. I will describe recent advances modern NGS technologies on a single cell level which allow the discovery of new cell types within a heterogeneous sample.

Conclusion: New NGS methods provide a close view on the establishment and maintenance of the immune response to pathogens and vaccines, pushing our understanding of the field on a higher level.

THE PACKAGING OF CHROMOSOMES, EPIGENOME IS A REGULATOR OF NUCLEAR PROCESSES

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Topics Overviewed The chromatin, the structure of DNA and protiens, is a major regulator of nuclear processes in cells. Recent studies have shown that several different chromatin states exist, from active states to repressed states. The states are constituted by different histone modifications and non-histone proteins and sometimes referred to as the epigenome of cells. The states over the DNA vary, according to cell type but also dynamically depending on cell state. These states affect the outcome of the nuclear processes, for instance, chromatin state determines the replication timing, the level of transcriptional initiation and recombination events. The chromatin states, in particular outside of the coding regions, varies between individuals, for instance opening different DNase hypersensitive sites, leading to a variation of chromosome associations.

DELIVERY AND IMMUNOGENIC PERFORMANCE OF NAKED DNA VACCINES VIZUALIZED BY IN VIVO IMAGING OF A NOVEL NEAR-INFRARED FLUORESCENT REPORTER

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Background Recently developed family of near infra-red fluorescent proteins that absorb and fluoresce in a tissue transparency “optical window” between 650-900 nm have the advantage over traditional bioluminescent luciferase reporters in following up the delivery and immunogenic performance of naked DNA vaccines by optical imaging of deep tissues. iRFP670 protein with excitation/emission maxima at 643nm/670nm was engineered introducing mutations in the PAS-GAF domains of the RpBphP2 bacterial phytochrome of the photosynthetic bacterium *Rhodopseudomonas palustris*. It shows high efficiency in deep imaging experiments, but the main goal of our study was to estimate immunogenicity of iRFP670 and to conclude if it’s suitable for using in DNA immunization experiments as reporter.

Methods The plasmids on the basis of pVAX1 vector expressing iRFP670 protein and luciferase were used in study. The expression of iRFP670 protein in cell culture was confirmed by Western Blotting, the fluorescent activity of expressed protein was detected by microscopy and flow cytometry. The BALB/c mice were inoculated with iRFP670 plasmid DNA by intradermal injection with subsequent electroporation performed using in *vivo* electroporator CUY21EDIT II (BEX Co., Ltd., Japan) with multineedle, two-needle, plate or plate-fork electrodes. Fluorescence and bioluminescence was detected *in vivo* and *ex vivo* using optical imaging by Spectrum CT. The immune response in mice was detected using ELISpot and Fluorospot assays for T-cells and ELISA for antibodies.

Results The fluorescence from the iRFP670 gene injection sites was detectable 1 h post injection and persisted for 4 weeks (day 27 post injection) while decreasing by that time by 30% only. During the same time-period, the expression of the firefly luciferase usually decreases by up to 90%, and of the luciferase gene delivered with a potent gene immunogen by >99%. During *ex vivo* studies the fluorescent activity of iRFP670 was detected in human skin explants in 48 hours’ post injection. Representative peptides predicted as iRFP670 T cell epitopes were synthesized and together with recombinant iRFP670 used for screening of immune response raised in iRFP670 gene recipients. A dominant epitope was localized at amino acids 224-257. Respective peptide induced the production of IFN-γ (100-300 SFC/10^6 splenocytes) in >50% of immunized mice. An anti-iRFP670 response was mainly cellular: the average titer of anti-iRFP670 antibodies did not exceed 10^3. The immunogenicity of iRFP670 was similar to that of firefly luciferase, and likewise not sufficient to clear the iRFP670-expressing cells from the sites of gene injection.

Conclusions The properties of iRFP670 protein, such as the NIR emission/excitation spectrum and relatively low immunogenicity and substrate independence, make it an attractive alternative to bioluminescent reporters, and suitable for a longitudinal follow-up of the labelled cells in a variety of applications including gene immunization.

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ADJUVANTS: 21ST CENTURY TOOLKIT FOR VACCINES AND IMMUNOTHERAPEUTICS, WITH A SPECIAL EMPHASIS ON MUCOSAL ROUTES

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Background: Thanks to the progress in immunology, microbiology, genetics, biotechnology, and structural biology, modern vaccine research and development should be harnessed by new toolkits. TLR ligands are considered attractive adjuvants for vaccines and immunotherapy. New generation vaccines are supposed to be improved in many areas: one of them should be associated with novel classes of vaccine adjuvants that can promote better protective humoral and cellular immune responses, the optimal presentation of antigens to the immune system in order to shape proper protective and therapeutic activities.

Aim: Successful protective immune responses could be generated as the result of a harmonious interaction between innate and adaptive immunity. Adjuvants mostly act at the interface in several nonmutually exclusive ways to augment the adaptive immune response and to generate effective immunological memory. TLRs are widely studied recently and their ligands/agonists are regarded as good adjuvants whose action mechanisms could be traced definitively by existing scientific tools. Mucosal vaccination is, while injected vaccines generally fails to do so, capable of inducing protective immune responses both in the mucosal and systemic immune compartments. Mucosal vaccination offers other advantages, but requires safe and effective adjuvants to overcome mucosal immune tolerance mechanisms.

Topics overviewed: Flagellin is the cognate ligand for TLR5 of host cells. TLR stimulation leads to activation of innate immunity and subsequently modulates adaptive immune responses. This presentation will try to convince that flagellin has a unique immunomodulating activity in the mucosal immune compartment. Mucosal co-administration of a *V. vulnificus* flagellin (FlaB) with microbial antigens induced significantly enhanced antigen-specific IgA responses in both mucosal and systemic compartments and IgG responses in the systemic compartment. Intranasally administered FlaB colocalized with CD11c as patches in DCs and caused an increase in the number of TLR5 expressing cells in draining lymph nodes. Furthermore, flagellin could serve an efficacious adjuvant for vaccines and immunotherapies against noninfectious intractable diseases in the mucosal compartments such as allergic asthma and cervical cancer. We found that therapeutic doses of flagellin together with allergens suppress allergic asthma by inhibiting pathogenic $T_{h1}/T_{h2}/T_{h17}$ responses while generating regulatory DCs (DC_{reg}) and T_{reg} cells. For cervical cancer, we examined whether flagellin can be used as an adjuvant for topical therapeutic cancer vaccine in a genital cancer model. Intravaginal (IVAG) co-administration of E6/E7 peptides with flagellin resulted in tumor suppression and long-term survival of the tumor bearing mice. IVAG immunization of E6/E7 peptide with flagellin induced accumulation of CD4+ or CD8+ cells and T cell activation in draining genital lymph nodes (gLNs). The co-administered flagellin elicited antigen-specific IFN-γ production in gLNs and spleen. The IVAG administered flagellin co-localzed with CD11c+ cells in the gLN T cell areas and enhanced TLR5 expression.

Conclusion: Flagellin serves a versatile adjuvant for mucosal vaccines and immunotherapeutics against intractable diseases.

SESSION VII:
MOLECULAR MECHANISMS BEHIND DNA VACCINATION, IMMUNE ASPECTS

Kilpeläeinen Athina	Murine breast adenocarcinoma cells expressing HIV proteins as a challenge model to test the efficacy of therapeutic HIV vaccines	short oral
Reche Pedro	Epitope-based vaccines and the educated search for epitopes	key-note
Westerberg Lisa	DNA vaccination to activate the adaptive immune system to fight cancer	key-note

MURINE BREAST ADENOCARCINOMA CELLS EXPRESSING HIV PROTEINS AS A CHALLENGE MODEL TO TEST THE EFFICACY OF THERAPEUTIC HIV VACCINES

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Background: There are currently no direct ways to evaluate therapeutic DNA vaccines against HIV in small animal models as mice cannot be infected with HIV. We have developed several gene immunogens targeting HIV enzymes harboring drug resistance mutations. The 4T1-luc2 mouse mammary gland cell line (Perkin Elmer) constitutively expresses luciferase and thus allows *in vivo* visualization using bioluminescence imaging (BLI).

Aim: The aims of the study were to generate 4T1-luc2 cell lines stably co-expressing HIV enzyme gene variants by lentiviral transduction, characterize the cells and their capacity to form tumors *in vivo,* and to assess whether immunization with DNA encoding luciferase prior to tumor cell implantation would have an effect on tumor growth.

Methods: 4T1-luc2 cell lines constitutively expressing HIV enzyme gene variants were generated by lentiviral transduction (Evrogen, Moscow). The cell lines were implanted subcutaneously into BALB/c mice and photon flux luminescence was followed using BLI. The tumors were removed and analyzed to confirm presence of the HIV gene insert or mRNA. To assess whether immunization with a DNA immunogen expressed by the 4T1luc2 cell line prior to implantation could impact or confer protection to tumor growth *in vivo*, mice were immunized with a plasmid encoding the luciferase or an empty vector as a negative control using different electroporation regimens with the DermaVax system (Cellectis) or CUY21 EditII (BEX Ltd) and subjected to a homologous boost one month later. Immunization sites were regularly monitored by BLI. Mice were then subcutaneously implanted with 4T1luc2 cells and followed up by BLI. 9 days later, when control mice had formed palpable tumors, mice were sacrificed. Splenocytes were harvested and stimulated with a murine CD8+ T-cell peptide antigen of luciferase.

Results: The cell lines expressing HIV enzyme gene variants along with their parental cell line 4T1-luc2, were characterized and their capacity to form subcutaneous tumors *in vivo* in BALB/c mice was confirmed, as well as gene expression in excised tumors. In mice immunized with DNA encoding luciferase, 4T1-luc2 cells implanted in mice immunized and electroporated with the DermaVax system (Cellectis) or CUY21 EditII (BEX Ltd) at 100V did not grow during the first week. Photon flux measured by BLI at the challenge sites revealed only 15% of the bioluminescence signal remained in mice electroporated with the DermaVax system and no significant increase in signal was observed in mice immunized with the BEX system, both using a 100V driving pulse. 9 days post implantation, endpoint immune assays revealed specific IFN-gamma secretion by CD8+T-cells in the luciferase immunized mice but not in the control group, confirming this response as the mechanism of resistance to early tumor growth.

Conclusions: We have demonstrated that DNA immunization using optimal electroporation regimens can confer resistance to initial tumor growth of breast adenocarcinoma cells stably expressing an antigen *in vivo*, with specific IFN-gamma secretion seen in resistant mice. The generated cell lines constitutively co-expressing HIV enzymes and luciferase allow detection by BLI, and will be used as a tumor challenge model following DNA immunization with candidate therapeutic HIV-1 DNA vaccines.

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EPITOPE-BASED VACCINES AND THE EDUCATED SEARCH FOR EPITOPES

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Vaccines are without doubt the most effective treatment against infectious diseases, having saved millions of lives. Most vaccines are based on life attenuated or dead pathogens and many were developed with little knowledge on how the immune system works. In contrast, our current understanding of the immune system has enabled a new vaccination approach that is based on the precise antigen fragments, epitopes, recognized by B and T cells. Epitope-based vaccines bring new hope for unmet medical needs such as cancer and pathogens like HIV that escape the immune response due largely to genetic plasticity. The development of epitope-based vaccines is facilitated by the availability of computational tools that readily predict epitopes within antigens. Here, we will overview key features of antigen recognition by B and T cells and will examine relevant methods for predicting epitopes. Since B cell epitope prediction remains problematic, we will concentrate on T-cell epitopes and more specifically on predicting peptide binding to Major Histocompatibility Complexes (MHC). T cells can only recognize peptides presented by MHC molecules and thereby prediction of peptide-MHC binding constitutes the principal basis for anticipating T cell epitopes. There are many methods to predict peptide-MHC binding, including those developed by our group, and we will review them systematically. We will also address issues such as HLA polymorphism that complicate the practical deployment of epitope-based vaccines. Overall, while prediction of peptide-MHC binding is quite precise only around 10% of predicted epitopes are immunogenic. Therefore, in order to accelerate epitope identification and translational epitope-vaccine research, we must improve epitope prediction methods and define rationales for prioritizing protein antigens.

DNA VACCINATION TO ACTIVE THE ADAPTIVE IMMUNE SYSTEM TO FIGHT CANCER

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Background: Vaccination against infectious diseases has been one of the success stories in elimination of mankind diseases. Therapeutic vaccination against established disease such as chronic infection and cancer has proven significantly more challenging since the vaccine has to activate specific parts of the immune system to avoid tolerant or skewed immune responses. An ultimate goal in cancer therapy is to activate the host immune cells to recognize the cancer cells as foreign to eliminate the cancer cells from the body. Cytotoxic T cells can directly kill the cancer cells and B cells can produce cancer cell-specific antibodies that will enhance the killing capacity of other cells including natural killer cells. The adaptive immune system is equipped with B cells and T cells that can recognize virtually any non-self antigen that we are exposed to. The generation of this vast B and T cell repertoire is random, and therefore the immune system has developed strong breaks to prevent activation of autoreactive B and T cells that can cause severe autoimmune disease.

Aims: Vaccine approaches to non-viral cancer have mainly relied on target molecules that are common to a particular cancer type and therefore also expressed on healthy cells in the body. In development of a vaccine for non-viral cancer, one of the major hurdles to overcome is the intrinsic breaks of the immune system. This topic will be discussed in the context of T cell development and T cell activation by dendritic cells.

Topics overviewed: T cells undergo extensive selection during development in the thymus to delete T cells that express a T cell receptor with high affinity for self antigen. This means that the cancer-specific T cells that we want to activate is eliminated.

- ✓ Can we modify peripheral T cells to efficiently recognize cancer cells?
- ✓ How can we steer dendritic cells to activate the correct T cells, and avoid activation of T regulatory cells that suppress the activation of effector T cells?
- ✓ Can we use DNA vaccines to activate presentation of tumor antigens by dendritic cells?
- ✓ Do the activated T cells reach the tumor?
- ✓ What type of cancer is likely to be successful using DNA vaccines for immune therapy?

Conclusion: The last 5 years have been a success story in combating cancer by usage of check point blockade therapy where the break for T cells activation is turned off and therefore allows effector T cells to efficiently kill cancer cells such as melanoma cells. The survival curves for the melanoma patients has increased significantly, however, a major side effect is development of severe autoimmune disease. Therefore, a major focus for therapy against non-viral cancer is now to develop more safe approaches including using DNA vaccines to target antigen presentation by dendritic cells.

SESSION VIII:
IMMUNE RESPONSE
TO INFECTIONS AND CANCER

Kichatova Vera	Analysis of prevalence of substitutions in amino acid positions 70 and 91 of viral nucleocapsid associated with sustained virologic response to interferon therapy in chronic hepatitis C	poster
Podschwadt Philip	Polarized macrophages use veiled surface structures to ingest HIV particles	poster
Prostova Maria	Immunogenicity of poliovirus non-structural proteins during natural poliovirus infection	oral
Sander Philip	Monitoring cell death of glioblastoma cells and immune cell interactions by IncuCyteZOOM	oral

ANALYSIS OF PREVALENCE OF SUBSTITUTIONS IN AMINO ACID POSITIONS 70 AND 91 OF VIRAL NUCLEOCAPSID ASSOCIATED WITH SUSTAINED VIROLOGIC RESPONSE TO INTERFERON THERAPY IN CHRONIC HEPATITIS C

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Background Combination therapy of chronic hepatitis C with interferon (IFN) and ribavirin is still current clinical practice in Russia due to the high cost and low availability of direct-acting antivirals (DAA). About 55% of HCV infections in Russia are caused by HCV 1b subtype, which is associated with lower IFN response rates. Amino acid substitutions Q/H70R and M91L in capsid protein in HCV subtype 1b (HCV-1b) can affect achieving sustained virologic response (SVR) on IFN and can also be associated with the development of hepatocellular carcinoma (HCC). Data on the prevalence of these polymorphisms among HCV-1b strains circulating in Russia are absent.

Aim of this study was to investigate the prevalence of Q/H70R and M91L substitutions in HCV strains circulating in Russia in comparison to other geographical regions.

Materials and methods Total 314 HCV core sequences amplified from serum samples collected in 2007 to 2014 from different regions of Russia were included in this study. Also 95 HCV core sequences from Russia deposited in Genbank were added to analysis. As a comparison group, core sequences from US Los Alamos database (LANL) were analyzed taking into account its origin: Asia (n = 374), North America (n = 1343), and Europe (n = 493). In addition to subtype 1b sequences of other HCV subtypes that are prevalent in Russia (1a, 3a, 2k/1b) were also included in analysis.

Results The rate of Q70R substitution in HCV-1b isolates from Russia was 26.7%, which was significantly lower compared to isolates from North America and significantly higher compared to ones from Europe (p<0.01), but similar to its prevalence in isolates from Asia. The variant H70R was found in 4.7% isolates from Russia, more frequently compared to HCV-1b isolates from Europe (p <0.01), but not from Asia and North America. Substitution M91L was observed in 80.6% of HCV-1b isolates from Russia, similar to its rate in strains from North America and Europe, but significantly higher compared to strains from Asia (p <0.01). Core sequences of 1a and 3a subtypes, and 2k/1b recombinant had similar distribution of amino acid variants in positions 70 and 91, except one variant: Q70R rates in HCV subtype 3a sequences from Russia was significantly higher compared to 3a strains from Asia (p <0.01).

Conclusions Comparative analysis of HCV-1b core variants distribution demonstrated higher prevalence of unfavorable Q/H70R variants in Russia compared to neighboring European countries. North America is the only region where Q70R mutation rates were higher compared to Russia and other regions studied. M91L mutation present in most HCV-1b strains circulating in all regions of the world, including Russia, with the exception of Asia, where the Leucin in position 91 is the predominant variant. Screening of HCV-1b infected patients for unfavorable mutations in core region could be a useful tool to identify those who are in greater need of IFN-free treatment. In future, after complete implementation of IFN-free regimens, analysis of polymorphisms in position 70 of HCV-1b core remain relevant to identify patients at higher risk for HCC development requiring immediate treatment.

Acknowledgements Supported by the grant of Russian Science Foundation nr 15_15_30039. Training of young researchers was supported by TP 09272_2013 of the Swedish Institute

POLARIZED MACROPHAGES USE VEILED SURFACE STRUCTURES TO INGEST HIV PARTICLES

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Background We established a culture system to characterize the inflammatory immune response of monocytes and dendritic cells in various diseases such as severe sepsis, diabetes type 2, autoinflammatory disorders such as macrophage activation syndromes (MAS), and from conditions with major tissue repair activities following trauma. After in vivo exposure to local growth factors, pro-inflammatory cytokines, and microbial compounds, these cells differentiate into macrophages and dendritic cells (Tacke and Randolph 2006). While these monocytes play a key role in eliminating invading bacteria, viruses, fungi, and protozoans, they can also play a role in the pathogenesis of inflammatory and degenerative diseases. In the present investigation we studied virus-uptake mechanisms by in vitro cultures macrophages and dendritic cells derived from patients. We used recombinant HIV-particles enriched from HEK-cell cultures and simultaneously tested the effect of a novel albumin-derived peptide, EPI-X4 (Mohr et al. 2015).

Methods In vivo activated antigen presenting cells were enriched by cell culture and subjected to novel culture conditions in the presence of absence of 2% human serum albumin. Before cell culture in 1µ-slide IV IBIDI dishes, the 14-28 day cultured cell preparations were characterized by flow cytometric analysis to distinguish inflammatory macrophages (designated M1, M2a-M2c) and anti-inflammatory macrophages termed M2d. After 72h, the cell cultures were inoculated with HEK derived HIV-1 particles. After 48h, the cell cultures were examined by standard fluorescence microscopy and then subjected to standard transmission electron microscopy.

Results Different macrophages and dendritic cell subtypes derived from the different diseases and disease states were all similar regarding a unique virus uptake structure characteristic for dendritic cells and composed of a pattern of veiled plasma membrane leaves. These protrusions were preferentially used as virus uptake machinery and remained intact when ingested into the phagocytic cell. Remnants of the extracellular fluid in the cytoplasm were regularly observed which indicates a mechanism described as macropinocytosis. When HSA-treated cultures (Fig. 2) were compared with non-treated cultures, the amount of morphologically identifiable particles was in all cases reduced. Most pronounced effects by HSA-treatment were observed in M2d type macrophage cultures (Fig. 1, 2).

Conclusion The current investigations imply the importance of macropinocytotic uptake mechanisms in macrophages and dendritic cells by veiled surface structures. Macropinocytosis must be further studied for vaccination protocols to be applied for virus infections and malignancies.

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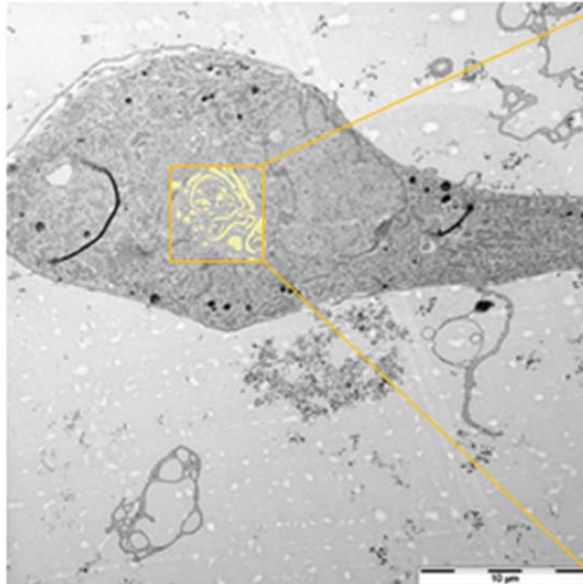
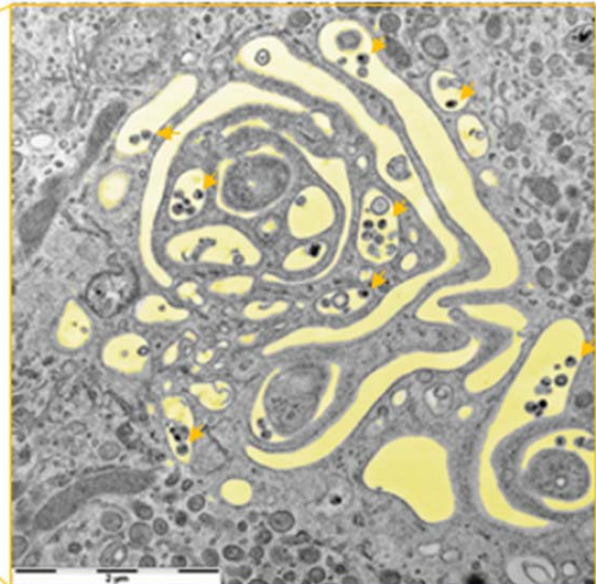


Figure 1: Control macrophage (M2d differentiation type) infected with rhHIV1



Enlarged insert area, note abundant virus particles (arrows) in ingested veiled membrane protrusions, fluid from extracellular medium has been colored in yellow

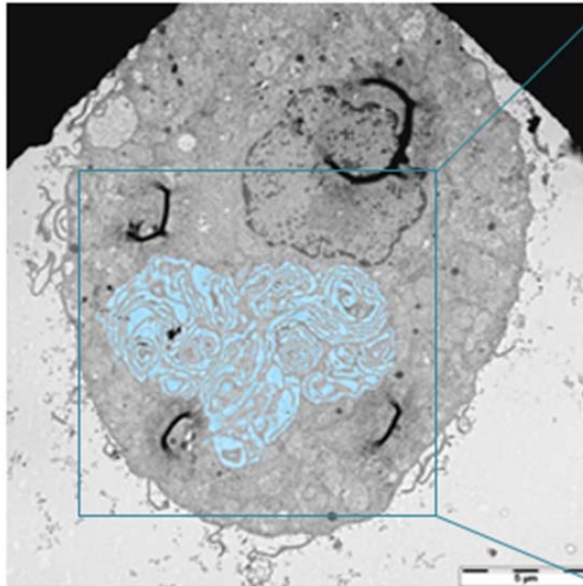
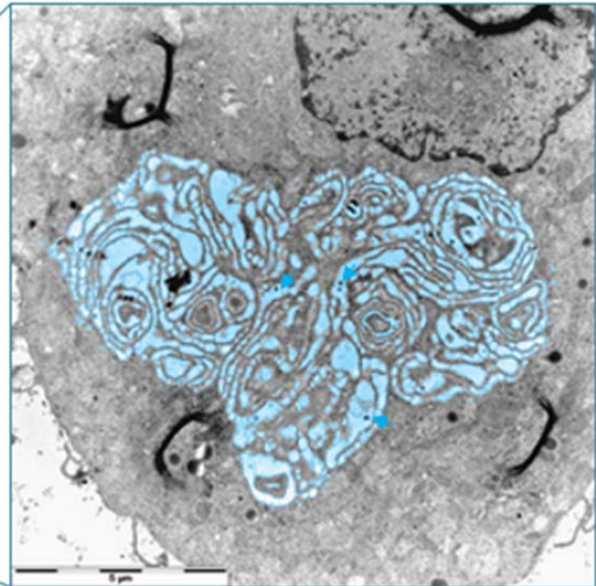


Figure 2: Human serum albumin- (HSA-) treated macrophage (M2d differentiation type) infected with rhHIV-1



Enlarged insert area, note the sparse virus particles (arrows) in ingested veiled membrane protrusions, fluid from extracellular medium has been colored in blue

IMMUNOGENICITY OF POLIOVIRUS NON-STRUCTURAL PROTEINS DURING NATURAL POLIOVIRUS INFECTION

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Antibodies against viral non-structural proteins are produced during poliovirus infection, but little is known about their role in adaptive immunity (1). To investigate the possible role of non-structural proteins during natural poliovirus infection we tested sera samples from polio cases during an outbreak of poliomyelitis in Tajikistan in 2010. During outbreak two wide spread co-circulating poliovirus lineages were identified as causative agents of polio cases. One of them had recombinant structure with the structural region belonged to co-circulating wild type 1 poliovirus imported from India while the region coding for non-structural proteins was acquired from non-polio Enterovirus C (2). 3CD amino acid sequences of Indian poliovirus strains and recombinant strains differ from each other in 9-15 residues, which, therefore, could represent potential antigenic epitopes. To test the hypothesis whether the antigenic structure of non-structural proteins of recombinant virus was different from that of parental virus, 24 sera samples from polio cases (7 sera samples from people infected with parental Indian poliovirus and 16 sera samples from people infected with recombinant poliovirus) were analyzed in Western Blot with lysates of infected with two different polioviruses RD cells. All sera samples reacted with capsid protein VP1. Almost all sera samples (20 out of 24) reacted with viral non-structural protein 3CD in both type of lysates. Additionally, the sera sample from patient, infected with the recombinant virus, equally immunoprecipitated protein 3CD from RD cell lysates infected with poliovirus isolates of Indian strain (3 viruses), with recombinant isolates (3 viruses) and with poliovirus type 1 strain Mahoney. These results indicate that viral protein 3CD is the most immunogenic in comparison with other non-structural proteins. However, we did not detect any difference in reactivity of tested sera with non-structural proteins contained in lysates of RD cells infected with different polioviruses. According to this data we could not suggest that recombination event significantly changed the antigenic structure of poliovirus non-structural protein 3CD. However, we cannot exclude that patients could contact with both poliovirus variants due to their wide co-circulation and elicit immune response to both types of non-structural proteins. Thus, possible immunological advantage for the virus resulting from non-structural proteins exchange could not be completely excluded, and should be investigated more accurately on animal models.

Acknowledgements. This work was founded by RSF grant No 15-15-00147

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MONITORING CELL DEATH OF GLIOBLASTOMA CELLS AND IMMUNE CELL INTERACTIONS BY INCUCYTEZOOM

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Objectives In an approach to establish rapid testing of immune sensitization against brain tumors, we established permanent cell lines from glioblastoma multiforme. Methods: Permanent cell lines were established from necrotic tumor tissues, following mechanical dissection, trypsin treatment and ficoll separation. The cell suspension was tested for contaminating hematopoietic cells and cytospins were made and examined after Wights-Giemsa-staining. GBM cell lines were analyzed by flow cytometry, then seeded into 96-well (flat-bottomed) microtiter plates and analyzed by microcopy as well as caspase 3/7, Annexin-V and PI-staining. About 10 different cell lines have been selected for more detailed testing against cytokine-activated, allogeneic and autologous immune cells, as well as xenogeneic viruses.

Results and Conclusion Theoretically, immune phenotypes of peripheral blood and functional properties of an individual patient's immune cells can be applied to predict immune recognition. These results are substantiated by survival characteristics (Mostafa et al. 2016). The majority of our cell lines expressed HLA- class I and -class II antigens only weakly, but patients whose cell lines had higher MHC-antigen densities presented with a better survival. When addressing agents which may influence the proliferative potential of individual tumor cell lines, we used an IncuCyteZOOM (Essenbioscience.com) device to monitor cell viability against cell death, caspase 3/7 activation, Annexin V surface expression and cell-cell interactions. In addition to screening xenogeneic viruses, we also tested the cell lines susceptibility to Vacquinol-1 (Kitambi et al. 2015). At 7 µM, Vacquinol-1 induced a rapid cell death in all GBM cell lines, occurring with massive cell blebbing. Morphological criteria of Vacquinol-induced cell death demonstrate that cell death is not related to apoptosis despite of caspase 3/7 activation. We currently study, whether cell death induced by activated T- and NK-cells is similar to the efficient cell death induced by Vacquinol-1.

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Sunday November 20, 2016

SESSION IX: MOLECULAR MEDICINE: CANCER

Alekseeva Ekaterina	Structural pathology of the 10q23.3-26.3 chromosome region in glioblastoma	poster
Glukhov A.	Analysis of telomerase activity in order to develop non-invasive diagnosis of bladder cancer	poster
Kalnina Lelde	The FABP5 gene expression is significantly higher in triple-negative sporadic than hereditary breast cancer tissues	short oral
Kazakova Svetlana	Identification of aberrant DNA methylation in paediatric acute myeloid leukaemia by multiplex methylation sensitive PCR	short oral
Kuznetsova Ekaterina	Methylation of matrix and transmembrane proteins encoding genes in breast cancer	poster
Mezale Dzeina	Expression of p53 protein in primary and secodary malignant liver tumors	short oral
Mikhaylenko Dmitry	Mutation and gene expression analysis of urine sediments in non-invasive molecular genetic diagnostics of the common oncourological diseases	short oral
Osminin Sergey	Abnormal methylation MGMT, CDH1, p16/CDKNA2, DAPK, RAR-B genes in patients with Barretts esophagus before and after surgical treatment	poster
Ustinova Monta	The impact of 11 low-penetrance allelic variants on breast cancer morbidity in population of Latvia	short oral

STRUCTURAL PATHOLOGY OF THE 10Q23.3-26.3 CHROMOSOME REGION IN GLIOBLASTOMA

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Loss of heterozygosity on chromosome 10q is the most frequent genetic alteration in glioblastoma and occurs in up to 80% of cases. Using microsatellite analysis we have determined the frequency of loss of heterozygosity in the 10q23.3-26.3 chromosome region, containing candidate genes *PTEN*, *FGFR2, MKI67* and *MGMT*, in 124 samples of glioblastoma. Frequency of loss of heterozygosity at the region under investigation equals 62.1%. We have used quantitative microsatellite analysis to establish a quantitative change of copy number of the 10q23.3-26.3 in 64 samples of glioblastoma with identified loss of heterozygosity in 10q23.3-26.3 area. In 37,5% of the samples only one copy of 10q23.3-26.3 chromosome region was found (deletion), in 25,0% two copies were detected (acquired uniparental disomy,). In 37,5% (24/64) of the samples areas of alternation of deletion and acquired uniparental disomy throughout the tested region were identified.
Higher frequencies of deletions were characteristic for the proximal part of 10q23.3-26.3 region (PTEN and FGFR2 genes), while acquired uniparental disomy and deletion were equally in the distal part (MGMT gene).

ANALYSIS OF TELOMERASE ACTIVITY IN ORDER TO DEVELOP NON-INVASIVE DIAGNOSIS OF BLADDER CANCER

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Purpose: The article is aimed to evaluate the possibility of using telomerase activity measurement in urine cell sediments for non-invasive diagnosis of bladder cancer. 48 urine cellular samples and 48 tumor tissue samples from bladder cancer suspected patients have been investigated during the research. 12 urine cellular samples and 12 bladder tissue samples from patients with chronic cystitis have been examined as a control group.
Measuring telomerase activity in samples involved the following methods: modified TRAP method; RT-PCR; PCR; electrophoresis. The sensitivity of the method is 93,75% and its specificity is 97,9%.
Results: According to the research, telomerase activity were detected in 45 out of 48 (93,75%) urine samples of the patients suspected of having bladder cancer in the preoperative period, and in 47 out of 48 tumor samples (97,9%). Bladder cancer histologically verified in all patients from this group. In the postoperative period telomerase activity was absent in all 48 urine samples as well as in all 12 samples of cellular material in conrol group. Statistically, difference between patients with bladder cancer and telomerase activity presence in the urine (p=0.001) is significant.
We have also determined the dependence of telomerase activity level in both urine and tissue samples from the degree of bladder cancer differentiation. In 15 urine samples from patients with G1 the average rate of telomerase activity was 0.61%, in 23 cellular samples from G2 bladder cancer patients, telomerase activity was 0.95 % in average and in 10 urine cellular sediments from G3 patients was 1.33% in average. We noted that the level of urine telomerase activity is increasing while the degree of differentiation is decreasing (p=0,155).
Conclusion. The preliminary data suggests the possibility of using telomerase activity measurement in cellular urine material for diagnosing and monitoring the recurrence of bladder cancer.

THE FABP5 GENE EXPRESSION IS SIGNIFICANTLY HIGHER IN TRIPLE-NEGATIVE SPORADIC THAN HEREDITARY BREAST CANCER TISSUES

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Introduction. Triple negative breast cancer (TNBC) is a heterogeneous disease characterized by the lack of oestrogen, progesterone and HER2 basal cell markers and has a tendency to be more aggressive than any other breast cancer subtype [1]. Sporadic TNBC patients have worse recurrence-free survival rate than hereditary TNBC patients [2]. Futhermore, sporadic cancer is not only larger in tumor size compered to *BRCA1* mutation carriers but are also less sensitive to chemotherapy [3].

Material and m ethods. Twelve sporadic and eleven hereditary TNBC patients were selected for this study. Sporadic TNBC group was selected to match hereditary TNBC group in count. All patients signed informed consent forms. RNA samples from formalin fixed and paraffin embedded tissues (FFPE) were extracted with RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion) according to the instructions of the manufacturer. Gene expression analysis was performed with Whole-Genome Gene Expression DASL Assay (Illumina) following the manufacturer's protocol. Data analysis was done with CLC Workbench (Qiagen).Gene expression data was verified with qPCR which was carried out using following conditions: 50°C for 2 min, 95°C for 10 min and followed by 45 cycles of 95 ^{o}C for 15 sec., 55 ^{o}C for 30 sec. and 72 ^{o}C for 30 sec. Data analysis was performed with ViiA 7 RUO software. *B2M* and 18S rRNA were used as endogenous controls. The ΔCt method was used to evaluate relative expression. Statistical significance level α was set at 0.05.

Results. Microarray gene expression analysis and qPCR showed that the *FABP5* is significantly more expressed in sporadic TNBC tissues than hereditary ones (P=0.002; Whitney-Mann test).

Conclusions. High expression of the *FABP5* gene in TNBC tissues may be the cause for patients to have worse disease specific survival than hereditary TNBC patients.

Acknowledgements. This study was funded by National Research Program BIOMEDICINE for Public Health. Horizon 2020 project VACTRAIN 692293 is acknowledged for training and mobility support.

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IDENTIFICATION OF ABERRANT DNA METHYLATION IN PAEDIATRIC ACUTE MYELOID LEUKAEMIA BY MULTIPLEX METHYLATION SENSITIVE PCR

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The aim of this study is to develop a system of DNA methylation markers of acute myeloid leukaemia (AML) in children. Aberrant methylation diagnostic potential can be used to identifying AML subtypes having different sensitivity to therapeutic regimens in particular with the use of epigenetic modifiers. Our study involves 53 bone marrow samples from AML patients before treatment and after courses of chemotherapy (CT) and demethylating therapy (DT). Primary identification of aberrant DNA methylation is carried out by an unbiased DNA differential methylation screening method developed within this study. We proposed a system of DNA methylation markers (belonging to the promoter regions of *ABCG4, AIFM3, CLDN7, CXCL14*, *DLK2*, *EGFLAM, GSG1L, KHSRP, MAFA, RXRA, SOX8, TMEM200B, TMEM176A /TMEM176B* genes) for the assessment of aberrant DNA methylation (using SmaI restriction enzyme site). The DNA methylation frequency is estimated using the system of 4 multiplex methylation sensitive PCR reactions with the internal controls. Methylation is determined at the BstHHI restriction enzyme site; it was used because having many restriction sites within the loci studied, to determine the homogeneity of the CpG methylation across the region. Methylation frequencies for genes show in table.

Gen	Before	Before	After 1-2	After 4-5
CLDN7, 17p13.1	31	28.3	66	0
CXCL14, 5q31.1	63	96	90.9	0
DLK2, 6p21.1	34.5	11.3	0	0
EGFLAM, 5p13.2	24.1	11.3	22.6	0
GSG1L, 16p12.1	48.3	9.4	3.8	0
SOX8, 16p13.3	34.5	43.4	0	0
TMEM176A/TMEM176B,7q36.1	27.6	64.2	47.2	0

The promoter regions of *ABCG4, AIFM3, KHSRP, MAFA, RXRA, TMEM200B* demonstrated high heterogeneity of methylation of CpG pairs and were not included in the analysis. It has been shown the statistically significant association between TMEM176A/176B methylation status and involvement of central nervous system in the pathological process in AML patients before treatment (p = ***0,0183***).
Differential methylation of these genes in the samples before and after treatment may be indicative of clonal evolution of malignant transformation.
Our study provides technical opportunities for profiling the epigenetic abnormalities for a given individual, promising the development of individualized approaches in the therapy of AML.

METHYLATION OF MATRIX AND TRANSMEMBRANE PROTEINS ENCODING GENES IN BREAST CANCER

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Background. Matrix and transmembrane proteins play fundamental role in cell's live regulation. They are involved in maintenance of tissue architecture, signals transduction, wound healing, adhesion and play significant role in tumor development.

Materials and methods. We evaluated epigenetic regulation of 12 laminin-encoding genes (*LAMA1, LAMA2, LAMA3A, LAMA3B, LAMA4, LAMA5, LAMB1, LAMB2, LAMB3, LAMC1, LAMC2, LAMC3*), 8 integrins (*ITGA1, ITGA2, ITGA3, ITGA4, ITGA6, ITGA7, ITGA9, ITGB1*), 2 nidogens (*NID1,NID2*), the dystroglycan gene *DAG1* and 10 matrix metalloproteinases-encoding genes (*MMP2, MMP14, MMP15, MMP16, MMP17, MMP21, MMP23B, MMP24, MMP25, MMP28*) and 4 genes of tissue inhibitors of metalloproteinases (*TIMP1*, *TIMP2*, *TIMP3*, *TIMP4*) in 186 samples of breast cancer, 186 paired adjacent nonmalignant samples and 6 samples of normal mammary gland from autopsy by methylation sensitive PCR and bisulfite sequencing.

Results. Promoters of the *LAMA3A, LAMB2, LAMB3, LAMC2, MMP14, MMP21, TIMP1, TIMP4* genes were constitutively methylated in breast tissues. Abnormal methylation frequencies at the promoter regions of 15 genes *LAMA1, LAMA2, LAMB1, LAMC1, ITGA1, ITGA4, ITGA7, ITGA9, NID1, NID2, MMP2, MMP23B, MMP24, MMP25, MMP28* in breast cancer accounted for 29,4%(50/170), 25,8%(48/186), 28,5%(51/179), 3,7%(7/186), 13,3%(20/150), 29,3%(44/150), 3,3%(5/150), 40,6%(61/150), 37,3%(56/150), 39,3%(59/150), 7,95%(14/176), 17,24%(30/174), 10%(15/150), 20,33%(24/118), 5,14%(9/175) respectively. In autopsy breast tissue samples these DNA regions were not methylated. In small number of cases genes *LAMA1, LAMA2, LAMB1, NID1, ITGA4, ITGA9* were methylated not only in BC, but in paired adjacent nonmalignant samples as well.
For some genes we found association of methylation with a number of clinicopathological features. So, the methylation of promoter regions of *LAMA2, LAMB1, ITGA4, ITGA9, NID1* and *MMP23B* genes was enriched in HER2 positive tumors, a highly aggressive form of breast cancer.

Conclusions. Complex alteration of the studied genes methylation can be important for understanding of the dramatic changes in tissue architecture and signal transduction during tumor growth and development.

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EXPRESSION OF P53 PROTEIN IN PRIMARY AND SECONDARY MALIGNANT LIVER TUMOURS

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Background Multiple genetic and epigenetic changes are involved in the molecular pathogenesis of primary malignant liver tumour – hepatocellular carcinoma. The relevant molecular pathways can include somatic mutations in the tumour suppressor gene *TP53* [1]. Since *TP53* is frequently mutated or inactivated in different cancer types, including colorectal, pancreatic and gastric cancer [2], the aberrant p53 protein is a highly attractive therapeutic target in oncology, hypothetically allowing comprehensive, yet still personalised approach. The success of targeting p53 by anti-cancer vaccination or other means is likely to depend on the frequency of pathological aberrations and availability of a predictive biomarker [3]. Thus, the aim of the present study was to evaluate p53 protein expression in hepatocellular carcinoma and selected representative secondary liver tumours including colorectal, pancreatic and gastric cancer metastases.

Materials and Methods In a retrospective study, 67 consecutive, morphologically confirmed cases of hepatocellular carcinoma (35 patients), as well as liver metastases of colorectal (15 cases), pancreatic (10 patients) and gastric (7 cases) carcinoma were included, based on the tissue availability in liver biopsy or resection material. p53 protein expression was evaluated by immunohistochemistry and assessed quantitatively by computer-assisted morphometry as the fraction (%) of positive neoplastic cells. Only intense nuclear reactivity was considered positive. Descriptive statistic evaluation was performed, including detection of 95% confidence interval (CI).

Results The hepatocellular carcinoma showed p53 expression in 16 (45.7%; CI = 30.5 – 61.8) cases. Among these, 11 (68.6%; CI = 44.4 – 85.8) tumours exhibited limited expression in only 30% or lower fraction of neoplastic cells. Regarding metastatic colorectal carcinoma, p53 expression was found in 12 (80.0%; CI= 54.8 – 93.0) cases, of which 8 (66.7%; CI = 39.0 – 86.2) had p53 levels higher than 80.0%. In pancreatic cancer, p53 expression was observed in 6 (60.0%; CI = 31.3 – 83.2) metastases, characterised by wide variations in the levels of p53 expression (25.0 – 100.0% positive cells). In metastatic gastric cancer, 4 (57.1%; CI = 25.0 – 84.2) cases showed positive p53 expression. The expression exceeded 20% of neoplastic cells in only 2 (50.0%; CI = 15.0 – 85.0) metastases.

Conclusions

1. The expression of aberrant p53 in hepatocellular carcinoma was limited both by frequency and by extent. Thus, p53 peptide vaccine, eliciting cytotoxic T lymphocyte immune response against p53 protein overexpressing cells, could be considered only within the frames of combined treatment for hepatocellular carcinoma.
2. There was a statistically insignificant trend to more frequent and widespread p53 expression in colorectal cancer. According to our data, vaccination against p53 protein could be recommended in case of metastatic colorectal cancer; however, further research is clearly needed.
3. Regarding gastric and pancreatic carcinomas, combined treatment should be considered again as expression of aberrant p53 protein was limited both by frequency and by extent.

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MUTATION AND GENE EXPRESSION ANALYSIS OF URINE SEDIMENTS IN NON-INVASIVE MOLECULAR GENETIC DIAGNOSTICS OF THE COMMON ONCOUROLOGICAL DISEASES

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Background. Prostate cancer (PCa) and bladder cancer (BC) are the most common oncourological diseases. The main method of laboratory diagnostics of prostate cancer is measurement of the level of prostate specific antigen (PSA) in the blood, but its concentration increase due to not only prostate cancer, but also inflammation or benign prostate hyperplasia (BPH). The sensitivity of urine cytology in BC patients is substantially limited in the first stage of the disease. In this regard, a search and characterization of new markers for non-invasive detection of PCa and BC are still actual.

The aim of our study is a comprehensive analysis of expression of *PCA3*, *TMPRSS2:ERG* genes and mutations *of FGFR3* gene in urine sediments as potential markers for common oncourological diseases.

Methods&Results. We have studied 29 urine sediments obtained from patients with PCa (10 samples), prostate adenoma / intraepithelial neoplasia - PIN - (16 samples) and prostatitis (3 samples). Expression of the *PCA3* and *TMPRSS2:ERG* genes was determined by real-time PCR with TaqMan-probes using the endogenous (*GAPDH*) and tissue-specific (*KLK3*) controls. *PCA3* overexpression was specific for PCa: diagnostic accuracy at the threshold ΔCt (PCA3-KLK3) equal to 0.98 was 84% for urine sediments. Expression of the chimeric oncogene *TMPRSS2:ERG* was detected in 50% of PCa and 17% of PIN. PCR and subsequent Sanger sequencing of the exons 7 and 10 of the *FGFR3* gene were applied to examine 16 samples of urine sediment from patients with BC, the control group consisted of 24 patients with cystitis and urolithiasis. Missense mutations were detected in 31% of BC cases: c.746C → G (p.S249C), c.1124A → G (p.Y375C), c.1144G → C (p.G382R) and 2 cases c.1156T → C (p.F386L).

Conclusion Thus, gene expression analysis and mutation detection in *PCA3, TMPRSS2:ERG* and *FGFR3* genes in urine sediments could be used as a component of non-invasive diagnostics of PCa and BC.

ABNORMAL METHYLATION OF MGMT, CDH1, CDKN2A, DAPK, RUNX3 AND RUR-B GENES IN PATIENTS WITH BARRETTS ESOPHAGUS BEFORE AND AFTER SURGICAL TREATMENT

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Introduction. Barrett's esophagus (BE) is a premalignant lesion that predisposes to esophageal adenocarcinoma (EAC). Risk of progression to EAC ranges up to 8% in patients with BE.

Materials and Methods. We examined DNA samples in endoscopic biopsies of patients with BE (60 patients: 32 intestinal metaplasia (IM) and 28 low-grade dysplasia (LGD)) taken from altered and non-altered esophageal epithelium before and after antireflux surgery. We also examined DNA samples of 34 patients with EAC. We performed endoscopic control and investigated methylation in long-term follow up (6-54 months). We divided patients in two groups - met+(>1 of 6 genes methylated in DNA sample) and met-(no methylation). Before surgery we observed abnormal methylation in 36/60(60%) patients with BE: 21/28(75%) with LGD, 15/32(46%) with IM and 22/34(64%) with EAC. Abnormal methylation was in 31/60(51%) cases in altered and in 5/60(8%) in normal epithelium.

Results. After operation 19/60 patients with BE (10/13 with IM and 5/5 with LGD) were in group met+. We found abnormal methylation in 15/60 cases in altered and in 4/60 in normal epithelium.

Conclusions. we showed reliable regression of methylation in investigated genes after surgery in patients with LDG from 21/60 to 5/60 (p=0,0007) and unreliable in IM patients from 15/60 to 10/60. We found reliable decreasing of methylated genes in altered epithelium from 31/60 before to 14/60 (p=0,0024) after surgery, but in cases of normal epithelium it was unreliable (5/60(8%) before and 4/60(6%) after). We suggest that patients with abnormal methylation in normal epithelium after operation are more likely for progression from BE to EAC, and need further surveillance.

THE IMPACT OF 11 LOW-PENETRANCE ALLELIC VARIANTS ON BREAST CANCER MORBIDITY IN POPULATION OF LATVIA

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Introduction. The impact of high-penetrance gene variants on breast cancer risk is widely described, nevertheless they account for only about 25% of the familial risk and less than 5% of total breast cancer predisposition [1]. It is suggested that remaining risk may result from a polygenic effect of common low-penetrance gene variants. There is a variable frequency and predisposing effect strength observed among low-penetrance variants associated with breast cancer development [2]. The aim of this study was to determine the association among 11 allelic variants and breast cancer morbidity in population of Latvia.

Methods. Genomic DNA from 2,609 breast cancer patients and gender-matched 694 healthy volunteer blood donors were analyzed in this study. Breast cancer patients with proven founder mutations in the *BRCA1* gene (exon 5 - c.181T>G, exon 11 - c.4035delA and 20. exon c.5266dupC) and/or positive family history of the disease, were excluded from the study group. Genotyping of 11 allelic variants was done by RFLP and TaqMan probes. Both analytical methods were verified using Sanger sequencing. The obtained data were processed in program R-3.1.2. (χ^2 test, likelihood ratio test, Mantel-Haenszel test, Kaplan-Meier analysis, Bonferroni correction).

Results. There was an association with breast cancer morbidity observed for seven out of eleven analyzed variants. Five of them: rs9693444 (OR 1.39; CI 1.13–1.71), rs1550623 (OR 1.40; CI 1.12–1.75), rs13329835 (OR 1.37; CI 1.12–1.67), rs3760982 (OR 1.35; CI 1.09–1.66), rs7072776 (OR 1.25; CI 1.03–1.50) showed a significant breast cancer risk-increasing effect, whereas rs17356907 (OR 0.73; CI 0.60–0.90) and rs1436904 (OR 0.86; CI 0.75–0.99) – protective effect. Rs132390, rs204247, rs616488 and rs11571833 showed no significant impact on breast cancer morbidity.
Rs13329835 (OR 1.69; CI 1.04–2.76) and rs3760982 (OR 1.87; CI 1.05–3.48) proved a stronger association with breast cancer risk among women under 40 years of age. Rs9693444 (OR 2.10; CI 1.45–3.04), rs17356907 (OR 0.56; CI 0.38–0.80), rs1550623 (OR 1.81; CI 1.20–2.80) and rs7072774 (OR 1.50; CI 1.06–2.14) showed a stronger effect among women aged 60 to 80 years.
We proved the polygenic effect of low-penetrance risk alleles - women who are carriers of at least three out of five risk alleles identified, has a significantly higher breast cancer risk than those with risk allele count less than three (OR = 1.49; CI = 1.17-1.91).
There was no significant impact of analyzed variants noticed on patients disease-specific survival rates.

Conclusions. Rs9693444, rs1550623, rs13329835, rs3760982, rs7072776 proved an association with increased breast cancer risk, whereas rs17356907 and rs1436904 has a protective effect. There is no significant impact of analyzed variants on patients survival. Breast cancer predisposition comprising of multiple low-penetrance allelic variants corresponds to the polygenic model.

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Monday November 21, 2016

MOLECULAR MEDICINE WORKSHOP

Belikov Sergey	Quantification of transcription factor--DNA binding affinity in a living cell	invited oral
Östlund Farrant AK	Differential response to malaria in two ethnic groups in Sub-Saharan Africa	invited oral
Manuylov Victor	Genetic and serological diversity of hepatitis B virus in Siberia, Russia	poster
Rhee Joon Haeng	Flagellin as a versatile adjuvant for vaccines against intractable diseases	invited oral
Shneider Alex	Anti-inflammatory gene therapy	invited oral
Westerberg Lisa	Understanding how Wiskott-Aldrich syndrome protein (WASp) stings in immune cell	invited oral

QUANTIFICATION OF TRANSCRIPTION FACTOR-DNA BINDING AFFINITY IN A LIVING CELL

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Background. Gene regulation involves a sequence-specific DNA binding by transcription factors (TFs). The modes of DNA binding of these TFs have been studied in detail *in vitro*. However, the estimation of the DNA binding affinity of a native TF to a defined DNA sequence *in vivo* in eukaryotes remains a challenge. In metazoans the organization of the DNA in chromatin is exploited to gain tissue-specific gene regulation from a common genome achieved by composite binding of different TFs to the regulatory DNA segments.

Materials & Methods. We decided to apply Xenopus oocyte system to address the DNA binding affinity for androgen receptor (AR) and glucocorticoid receptor (GR) in vivo. These gigantic oocytes allow protein(s) to be expressed at will by injection of corresponding *in vitro* transcribed mRNAs. The specific DNA binding sites, i.e. prostate-specific androgen responsive DNA element, can be introduced by intranuclear injection of circular single-stranded (ss) DNA, which in our case yielded ~0.3–3 × 10^9 gene copies. This injected DNA constitutes more than 99% of the total nuclear DNA since the oocyte nucleus contains only 37 pg of endogenous genomic DNA. Importantly, the introduction of ssDNA leads to second strand synthesis concomitantly with replication coupled chromatin assembly.

Results. The apparent dissociation constant (Kd) for specific binding of GR and AR to DNA was determined *in vivo*. The total nuclear receptor concentration was quantified as specifically retained [(3)H]-hormone in manually isolated oocyte nuclei. The fraction of DNA sites occupied by the expressed receptor was determined by dimethylsulphate *in vivo* footprinting and used for calculation of the receptor-DNA binding affinity. The forkhead transcription factor FoxA1 enhanced the DNA binding by GR with an apparent Kd of ~1 μM and dramatically stimulated DNA binding by AR with an apparent Kd of ~0.13 μM at a composite androgen responsive DNA element containing one FoxA1 binding site and one palindromic hormone receptor binding site known to bind one receptor homodimer. FoxA1 exerted a weak constitutive- and strongly cooperative DNA binding together with AR but had a less prominent effect with GR, the difference reflecting the licensing function of FoxA1 at this androgen responsive DNA element.

Conclusions. Using Xenopus oocytes *in vivo* system we can for the first time report the sequence-specific TF-DNA binding affinity, specifically the apparent Kd, in a living cell. Furthermore, we demonstrated a strong selectivity for AR over GR binding to a previously described androgen response element involved in prostate-specific gene expression in vivo. This selectivity relies on the arrangement of the binding site of pioneer factor FoxA1, a known master regulator of normal and cancer prostate development, in relation to the bound hormone receptor.

DIFFERENTIAL RESPONSE TO MALARIA IN TWO ETHNIC GROUPS IN SUB-SAHARAN AFRICA

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Background and Aims Malaria is caused by species of the parasite *Plasmodium*, the most severe one being *Plasmodium falciparum*, which is prevalent in Africa. The ethnic group Fulani exhibits less symptoms and clears the parasite much quicker than sympatric groups. The underlying mechanism is not known, and several investigations into differences in immune response, cytokines, and in set-up have been performed but have not come to any conclusive results.

Results and Conclusions We have taken a different approach and have studied expression patterns and DNA-methylation patterns between Fulani and the neighbouring ethnic group Mossi. Both CD14+ (monocytes) and CD14- (T-cells and B-cells) were isolated, but only between the CD14+ from infected individual from Fulani and CD14+ from non-infected individuals from Fulani could differential expressed genes be detected.

GENETIC AND SEROLOGICAL DIVERSITY OF HEPATITIS B VIRUS IN SIBERIA, RUSSIA

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Background Hepatitis B is a global health problem. In Russia, despite of the existing activities for common vaccination, the officially reported incidence of the acute hepatitis B remained at the level of 1,1 cases per 100 000 of population in 2015 whereas the incidence of newly reported cases of chronic and asymptomatic hepatitis B was 24,7 / 100 000. General prevalence of hepatitis B virus (HBV) carriers in Russia is estimated at the level of 2-4% of population by different authors that means 3-6 millions of infected persons. HBV is a highly variable DNA-virus that is classified genetically at 10 major genotypes and at least 34 subgenotypes that complicates its molecular diagnostics and is significant for proper clinical treatment and outcome. Moreover, HBV has at least 9 main serotypes (subtypes) of its surface antigen – HBsAg, and may affect the efficacy of the immunization by recombinant vaccines and the sensitivity of the ELISA tests. Thus, the geographical distribution of the genotypes and HBsAg subtypes has an important epidemiological significance.

Methods&Results In this study we investigated genetic and serological variability of HBV among multiple groups of native population of Siberia: Kazakhs, Altaians, Teleuts and Tyva at the South-West of Siberia; Nenets, Komi, Khants and Selkups (Yamalo-Nenetsky Autonomous Region, YNAR), Dolgans and Nganasans (Taimyr Peninsula) and Kets in the Northern Siberia; Buryats, Russian (rural), Yakuts and Chikchi at the East of Siberia. It was shown that these groups differ statistically by the prevalence of HBsAg-positive persons with levels range from 2-4% of population of YNAR and Russians to 10-13% in Altaians, Teleutes, Tyva, Taimyr, Yakuts and Chukchi.
Total 340 HBV DNA sequences (Pre-S/S genome region) were studied. Most of isolates in the total group belonged to the genotype D (82%), although genotypes A (5%) and C (13%) were also found. Prevalence of the main subgenotypes of the genotype D – D1, D2 and D3 and HBsAg subtypes were dissimilar in different groups of native populations: D1 (mainly HBsAg subtype ayw2) prevailed in the groups of

Kazakhs (89%), Russians (88%), Teleutes (100%), joint group of Taimyr (72%) and Tuva (100%); D2 (subtype ayw3) – in Yakuts (50%) and group of YNAR (48,5%); D3 (ayw2) – in Altaians (76%), Buryats (40%) and Chukchi (51%). Subgenotype A2 (adw2) was common for Yakuts (22%), while C1 (adrq+) often occurred among Chukchi (27%) and Taimyr peoples (19%).

Conclusions Discovery of genotype C and adrq+ subtype (which are endemic for the Southeastern Asia and previously were not reported as a common HBV types in Russia) at the far North and East of Siberia is an important result since the clinical outcome for the genotype C is quite different comparing to the genotype D that is the most prevalent genotype in Russia. The other important result was that statistical analysis of the obtained data has shown that the studied groups were epidemiologically isolated from each other and may be considered as local epidemiological reservoirs with circulation of different HBV variants. These results should be noted by the specialists in the regional medical care services.

FLAGELLIN AS A VERSATILE ADJUVANT FOR VACCINES AGAINST INTRACTABLE DISEASES

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Background: TLR ligands are considered attractive adjuvants for vaccines and immunotherapy. Flagellin is the cognate ligand for Toll-like receptor 5 (TLR5) of host cells. TLR stimulation leads to activation of innate immunity and subsequently modulates adaptive immune responses.

Aim: In this presentation, we show that flagellin has a unique immunomodulating activity in the mucosal immune compartment.

Methods and Results: (1) Flagellin c ould be used as an adjuvant for mucosal vaccines. Mucosal co-administration of a *V. vulnificus* flagellin (FlaB) with microbial antigens induced significantly enhanced antigen-specific IgA responses in both mucosal and systemic compartments and IgG responses in the systemic compartment. Intranasally administered FlaB colocalized with CD11c as patches in DCs and caused an increase in the number of TLR5 expressing cells in draining lymph nodes. Further, we tested whether FlaB could serve as an effective mucosal adjuvant for an inactivated trivalent influenza vaccine (TIV), *Streptococcus pneumonia* antigen PspA, and Norovirus P domain antigens. In those vaccine formulations, flagellin exerted excellent adjuvanticity in combination with antigens. **(2) Flagellin could serve an effi cacious adjuvant for vaccines and imm unotherapies against noninfectious intractable diseases in t he mucosal compartments such as allergic asthma and cervical cancer.** We found that therapeutic doses of flagellin together with allergens suppress allergic asthma by inhibiting pathogenic $T_{h1}/T_{h2}/T_{h17}$ responses while generating regulatory DCs (DC_{reg}) and T_{reg} cells. Adoptive transfer of FlaB/allergen mixture-induced DCs effectively inhibited asthma. In the peripheral blood from allegic asthma patients, FlaB treatment induced DC_{reg}, which subsequently induced allergen-specific Foxp3^{+} T_{reg} cells in a lymphocyte co-culture while inhibiting T_{h1}/T_{h2} responses in an IL-10-dependent manner. For cervical cancer, we examined whether flagellin can be used as an adjuvant for topical therapeutic cancer vaccine in a genital cancer model. Intravaginal co-administration of E6/E7 peptides with flagellin resulted in tumor suppression and long-term survival of the tumor bearing mice. IVAG immunization of E6/E7 peptide with flagellin induced accumulation of CD4+ or CD8+ cells and T cell activation in draining genital lymph nodes (gLNs). The co-administered flagellin elicited antigen-specific IFN-γ production in gLNs and spleen. The IVAG administered flagellin co-localzed with CD11c+ cells in the gLN T cell areas and enhanced TLR5 expression.

Conclusion: Flagellin serves a versatile agent for various intractable infectious and non-infectious diseases.

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TWINNING ON DNA-BASED
CANCER VACCINES

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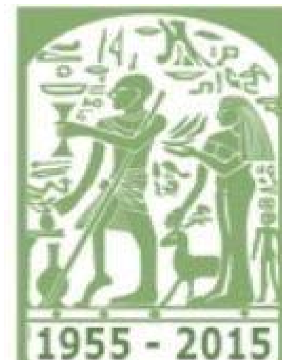

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