VACTRAIN

TWINNING ON DNA-BASED CANCER VACCINES

This project has received funding from the European Union's Horizon 2020 Research and innovation programme under grant agreement No 692293

TWINNING ON DNA-BASED CANCER VACCINES

RĪGA STRADIŅŠ UNIVERSITY

KAROLINSKA INSTITUTET ANNO 1810 Karolinska Institutet

VERITAS ET LIBERTAS UNIVERSITAS LODZIENSIS University of LODZ

OECI Organisation of European Cancer Institutes

R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology

http://vactrain.lv/

This project has received funding from the European Union's Horizon 2020 Research and innovation programme under grant agreement No 692293

TWINNING ON DNA-BASED CANCER VACCINES

The Coordinator:

Rīga Stradiņš University, Latvia

Project partners:

Department of Microbiology, Tumor and Cell Biology (MTC) at Karolinska Institutet, Sweden

Department of General Biophysics, University of Lodz, Poland

R.E.Kavetsky Institute of Experimental Pathology, Oncology and radiobiology of National Academy of Sciences of Ukraine, Ukraine

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PROJECT OBJECTIVE:

to significantly strengthen the research in Riga Stradiņš University in the smart specialization area of Latvia: biomedicine, medical technologies, and biotechnologies, with application to immunotherapy of cancer.

PROJECT TASKS:

Train essential steps of research aimed to deliver new anti-cancer remedies based on the novel promising vaccine platform employing naked DNA:

- Defining the target(s) of cancer vaccine,
- Designing respective immunotherapeutics,
- Construction and characterization,
- Delivery to vaccine recipients
- Testing of activity in preclinical trials
- Connecting to a clinical trial.

PROJECT ACTIVITIES:

- Group trainings and experimental laboratory courses
- Expert visits, short- and long-term on-site trainings
- Conferences, workshops, schools
- Training presentation on conferences and via publications
- Dissemination and outreaching activities
- Coordination of trainings, staff exchange, project management

Project acronym: VACTRAIN

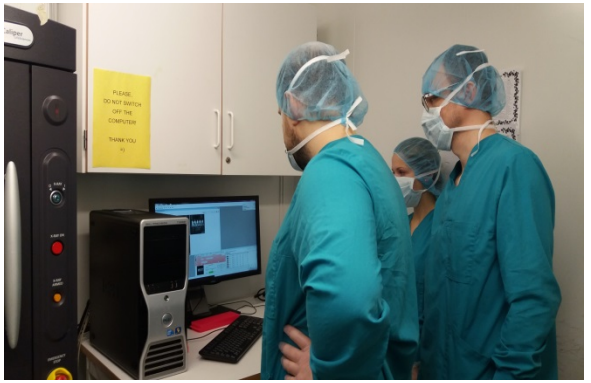
Grant Agreement No 692293

Contribution of EC : 999 475 EUR

Duration : 01.01.2016. – 31.12.2018.

Coordinator – Assoc. Prof. Maria Issagouliantis

Maria.Issagouliantis@rsu.lv



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RĪGA STRADIŅŠ UNIVERSITY

RSU is an advanced institution of higher medical education in Latvia and also one of the leading in Europe. RSU offers several levels of education, starting from Bachelor and Master lines up to PhD Programs in all fields of medicine. RSU has an excellent teacher-student ratio with approximately 6500 students to 400 academic staff members. 21% students are international, with 89 in ERASMUS out-going, and 56 in ERASMUS in-going projects. The majority of students are enrolled in the faculties of Medicine, Law, Rehabilitation and Public Health and Social Welfare. Another key RSU activity is the continuation of education and professional development programs for the medical doctors and healthcare practitioners. There are five doctorate study programs operating at RSU – medicine, pharmacy, sociology, law, political science and seven doctorate councils: with bases in medical science, internal medicine, surgery, medicinal biomechanics, dentistry, pharmacy, and sociology. RSU hosts the 2nd level professional education program "Residency in Medicine" in the form of full-time 3 to 6 year studies, depending on the chosen program or specialty. The teaching personnel are the country's leading specialists with remarkable experience and broad cooperation with their colleagues in many European countries. Teaching is offered in the recent theories of disease pathogenesis, modern methods of early diagnostics and modern principles of complex treatment.

Rīga Stradiņš University holds a unique place in Latvia's scientific field, providing a full research cycle from laboratory to hospital bed. This applies particularly to such research fields of public importance as oncology, infections, pediatrics, rehabilitation and dentistry. RSU is a leading academic research institution in the fields of medicine, pharmacy, dentistry, rehabilitation and nursing sciences. Scientific activity is centered around 62 professors and 30 leading researchers. RSU processes are ISO 9001:2008 certified (Bureay Veritas). Due to this, RSU possesses a managerial capacity required by complex projects.

Since 2008, Rīga Stradiņš University research activities have been organized within the following RSU priority research directions: Ageing: biological ageing factors and quality of life in Latvian population; Structural biology, functional and biomechanical studies for new diagnostic tools and new therapeutic interventions; Endogenous and exogenous risk factors in Latvia, comprehensive analysis of public health determinants; Modern approaches to infection agents, their role in infections relevant for Latvia; Mortality and disability factors during childhood; Immunochemical, radiological and clinical methods in research on dentofacial and maxillofacial deformities and anomalies; Clinical epidemiology of functional limitations caused by health disturbances; Social dimension of medicine, effects of globalization. One specific field is engulfing the topic of current project: research on the clinical and molecular characteristics of cancer and their advancement for prevention, early diagnosis and improvement of treatment strategies. Project is coordinated by the Research Department of RSU.

RD/RSU coordinates, monitors and supports research activities under the direct jurisdiction of the Vice-Rector for Science. It is responsible for the development of university science. Department supports the effective research activity strategies and the implementation of RSU science policies. An ever-increasing attention is paid to the transfer of knowledge and technologies by integrating knowledge in the basic functions of the University.

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Karolinska Institutet

KI/MTC focuses the research on immunology, infection biology and cell and tumor biology. The research interests are centered on the genetics and molecular biology of virus, bacteria and parasite replication, the role of gene products in abnormal cell growth, the use of microbes as probes for the study of signal transduction, transcription, translation, cell cycle regulation, cell differentiation, and cell death. Specific research areas include the biochemical mechanisms of cell growth control, transformation, signal transduction, and transcriptional regulation, the molecular genetics, molecular biology and molecular pathogenesis of latent, persistent and cytolytic infections, the characterization of receptor interactions and the mechanisms of cell entry, the interaction with cells involved in innate and adaptive immune responses, the pathogenesis of infection and rational drug design. Research at MTC is carried out in several scientific areas, this generates a diversified environment where cross-fertilization between different fields has the potential to generate novel and groundbreaking concepts. Specific area in focus with the current proposal is Cancer, and Tumor Biology. It consolidates the research on the basic mechanisms causing tumors and tumor progression, host responses to tumor, genetic factors predisposing to or on contrary preventing development, protein products of these factors, such as oncogenes, cell cycle regulating genes, signal transduction genes, growth factors, apoptosis and cell aging controls, and suppressor genes and susceptibility genes. This area also includes studies on genetic cancer susceptibility and familiar cancers, related molecular epidemiology, on the interplay between malignant and normal cells, cell adhesion, angiogenesis, metastasis formation, aspects on tumor regression, and research on the relationship between infections and tumor development, as well as related bioinformatics and biome complexity.

The application of this knowledge in the development on experimental treatments, such as gene and immunotherapy, and preventive measures is also included in this area of research. MTC offers several levels of education. Today MTC offers Third cycle (doctoral) education programs in the research areas, including Immunobiology, Infection Biology, Cell Biology, Tumor Biology, Vascular biology, Infectious Diseases and Microbiology. It provides a dynamic research environment for approximately 100 or so postgraduate students engaged in many research activities. All students are registered for a PhD in Medical Science. MTC also leads bachelor student education in Biomedicine including basic immunology covering morphology and general functions of the immune cells, proteins and organs of the immune system; maturation, interactions and regulation of innate and adaptive immune responses. It includes also clinically orientated immunology covering autoimmunity, allergy and transplantation immunology, morphology, taxonomy, genetics and metabolism of bacteria.

Current project is driven by a laboratory at MTC working on genetic vaccines against HIV and cancer. The laboratory conveys studies and educational activities in the field of the design, preclinical and clinical testing of DNA vaccines.

Genetic vaccines have generated wide-spread interest for many applications, since DNA encoding a microbial or tumor-specific gene results in in vivo expression of the desired gene, protein production and presentation as an endogenous foreign antigen. Since then, the laboratory has tested two genetic CEA vaccines (wild type and mutated) and one glycoprotein vaccine in patients who are radically operated against colorectal cancer, with a primary surgical finding of Dukes B or C, diagnoses that confer around 50% risk for progression within 2-5 years. The laboratory also develops new tools for *in vivo* evaluation of passive-active vaccination efficiency

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University of LODZ

The University of Lodz (LU) is one of the strongest scientific and research centers in the country. The University is repeatedly ranked among the top higher education institutions in Poland, and it especially excels in such fields of study as management, biology, law and economics . The 12 faculties of the University provide programs in 40 fields of study and 170 specializations for more than 40,000 students. LU has gained experience and reputation as a reliable project coordinator and project partner, as well as a strong international science and research center.

The Faculty of Biology and Environmental Protection is one of the strongest faculties of LU. Research carried on at the faculty is of pure, applied and methodological character. The scientific problems studied by the research staff focus on three main themes: biological environment, structure and functions of plant, animal and microorganism cells, and biomedical and biotechnological topics. Scientific schools created by faculty professors include among others: biochemical, biophysical, zoological, ecological, anthropological, neurophysiological, microbiological and immunological.

Researchers at the department of general biophysics have been studying a novel group of polymers called dendrimers for over 15 years, being actively present in this field from the very beginning. The main areas of interest are: (i) application of dendrimers in medicine, especially as carriers of anti-cancer drugs and antiamyloid agents; (ii) interaction of dendrimers with various molecules like proteins, nucleic acids, membranes; (iii) biophysical characterization of complexes of dendrimers with biomolecules (dendriplexes); (iv) *in vitro* toxicity studies of dendrimers and dendriplexes. Members of the Department are authors of over 280 scientific papers published in peer reviewed journals.

Main research topics of the Department:

- Interactions of various dendrimers (PAMAM, PPI, PPI modified with sugar residues, viologen-phosphorus, phosphorus, carbosilane) with biomolecules: nucleic acids, proteins, model and biological membranes;
- Characterization of formed dendriplexes, their physico-chemical properties, morphology, size, zeta potential and stability in time, temperature, pH;
- Toxicity of dendrimers and dendriplexes against various cell lines and blood cells. Applications of dendrimers in medicine, especially as: carriers of anticancer drugs, factors influencing the process of fibril formation in neurodegenerative diseases and carriers of antisense oligonucleotides and siRNAs (HIV infection, cancer).

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TWINNING ON DNA-BASED CANCER VACCINES

R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology

KIEPOR of National Academy of Science of Ukraine (NASU) is a strong academic center with more than 50 years of experience in the field of experimental and clinical oncology. The main topic of research at KIEPOR is a work on biology of tumor cell and the tumor microenvironment, and a study on molecular mechanisms of oncogenesis with the aim to develop personalized medicine. Another aspect of the work is an investigation of molecular features of metabolic regulation in cancer with the aim to develop the biotechnological approaches and sorption-based technologies for the anti-cancer treatment. Yet another field of research is to find out the molecular and cellular markers of the initiation, promotion and progression of tumors, and create panels of markers for the early diagnostics and prognosis of the course of disease. Among important directions of research are studies on the influence of potentially carcinogenic environmental factors (chemical carcinogens, ionizing and non-ionizing radiation) on carcinogenesis and development of the agents that can counteract these factors. Under the umbrella of translational medicine, researchers are working on innovations in diagnostics and anti-cancer treatment, including the development of anti-cancer vaccine.

The Scientific Council and the Specialized Dissertation Advisory Council (D.26.155.01) at KIEPOR has the right to grant degrees of Doctor of Science (Dr Sci, Dr Hab) and PhD in Medical and Biological Sciences with the specialty 14.01.07 (Oncology). The international journals "Experimental Oncology" (founded in 1979) and "Oncology" (founded in 1999) are issued by KIEPOR. At KIEPOR, the bank of cell lines is created that consists of more than 35 000 cell cultures. This bank has the status of the National Heritage of Ukraine since 2001. KIEPOR is a member of the Organization of European Cancer Institutes. Leading researchers of KIEPOR are members of numerous international organizations, such as American Society of Clinical Oncology, the British Association for Cancer Research, the European Association for Cancer Research European Society For Medical Oncology, European Society for Hyperthermic Oncology, International Network For Cancer Treatment and Research, European Society Gynaecologic of Oncology, and EBV-association. KIEPOR has hosted numerous scientific forums.

The Ukrainian team leads research in the field of the molecular and cell biology of cancer, with the aim to reveal molecular mechanisms of oncogenesis. Team members in collaboration with Prof G Klein, Karolinska Institutet, demonstrated that the human mitochondrial ribosomal protein MRPS18-2 (S18-2) could bind RB1 protein, preventing association of RB1 with E2F1 and promoting the S-phase (Snopok et al., 2006; Kashuba et al., 2008). Overexpression of S18-2 protein led to immortalization of primary rat embryonic fibroblasts and induced expression of embryonic stem cell markers (Kashuba et al., 2009). They have shown up-regulation of pathways characteristic for rapidly proliferating cells, in S18-2 immortalized cells overexpressing the protein. This and elevated expression of S18-2 in stem cells indicate that it can be a promising target for anti-cancer vaccine. Researchers from KIEPOR also look for other new markers for early diagnostics and/or prognosis of the course of cancer disease.

The Twinning project gives Ukrainian partners an opportunity to widen the expertise of our group towards the field of the immunotherapy of cancer. In the result of the Twinning project the members of Ukrainian team will get the possibility to extent their research network, take part in the trainings, and exchange their expertise during workshops held together with Twinning partners.

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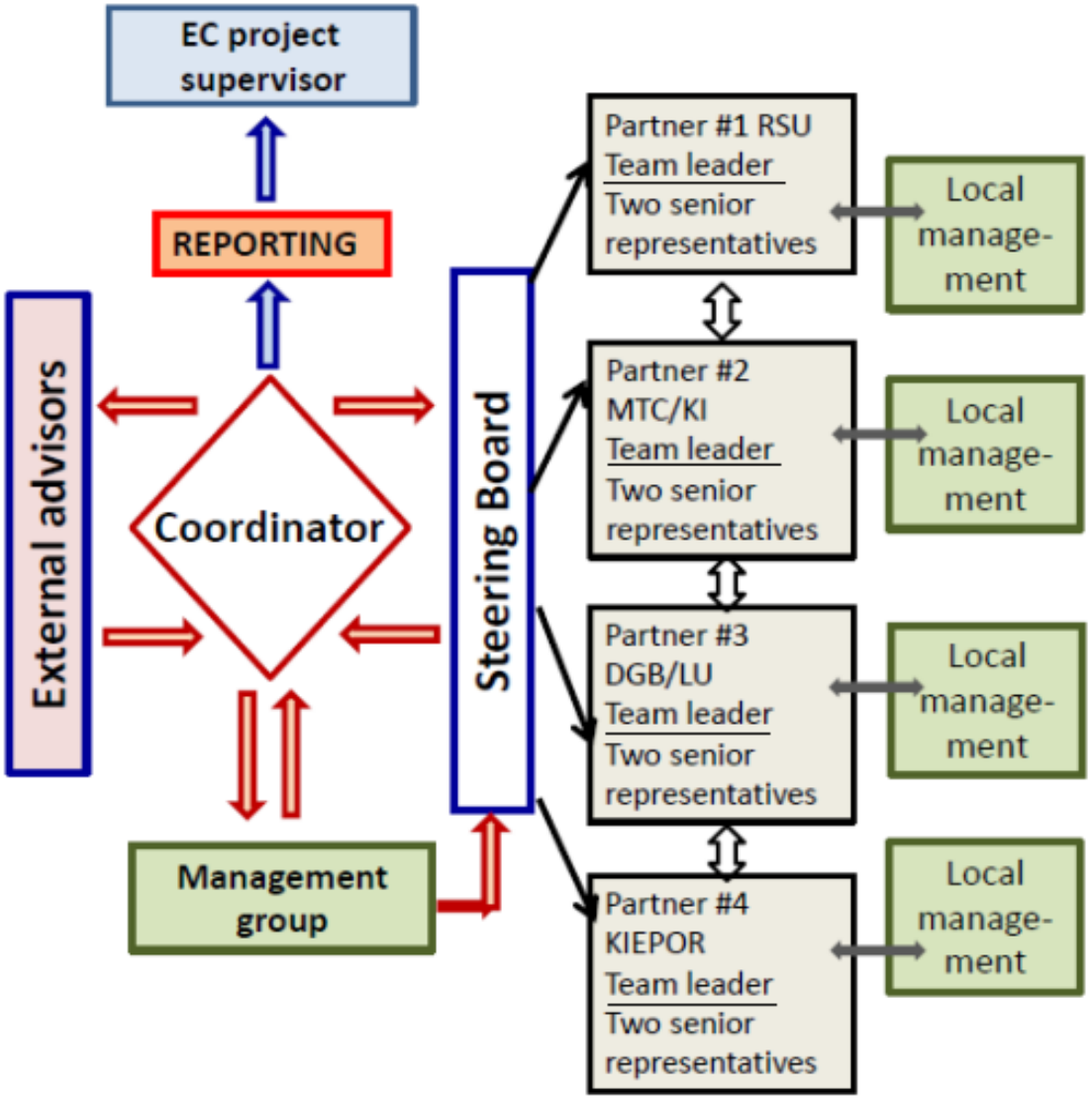

Steering of the Consortium

The overall management of VACTRAIN will be run by the **Steering Board**. Steering Board is formed by co-opting the team leader and one senior team member (reserve) with complementary research and educational experience apt to jointly make strategy-forming decisions. Steering Board meets every 6 month. Intermediate meetings are held monthly as Skype conferences.

The steering Board tasks are to:

- ✓ Make key decisions on the work to be performed within the work packages and will serve as an ultimate decision making body in case of any conflict or dispute between the partners;
- ✓ Prepare, update and manage the agreement among the partners, approve changes in the partnership and composition of partner teams;
- ✓ Ensure smooth interaction between partner teams from team leaders to PhD students, to achieve fluent knowledge transfer;
- ✓ Coordinate innovation-related activities, intellectual property related issues and their commercialization.

Day-to-day management of the project will be done by the project Manager, Ms Asja Lunga.

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Steering Board Members:

1. Coordinator Assoc Prof Maria Issagouliantis, Rīga Stradiņš University, Latvia

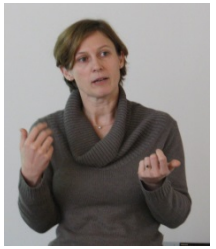
2. Prof Uldis Berkis, Rīga Stradiņš University, Latvia

3. Prof Francesca Chiodi, Department of Microbiology, Tumor and Cell Biology (MTC), Karolinska Institutet, Sweden

4. Prof Maria Bryszewska, Department of General Biophysics, University of Lodz, Poland

5. Assoc Prof Elena Kasuba, R.E.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of National Academy of Sciences of Ukraine, Ukraine

Advisors:



Dr Birke Bartosch, Cancer Center, Lyon, France

Prof Manuel Patarroyo, Karolinska Institutet, Stockholm, Sweden

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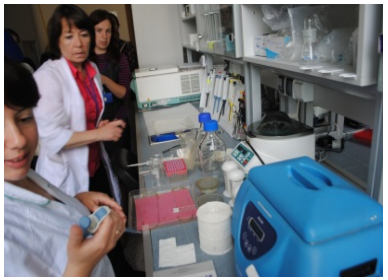
Group Research Training

"GENE EXPRESSION" Course, June 14-15, 2016, KIEPOR, Kyiv, Ukraine

COACHES:

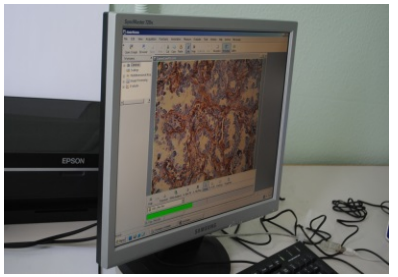
Researchers from the MTC/Karolinska Institutet and University of Lodz



TRAINEES:

From Riga Stradins University, Riga, Latvia:
Dzeina Mezale, MSi, PhD student;
Lelde Kalnina MSi, PhD student;
Monta Ustinova, MSi, PhD student.

From IEPOR of NASU, Ukraine:
Glushchenko N, MSci;
Lozovska Yu, MSci;
Lykhova O, MSci;
Paliichuk O, MSci;
Pavlova A, PhD student;
Storchay D, PhD student ;
Yurchenko N, Msci.



AIMS:

By demonstration and active participation in training, trainings aim to ease up the incorporation of modern innovative biomedicine and biotechnology methods into the on-going research projects, share experience, and transfer critical know-how to increase the research capacities of Latvian and Ukrainian researchers of all levels.

TRAINING FORM:

The training was organized as presentations of the methods in the form of a conference, and a series of experimental/wet trainings on several modern research techniques used to detect gene/protein expression, namely: quantitative real-time polymerase chain reaction; single cell gel electrophoresis assay; fluorescence activated cell sorting; and immunohistochemistry.

CONTENT OF THE WET LABS

Quantitative real-time PCR (q-PCR) Twenty six people were trained in two groups, due to the high interest from public. Teachers: Larysa Kovalevska, PhD (IEPOR of NASU, UA) and Muhammad Mushtaq, PhD student (MTC, KI, SE).

Fluorescence activated cell sorting (FACS) Twenty one persons have signed for this wet lab, therefore two rounds of training were performed. Teachers: Yulia Shvets, PhD (Taras Shevchenko National University of Kyiv, UA) and Daria Storchay, PhD student (IEPOR of NASU, UA).

Method of immunohistochemistry Due to the high number of participants (20 individuals), method was trained in two groups, 10 people each. Teachers: Lilia Gumenyuk, PhD and Natalia Iurchenko (both from IEPOR of NASU, UA).

Single Cell Gel Electrophoresis assay (SCGE) Training was performed for 15 persons. Teachers: Olga Bryeyeva, PhD student and Dmytro Demash, PhD (both from IEPOR of NASU, UA).

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Karolinska Institutet

Group Research Training

"IN VIVO GENE DELIVERY", November 16 - December 22, 2016, MTC/Karolinska Institutet, Stockholm, Sweden

COACHES

Ilya Gordeichuk, PostDoc
Stefan Petkov (MTC/KI);
Anastasia Latanova, assisting MSi

TRAINEES Researchers from Riga Stradins Universitet

Martins Kalis (researcher, PhD; 5 weeks)
Dace Skrastina (researcher, PhD; one week)
Laura Hippe (lab assistant, Master student; 5 weeks)
Juris Jansons (researcher, PhD; 2 weeks)
Maria Issagouliantis (Researcher, PhD, 4 weeks)

TRAINING WAS FOCUSED ON

DNA delivery skills: Trainees participated in DNA injection in mice, which was done by staff in concordance with ethical permits. They also learned to operate a CUY21EditII electroporator used for *in vivo* cell transfection.

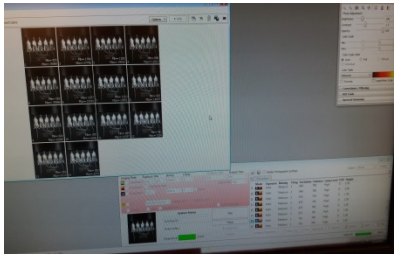
***In vivo* imaging:** Participants were trained in detection of the fluorescent signal emitted by iRFP670, which was expressed in murine skin. After the signal was acquired the trainees were taught methods of signal analysis and visualization using the Living Image software suite by Perkin Elmer.

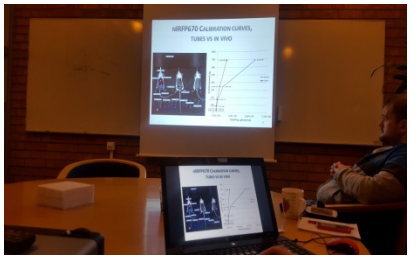
Immune tests by FACS and Fluorospot: Training of methods for detection of immune response against the reporter. Screening of cellular responses was performed by IFNγ ELISpot,IFNγ/IL-2 Fluorospot and intracellular flow cytometry.

Education & presentation skills: Trainees delivered oral and poster presentations during a conference on DNA immunization. The training continues during weeks 3-5 with weekly seminars.

RESULTS AND OUTCOME

- Mastering of the technique of *in vivo* DNA transfection by electroporation;
- Mastering of the technique of *in vivo* fluorescent imaging and processing of imaging data;
- Training of cellular immune assays;
- Improving data digestion and data communication/reporting techniques by repeated seminar;
- Obtaining certificates confirming mastering of *in vivo* imaging and flow cytometry techniques





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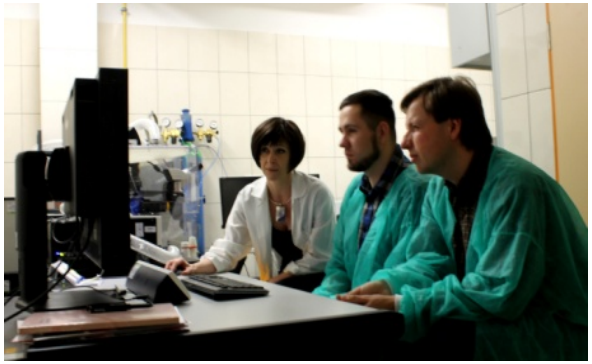
Uniwersytet ŁÓDZKI

Group Research Training

"DENDRIMERS & SMALL MOLECULE APPLICATIONS"

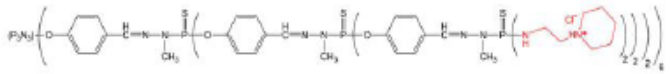
February 7 - May 28, 2017, Department of General Biophysics, University of Lodz, Poland



LEARNING OBJECTIVES

Part 1: Formation of complexes between plasmid DNA and different kinds of cationic dendrimers; Mastering methods of complex characterisation.

Part 2: Application of complexes for transfer of nucleic acids into cells in cell culture; Mastering methods of assessing the efficacy of transfection in vitro: Expression of bioluminescent reporters/luminometer; Detection of fluorescence from near-infrared reporterd/confocal microscopy; Assessment of cytotoxicity of the complexes/MTT test



TRAINEES & TIME FRAME

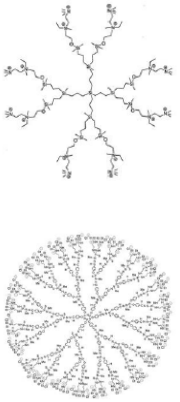
Part I Time Frame: February 07-25, 2017.

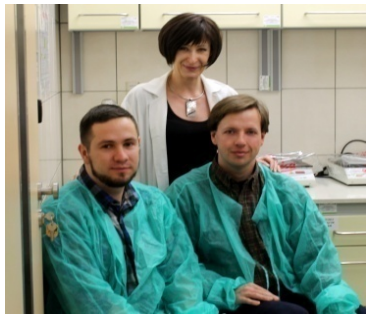
TRAINEES: Researches from KavetskyInstitute of the Experimental Pathology, Oncology and Radiobiology, Kyiv, Ukraine(KIEPOR); Dr. Dmytro Demash (researcher, Ph.D. 2.5 weeks), Mgr. Taras Zadvornyi (researcher, Ph.D. student, 2.5 weeks)

Part II Time Frame: May 08-28, 2017.

TRAINEES: Researches from KavetskyInstitute of the Experimental Pathology, Oncology and Radiobiology, Kyiv, Ukraine(KIEPOR); Mgr. Storchai Dariia (researcher, Ph.D. student, 3weeks); Mgr. Taras Zadvornyi (researcher, Ph.D. student, 3 weeks); Mgr. Borikun Tetiana (researcher, Ph.D. student, 3weeks)

COACHES: Researchers from the DGB/UL , University of Lodz Maksim Ionov, Sylwia Michlewska (DGB/UL)





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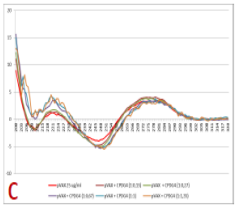
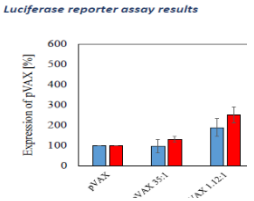
TWINNING ON DNA-BASED CANCER VACCINES

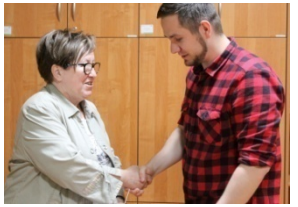
Uniwersytet ŁÓDZKI

RESULTS Part I

Trainees tested the formation of different dendrimer-DNA complexes and characterized formed nanosystems using circular dichroism and agarose gel electrophoresis methods. The ratios (w/w) between complexes components have been established. The seminars were organized allowing trainees to present and discuss the results of the trainings, overviw mastered methods and their applications. The trainees examination in the end of part I was performed to verify the level of the skills obtained during the training.

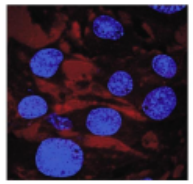




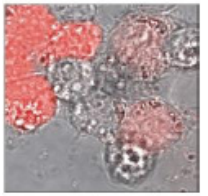


RESULTS Part II

Training included the experimental "wet" and theoretical "dry" parts. During wet part trainees obtained methodological skills in the field of nanobiophysics. To characterize manosystems formed on the base of dendrimers as potential anticancer gene material. Using the fluorescence technique the binding properties of dendrimers were analyzed. By the dynamic light scattering method the surface potential and size of dendriplexes were evaluated. Dry part introduced trainees to a paraply of theoretical aspects and the analysis of publshed literature. PART II was completed by examination.



OUTCOME New skills were leant. KIEPOR and RSU teams got introduced to the biophysical complexation techniques and approaches to the formation of stable dendrimer-based nanosystems considered as powerful delivery vehicles for drugs, also in anti-cancer therapy. Training helped to creat links between DGB/LU, KIEPOR and RSU institutions and upraise the research profile of the KIEPOR staff through the interaction with the leading partners in the field of bionanotechnology.



VACTRAIN
TWINNING ON DNA-BASED CANCER VACCINES
This project has received funding from the European Union's Horizon 2020 Research and innovation programme under grant agreement No 692293

TWINNING ON DNA-BASED CANCER VACCINES

RĪGA STRADIŅŠ UNIVERSITY

Group Research Training

"DENDRIMERS & SMALL MOLECULE APPLICATIONS, *ex vivo* STAGE", October 26 - November 14, 2017

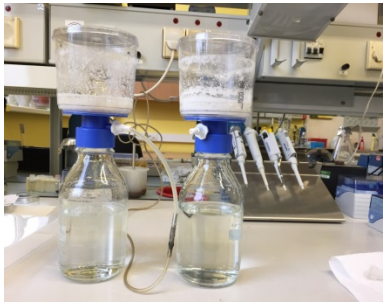
Riga Stradins University, and Latvian Biomedical Research and Study Center

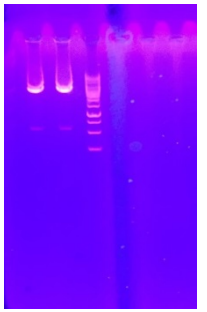
TRAINEES

Monta Ustinova (RSU/LBMC, Latvia)
Dzeina Mezhale (RSU, Latvia)
Anita Berzina (RSU, Latvia)
MSc Sylwia Michlewska (LU, Poland)
Dr Volha Dzmitruk (Institute of Biophysics and Cell Engineering, Minsk, Belarus)

COACHES

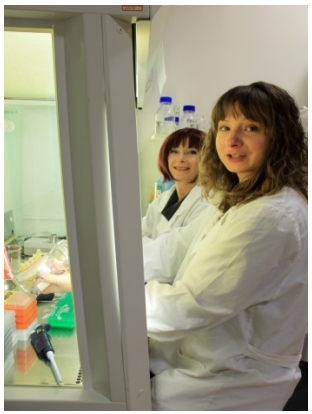
Dr Juris Jansons (RSU/LBMC, Latvia)
Dr Dace Skrastina (RSU/LBMC, Latvia)
Dr Maksim Ionov (LU, Poland)





SUMMARY

Dendrimers, highly branched star-shaped macromolecules with nanometer-scale dimensions, are powerful tool for delivery of drugs and genetic material into the cells. Different types of dendrimers were studied as transfection agents for the plasmid expressing near-infrared fluorescent reporter protein iRFP670 (kind gift of Prof Vlad Verkhusha, USA). The complexes of carrier dendrimers and reporter plasmids were analyzed by agarose gel electrophoresis and by photon correlation spectrometer Malvern Zeta-Sizer. The toxicity of the dendrimer/DNA complexes was evaluated by MTT cell viability assay. The ability of the dendrimer/DNA complexes to permeate the cell membrane was tested on the eukaryotic cell lines HEK293/T17 (human embryo kidney), SK-Mel-28 (human skin malignant melanoma) and MCF-7 (human breast adenocarcinoma) in comparison with the commercial transfection reagents. The number of cells expressing reporter gene iRFP670 was detected by flow cytometry.



Together with INNVOIMMUNE project of the Swedish Institute.

LEARNING OUTCOMES:

- ✓ The theoretical and practical knowledge of the capacity of dendrimers to serve as transfection agents in cell lines and in vivo;
- ✓ Plasmid DNA purification;
- ✓ Agarose gel electrophoresis ;
- ✓ Practice with Malvern Zeta-Sizer machine to determine size of the complexes;
- ✓ Training in the work with cell cultures: Determination of cell viability Techniques of cell transfection;
- ✓ Training in work with flow cytometer.

VACTRAIN
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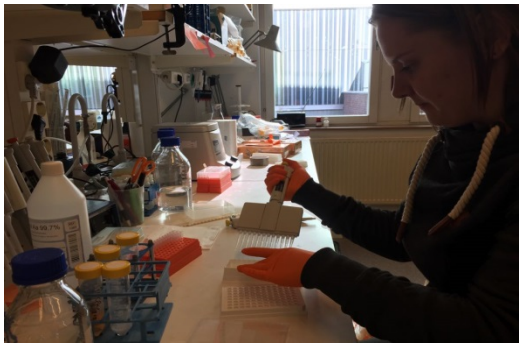
Karolinska Institutet

Group Research Training

"SCREENING IMMUNE RESPONSE, ANTI-CANCER EFFICACY ", March 1-31, 2018, MTC/Karolinska Institutet, Stockholm, Sweden



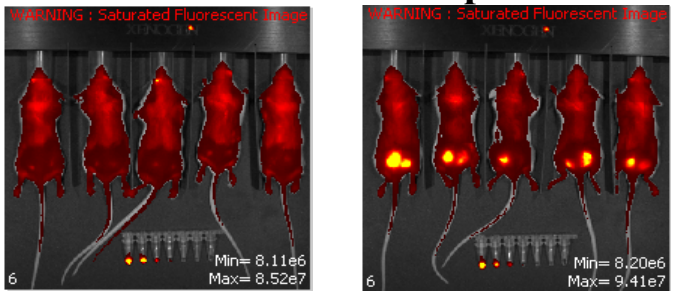
TRAINEES Ilze Fridrihsone (RSU; MD, PhD student; 4 weeks); Dr Volha Dzmitriuk (Institute of Biophysics) and Cell Engineering, Minsk, Belarus, associated through the Swedish Institute project).

COACHES Dr Stefan Petkov, Francesca Chiodi (Karolinska Institutet); Dr Maria Issagouliantis (RSU, and Karolinska Institutet); Dr Maksim Ionov, (Lodz University).

AIMS Train delivery of plasmid DNA by "golden standard" method by injection and electroporation compared to chemical transfection, and assess resulting immune response to encoded protein.

METHODS Training used plasmid DNA encoding near-infrared reporter protein iRFP670 (kindly provided by Prof Vald Verkhusha, Albert Einstein University, USA). Plasmid DNA, obtained by cultivation of E coli, was purified from lyzed bacterial cells using Quigene column chromatography. Complexes of DNA with dendrimers were formed using protocols during Riga training 2017. Complexes were injected into BALB/c mice, and near-infrered signal resulting from expression of iRFP670 was assessed by imaging (Spectrum). Control mice received injections of plasmid DNA followed by electroporation. At experimental end-point, splenes were processed to obtain splenocytes. Cellular immune response to iRFP was assessed by IFN-g ELISpot.

Group 1 **Group 2**



Detection of fluorescence in positions of injections of plasmid encoding infrared reporter protein iRFP670 in mice receiving injection of iRFP670 encoding plasmid piRFP670 complexed to Generation IV dendrimer (group 1) versus mice receiving piRFP670 injection followed by electroporation (group 2). Ilze Fridrihzone, Maksim Ionov and Stefan Petkov (unpublished).

Together with INNVOIMMUNE project of the Swedish Institute.

OVERALL RESULTS

Trainees obtained theoretical and practical knowledge on DNA delivery using intradermal injection and electroporation, visualization using Caliper IVIS device with quantification techniques using Perkin Elmer Living Image software 4.5.2, and insights into ELISA and ELISpot. *In vivo* results confirmed observations made in cell lines. Most efficient delivery of plasmid DNA encoding iRFP670 was supported by electroporation, followed by DNA complexed to Generation III dendrimers. DNA complexed to dendrimers of generation IV was was not delivered. Introduction of iRFP670 DNA induced no anti-iRFP67 immune response. This demonstrated the applicability of iRFP670 as a reporter of tumor growth in immuno-competent mice.

VACTRAIN
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Group Research Training

First summer school "INNOVATIVE VACCINES AND VACCINATIONS ", May 11-12, 2017

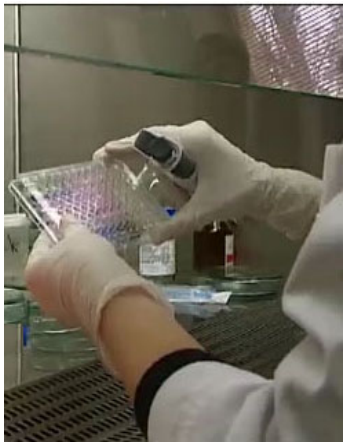
Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

The summer school was attended by the RSU trainees Monta Ustinova, Lelde Kalnina and Dzeina Mezale and also by Olga Dmitruk, a young researcher from Belarus (associated with Lodz University). Participants from the Ukrainian side were young researchers from KIEPOR, and students and PhD students of the Bogomolets National Medical University (Kyiv), Vinnyzja Medical University, Palladin IBC of NASU, and IMBG of NASU.

Coaches at the Summer school were staff members of KIEPOR and also by Bogomolets Medical University of Kyiv.

Professor Grigory Potebnja and Dr. Olga Karaman (KIEPOR) introduced trainees to two animal models of human cancer established at KIEPOR – undifferentiated Guerin carcinoma in rats and also Lewis lung carcinoma in mice.

These models were used to preclinically test the efficacy of tumor vaccines developed at KIEPOR, produced from autologous tumor cells and lectins of *Bacillus subtilis B-7025*



Trainees have learnt about clinical trials of the phase I-III that were performed at KIEPOR and certified for clinical use in Ukraine. Tumor vaccines were applied for patients with colorectal, lung, stomach, breast, kidney and ovarian cancer, and also malignant brain tumors.

More information on the tumor vaccines developed by KIEPOR was made available by a short video made by Ukrainian TV channel ICTV https://www.youtube.com/watch?v=yfs5bq5QGYQ&feature=youtu.be

Young researchers trained in presenting and discussing their data em with the specialists in different fields of oncology, vaccinology and molecular medicine. This allowed them to gain new skills in both analysis and presentation of their data and promoted establishment of collaborations young scientists promoting their carrier development.

Dzeina Mezale and Monta Ustinova (RSU)

VACTRAIN
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Group Research Training

Second summer school "FUNDAMENTAL PRINCIPLES OF CANCER BIOTHERAPY" May 23, 2018

Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

A summer school "Fundamental principles of cancer biotherapy" was organized at KIEPOR on May, 23, 2018. This event was addressed to young researchers at a PhD and Master student levels. The main aim was to train young motivated researchers in methods of modern biomedicine, to enable their active participation in the immunotherapy task force.

Julita Pietrzak , Agnieszka Robaszkiewicz (Lodz), Džeina Mežale, Ilze Fridrihsone (Riga) with Dr Elena Kashuba (KIEPOR, Kyiv)

This summer school was attended by 37 participants. Two researchers were from Lodz' University, Poland (Julita Pietrzak and Agnieszka Robaszkiewicz), two – from RSU, Riga, Latvia (Džeina Mežale and Ilze Fridrihsone). School was attended by 33 representatives of the Ukrainian universities, and research institutes.

Due to many students, they were divided in two groups. Each group took part in all 4 activities (see an enclosed program). One group was taught in English, and one – in Ukrainian

MAIN TOPICS OF THE TRAINING

- Quantitative PCR using BioRad machine 7500;
- Introduction into immunohistochemistry;
- FACS analysis of patient peripheral blood samples;
- Immuno-fluorescent microscopy.

Dmytro Shaposhka and students in the CSD Health care lab

VACTRAIN
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TWINNING ON DNA-BASED CANCER VACCINES

RĪGA STRADIŅŠ UNIVERSITY

Group Training with external experts

Workshop "OPENINGS FOR CANCER IMMUNOTHERAPY" with training in data presentation and grant application writing,

May 28 – 30, 2018, Riga, Latvia

14.00	**MAY 28, 2018 RSU, Dzirciema iela 16, D- 512** Opening
14.00 – 14.20	PROGRESS OF VACTRAIN PROJECT Dr. Maria Issagouliantis, VACTRAIN coordinator Rīga Stradiņš University, Latvia, and MTC, Karolinska Institutet, Stockholm, Sweden
14.20 - 15.10	MURINE MODELS OF HUMAN CANCER IN VACCINE TRIALS Dr. Maria Issagouliantis, Rīga Stradiņš University, Latvia, and MTC, Karolinska Institutet, Stockholm, Sweden
15.30 - 16.00	BURDEN OF LIVER METASTASES IN MODIFIED MURINE BREAST CARCINOMA Dzeina Mezale, Rīga Stradiņš University, Riga, Latvia
16.00 - 16.30	BIOLUMINESCENT IMAGING – OPENINGS FOR OPTIMIZATION OF DNA VACCINE DELIVERY Juris Jansons, Rīga Stradiņš University, and BMC, Riga, Latvia

Tuesday, MAY 29, 2018 RSU, Dzirciema iela 16, D- 512	
11.00 - 11.30	NANOPARTICLE-MACROPHAGE INTERACTIONS IN VITRO: SAFETY, MECHANISMS AND IMPLICATIONS IN NANOMEDICINE. Dr Olesja Bondarenko, National Institute of Chemical Physics and Biophysics, Laboratory of Environmental Toxicology, Tallinn, Estonia
11.30 – 12.30	Room B203: Training in research project design – round table and demonstration. Discussants: Maria Isaguliants, Javier Sánchez-Nieves Fernández, Juris Jansons, Olesja Bondarenko, Maxim Abakumov Trainees: Juris Jansons, Anita Berzina, Laura Hippe, Dzeina Mezale, Ilze Fridrihsone, Monta Ustinova, Martins Kalis.

Tuesday, MAY 30, 2018 RSU, Dzirciema iela 16, K-201	
10.30 – 11.00	MAGNETIC NANOPARTICLES IN TUMOR THERAPY AND DIAGNOSTICS Dr Maxim Abakumov, Pirogov University Moscow, Russia
11.00 – 11.30	GOLD NANOPARTICLES FOR NUCLEIC ACID DELIVERY Dr Javier Sánchez-Nieves Fernández, Organic and Inorganic Chemistry Dpt, Alcala University, Alcalá de Henares, Spain
11.30 – 12.30	Room K-201. Training in research project design - write your project draft. Trainees work in small groups on three projects.
13.30 – 14.00	TARGETING ANTIGEN PRESENTING CELLS IN GLIOBLASTOMA AND INFLAMMATORY DISEASES BY DIAGNOSTIC NANOGOLD- AND NANODIAMOND miRNA CONSTRUCTS Prof. Dr. Marion Schneider , Sektion Experimentelle Anästhesiologie, Universitätsklinikum, Ulm, Germany
14.00 – 15.00	Room K-201. Training in research project design - write your project draft. Trainees work in small groups on three projects.
15.30 - 16.30	Training in research project design – project presentations by training participants (3 presentations; 20 min each)

VACTRAIN
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Karolinska Institutet

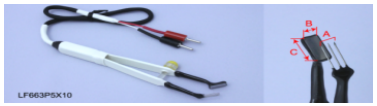
Individual Research Trainings

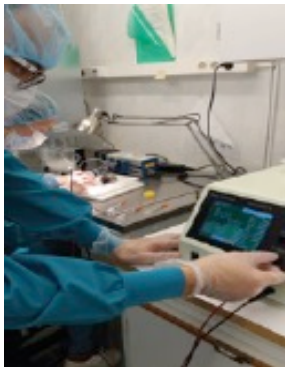
"DNA IMMUNIZATION AND FOLLOW UP OF IMMUNE RESPONSE IN MICE BY DIRECT IMMUNE AND INDIRECT BIOLUMINESCENCE-BASED METHODS"

May 26-August 5, 2016, MTC/Karolinska Institutet, Stockholm, Sweden

COACHES

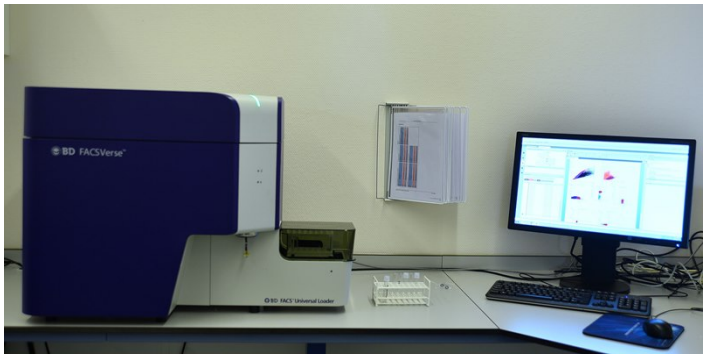
Stefan Petkov, (MTC/KI);
Elizaveta Starodubova (MTC/KI);
assisting, MSi Anastasia Latanova





TRAINEES

Juris Jansons, PhD (RSU),
Anda Vilmane, MSi (RSU).



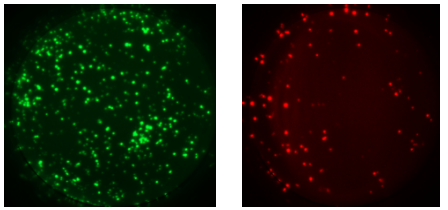
AIM OF THE TRAINING

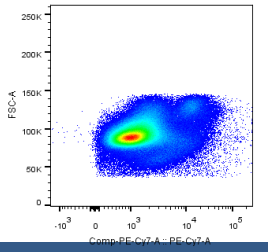
To master DNA immunization technique with injections and electroporation, and methodologies of screening of immune responses. Using these technologies to make a comparative assessment of immunogenicity of nine plasmid immunogens.

To understand the methods of DNA immunization, *in vivo* bioluminescent imaging (BLI) as a way to control DNA delivery and indirectly monitor the immune response development, and by the end of the tests, also actual methods of monitoring of the immune response, such as ELISpot, IFN-g/IL-2 Fluorospot, indirect ELISA and multi-parametric flow cytometry were trained.

RESULTS AND OUTCOMES

- Mastering of the technique of *in vivo* DNA transfection by electroporation.
- Mastering of the technique of *in vivo* bioluminescent imaging and processing of imaging data.
- Supplementary: first round of training of immune assays (all under supervision).





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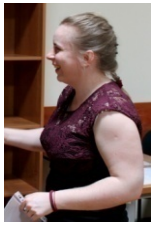
Uniwersytet ŁÓDZKI

Individual Research Trainings

"DENDRIMERS & SMALL MOLECULE APPLICATIONS" 1st round:

May 22- June 10, 2017 Department of General Biophysics, University of Lodz, Poland (DGB/LU)

TRAINEES Researcher from Riga Stradins University, Latvia, Riga (RSU) Anita Berzina (3 weeks); Researcher from Kavetsky Institute of the Experimental Pathology, Oncology and Radiobiology, Kyiv, Ukraine (KIEPOR) Mgr. Borikun Tetiana , Ph.D. student (3 weeks)



COACHES Researchers from the DGB, University of Lodz.

AIMS

By the presentation of new technologies in the field of *in vitro* transfection teach Latvian and Ukrainian trainees the ways of analyzing the nanobiomolecules internalization parameters. Training consisted of tests *in vitro* in cell lines to define which nanoparticle-packed pVax-Luc and iRFP670 reporter plasmid complexes provide the best reporter expression in cell culture.

METHODS Complex formation. Cells transfection. Confocal Microscopy. Reporter protein expression

OVERALL RESULTS

During this part of the training (third) the trainees from Latvia (RSU) and Ukraine (KIEPOR) have learnt new *in vitro* methods aimed to conduct the transfection capacity of the selected nanoparticles to transfect different types of cells. Two cell line models were used in this study, N2a, mouse neuroblastoma and mHippoE-18, embryonic mouse hippocampal cell line. Moreower participants of training have studied the cell cytotoxicity methods like MTT test. On the base of results obtained the CBD dendrimer have been suggested as potential carrier for delivering anticancer gene material into the cancer cells. Training was followed by public seminar with the presence of Ph.D. and Master students of Lodz university and participants from Latvia, Ukraine, Poland, Kazakhstan, Russia, India, and Belarus. The training certificates were handed at the end of the training.

VACTRAIN
TWINNING ON DNA-BASED CANCER VACCINES
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Karolinska Institutet

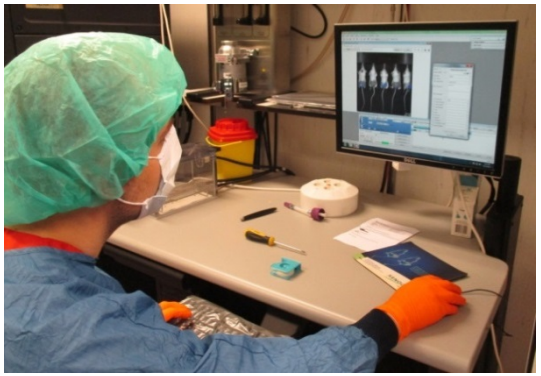
Individual Research Trainings

"DENDRIMERS & SMALL MOLECULE APPLICATIONS" 2nd round:

June 10 – July 9, 2017 MTC/Karolinska Institutet, Stockholm, Sweden

TRAINEES Researcher from Riga Stradins University, Latvia, Riga (RSU) Anita Berzina (M.Sc. Student, 4 weeks);
From INNVOIMMUNE project: Dr Maxim Abakumov, Pirogov Medical Research University, Moscow; Philip Podshwadt, Master student (Ulm University)

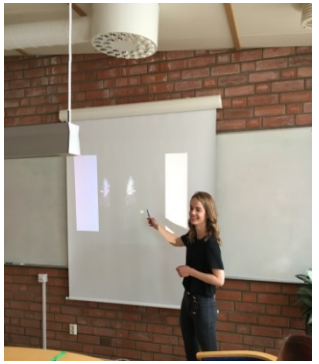
COACHES
Lodz University – Maksim Ionov, lector;
Karolinska Institutet – Stefan Petkov, research assistant; Ilya Gordeychuk, PostDoc.

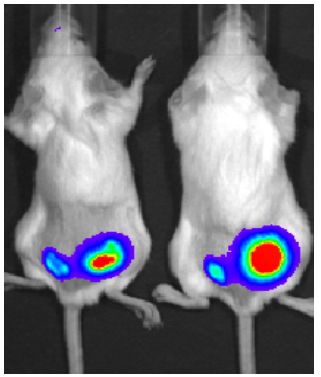


AIMS
By the presentation of new technologies in the field of *in vitro* transfection teach Latvian and Ukrainian trainees the ways of analyzing the nanobiomolecules internalization parameters. Training consisted of tests *in vitro* in cell lines to define which nanoparticle-packed pVax-Luc and iRFP670 reporter plasmid complexes provide the best reporter expression in cell culture.

METHODS
Methods of introduction of plasmid DNA into mice. Monitoring efficacy of reporter expression in vivo. Luminescence imaging and quantification.

OVERALL RESULTS
Dendrimers are noncytotoxic and can be used for DNA introduction into cells *in vivo* and *in vitro*. The transfection efficacy is lower than the transfection by lipofection or electroporation. Increase of transfection efficacy requires further optimization of DNA-dendrimer formulations. Data will be presented by Anita Berzina and Maksim Ionov on the international conference "Vaccines & Vaccination" in Moscow www.onlineregru/VAC&VAC2017 .





Together with INNVOIMMUNE project of the Swedish Institute.

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TWINNING ON DNA-BASED CANCER VACCINES

Karolinska Institutet

Individual Research Trainings

"TUMOR CHALLENGE" 1st round, June 26 - July 27, 2017

MTC/Karolinska Institutet, Stockholm, Sweden

Weekly seminars on which trainees presented and discussed their results.

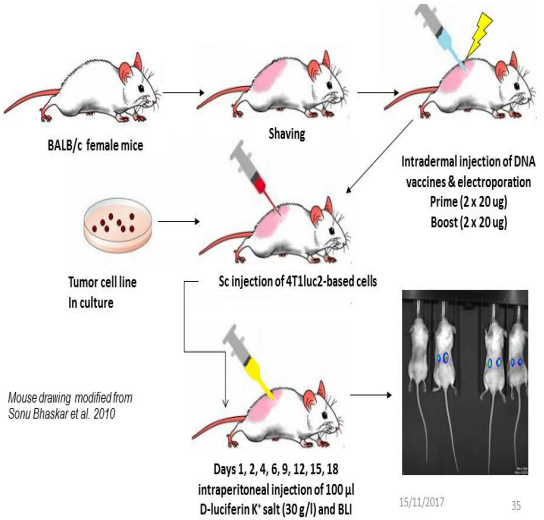
TRAINEES Researchers from Riga Stradins University, Latvia, Riga (RSU) Dzeina Mezale , MD, PhD student, VACTRAIN trainee; and Dr Juris Jansons.
From INNVOIMMUNE project: Ekaterina Pankova, Philip Podshwadt (Master students)

COACHES Prof Britta Wahren, MSc Stefan Petkov, Dr Ilya Gordeychuk, Dr Mohammad Mushtaq.
Assisting: Mina Saleem, Urszula Rykaszewska

ELEMENTS TO TRAIN

1.DNA immunization and follow up of expression of introduced gene by *in vivo* imaging (1 week);
2.Implantation of tumor cells and follow up of tumor growth by morphometric measurements and in vivo imaging (2-3 weeks);
3.Euthanasia of mice, organ collection, fixation of tissues, preparation of paraffin blocks, sectioning, histochemical and immune histochemical staining (2-3 weeks;)
4.From spleens, isolation of splenocytes, characteristics of cell viability, staining of cell surface receptors, intracellular staining for cytokine production; same procedures ofter thawing of frozen splenocyte; operation of flow cytometer, collection and digestion of data (2 weeks).
5.From spleens and purified splenocytes, characteristics of specific recognition of antigens by ELISpot and mono- and di-cytokine production by Fluorospot (1 week).

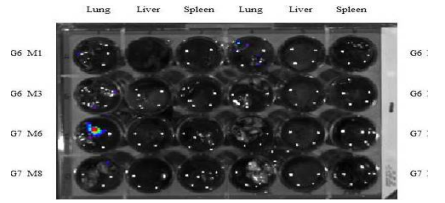
AIM Induction of resistance to tumors by DNA-immunization against tumor-expressed foreign antigens



Tumor challenge experiment in laboratory mice.
Such experiments are needed to define the protective and curative potential of DNA vaccines against virus-induced cancer.

OVERALL RESULTS

Trainees leant the methods for experimental challenge of small animals with tumor cells, methods of optical, physical assessment of tumor growth, assessment of tumor growth by in vivo imaging, tried different approaches to quantify metastases, and practiced methods of histological assessment of tumor tissues (Elements 1-3).



The team developed a rapid *ex vivo* assay of metastatic cells after spontaneous challenge of mice with Luc-expressing tumor cells . Method was used to characterize metastatic activity of cells used in the training.

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TWINNING ON DNA-BASED CANCER VACCINES

RĪGA STRADIŅŠ UNIVERSITY

Individual Research Trainings

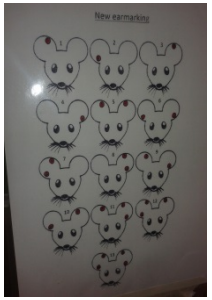
"TUMOR CHALLENGE" 2nd round, April 23 –July 28, 2018

Riga Stradins University, Riga, Latvia

TRAINEES Researchers from Riga Stradins University, Riga (RSU) Dzeina Mezale , MD, PhD student; Ilze Fridrihzone, MD, PhD student; new trainee Alica Kurlanda, Bachelor student at the Latvian University.

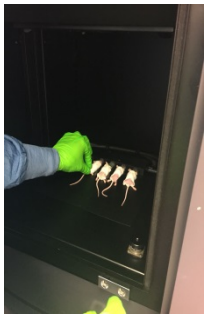
COACHES MSc Stefan Petkov (MTC/KI); Dr Ilya Gordeychuk (MTC/KI); Prof Ilze Strumfa (RSU)
Assisting in animal exeperiments: Dr Dace Skrastina (RSU/LBMC, Riga); Dr Maxim Abakumov (Pirogov Medical University, Moscow)

In this training, partner MTC/Karolinska Institutet, helped RSU team to perform locally a typical experiment on DNA-immunization of laboratory mice with plasmids encoding viral proteins, with subsequent challenge of immunized animals with tumor cells expressing these proteins . Training required >2 month preparations.



1. Stefan Petkov prepared tumor cells for transfer to RSU.

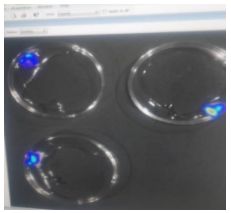
2. Mice were purchased, and accommodated at the animal facility of LBMC, Riga, by May 2018 equipped with bioluminescent imager Spectum CT. Prior to experiment, mice were divided into groups, and labelled.



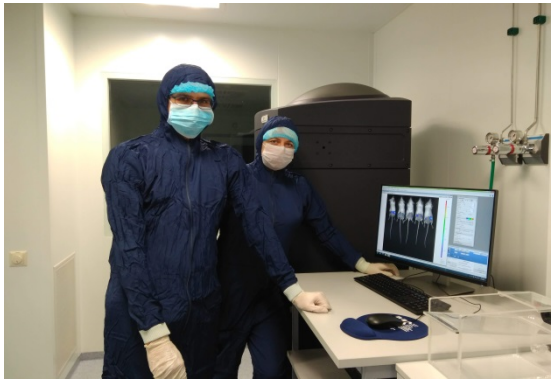
3. Mice were DNA immunized with plasmid encoding antigen expressed by tumor cells by two injections done with one month interval. Control animals were left untreated. Immunizations were done during expert visits of S Petkov to RSU, Riga.

4. Actual tumor challenge started May 31, 2018. Tumor growth was followed every 2-3 days by in vivo imaging.

5. After 3 weeks, mice were sacrificed, tumors excised, imaged and subjected to immuno-histochemical evaluation.



6.Splenocytes were purified and frozen for futher immune analysis



Dr D Skrastina and MD Dzeina Mezale monitor tumor growth in mice

OVERALL RESULTS

Trainees successfully practiced methods of DNA immunization; of experimental challenge of mice with tumor cells; methods of assessment of tumor growth; different approaches to quantification of metastases; and were trained in histological assessment of tumor tissues (Elements 1-3). Training of immune methods on frozen cells would follow in September-October 2018.

With INNVOIMMUNE project of the Swedish Institute.

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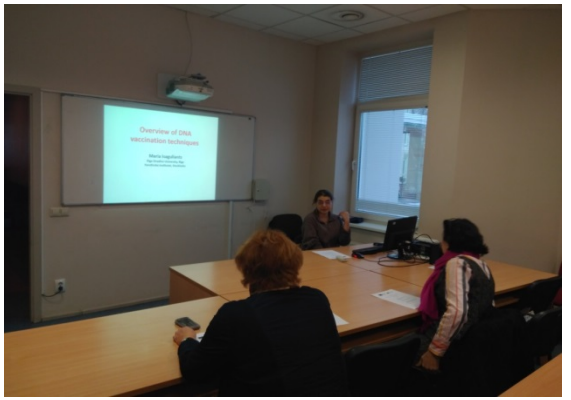
Dissemination of project results- Public Reports



Seminar "DNA VACCINATION PROGRESS IN VACTRAIN 2017" Riga Stradins University, Riga, December 20, 2017

- ✓ **Dr Maria Issagouliantis** (RSU) Overview of DNA vaccination techniques (project development and interactions)
- ✓ **Dr Juris Janosns (RSU, and LBMC, Riga)** Dendrimers as DNA delivery agents (results of expert visits and trainings in Latvia, autumn 2017
- ✓ **Dzeina Mezale (RSU)** Assessment of tumorigenic and metastatic potential of HIV expressing murine adenocarcinoma cells (results of training at Karolinska Institutet, summer 2017).

Workshop "VACTRAIN PROGRESS" Riga Stradins University, Riga, February 16, 2018

- ✓ **Margaret Liu**, ProTherImmune, University of San Francisco, USA) Cancer Vaccines and Immunotherapeutics: Challenges and Prospects
- ✓ **Maksim Ionov** (University of Lodz, Poland) Dendrimers for in vitro and in vivo delivery of oligo- and polynucleotides
- ✓ **Ilya Gordeychuk** (Chumakov Federal Center for Research and Development of Immune and Biological Products, Russia) Marmoset model for testing efficacy of human vaccines.
- ✓ **Maria Issagouliantis**, (Riga Stradiņš University, Latvia) Murine tumor models for testing efficacy of HIV vaccines
- ✓ **Džeina Mežale** (Riga Stradiņš University, Latvia) Liver metastases of HIV-expressing murine adenocarcinoma cells, and training report
- ✓ Perseption of VACTRAIN trainings by the trainees
- ✓ **Anita Bērziņa, Laura Hippe, Juris Jansons, Mārtiņš Kālis, Dace Skrastiņa, Monta Ustinova, Lelde Kalniņa**

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Dissemination of project results- Expert seminars

"Chemically driven transfections of eukaryotic cells in culture" Riga Stradins University, Riga, November 10, 2017

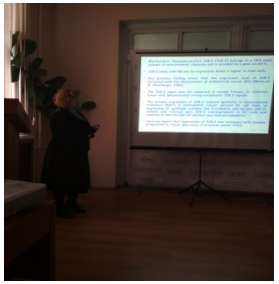
- ✓ **Dr Maksim Ionov (Lodz University, Lodz, Poland**) lectured in the design and applications of dendrimers for the delivery of nanomedicines.
- ✓ **Dr Volha Dzmitriuk (Institute of Biophysics and Cell Engineering NASB, Minsk, Belarus)** presented results of the training on delivery of DNA into cultured cells using dendrimers of III and IV generations.

"Present and future of genetic vaccines, can they cure cancer?" KIEPOR, Kyiv, Ukraine, March 29-30, 2018

Seminar addressed the principles of design and testing of novel tumor vaccines, and was attended by 27 participants.

- ✓ **Dr Sergey Belikov (the Wenner Gren Insitute, Stockholm, Sweden**) presented the state of art on DNA research, including chromatin structure, regulation of transcription and molecular determinants of gene expression efficacy.
- ✓ **Dr Maria Issagouliantis (RSU, Riga, Latvia)** gave a lecture on the development of DNA vaccines. with examples of successful DNA vaccines in veterinarian applications.
- ✓ **Dr Maria Yurchenko (Norwegian University of Science and Technology, Trondheim, Norway)** told about her latest work on Toll-like receptors and macrophages, as the first innate round of body reaction to vaccination.

- ✓ **Dr Elena Kashuba (KIPEOR)** presented the latest data on the role of MRPS18-2 in immune response, showing, that overexpression of this protein makes such cells a better target for the immune system.
- ✓ **Dr Olga Karaman (KIEPOR)** lectured on the application of lectin from *B. subtillis* as for cancer vaccines adjuvant
- ✓ **Dr Nataliya Fedosova (KIEPOR)** described the immunological parameters that should be measured upon vaccination, to assess the usability of the particular cancer vaccine.
- ✓ **Dr Gennadiy Didenko (KIEPOR)** presented his latest data on development of xenogenic vaccine, with the use of embryonic chicken proteins on mice.



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Dissemination of project results – *Internal Scientific Events*

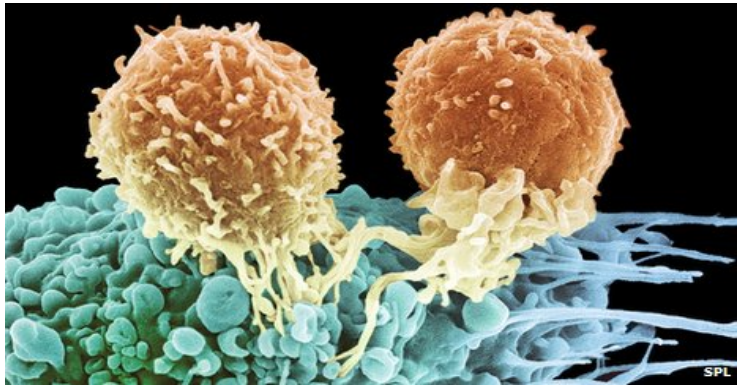
Symposium "TARGETS OF IMMUNOTHERAPY OF CHRONIC VIRAL INFECTIONS AND CANCER"

Rīga Stradiņš University, Riga, Latvia

May 24-26, 2016

Main Topics

TARGETS OF IMMUNOTHERAPY OF CHRONIC VIRAL INFECTIONS
EXPRESSION OF p53 IN HUMAN CANCER
INVASIVE PROPERTIES AND PROGNOSIS OF CANCER
OVERVIEW OF THE CANCER FIELD
TARGETS OF CANCER VACCINES
CANCER VIROTHERAPY
DESIGN AND DELIVERY OF VACCINES AND BIOPHARMACEUTICALS



http://vactrain.lv/events_TARGET_program.html

http://vactrain.lv/TARGET_SYMPOSIUM_ABSTRACT_BOOK_ed.pdf

http://vactrain.lv/events_TARGET_presentations.html

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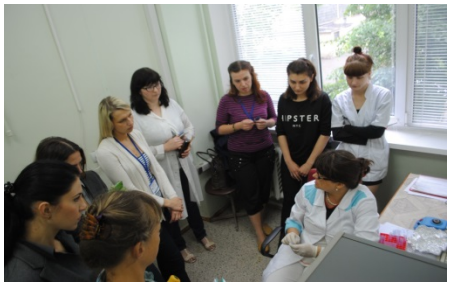
Dissemination of project results - *Internal Scientific Events*

Symposium "INTEGRAL CLINICAL AND PATHOGENETIC APPROACHES IN DIAGNOSIS AND THERAPY OF CANCER" KIEPOR, Kyiv, Ukraine, June 13-15, 2016

The focus of the conference was a search for the new fundamental, translational and clinical approaches in cancer research, prevention, diagnosis and treatment. At the conference we have learned a lot of new data presented by researchers from many countries, namely Ukraine, Sweden, Latvia, Lithuania, Poland and Belarus.

The Director of IEPOR, Academician Vasyl Chekhun gave an overview on the research that was and is performed at IEPOR, and also the future directions important to combat the cancer diseases. He discussed the concept of cancer stem cells, the importance of the multimeric interactions between cancer cells and normal cells, and microenvironment. Academician V. Chekhun highlighted that the research on stem cells were initiated at our Institute about half century ago by Academicians R. Kavetsky and Z. Butenko. And the First International Conference "Role of stem cell in leukemo- and carcinogenesis" summarizing the early developments in the field was held at our Institute at that time. The new level of the cancer problem was presented by Professor Ingemar Ernberg from Karolinska Institutet (Sweden) who presented his recent concept of cancer cell "attractor", at the edge of tumor biology, mathematical modeling and systems biology.



The keynote reports were presented also by Professor Ninel Berezhna, Doctor of Medical Sciences Victor Zhylchuk, Doctor of Sciences Iryna Kozeretska, Doctor of Sciences Olena Kashuba and Doctor of Sciences Denys Kolybo. The lecturers presented the up-to-date information on the role of connective tissue as the key factor of tumor microenvironment, the metastatic bone lesions and disseminated cancer cells in bone marrow, genetic aspects of cancer in Ukraine, the role of MRPS18-2 oncoprotein in regulation of cell differentiation, HB-EGF as potential oncomarker and target for anticancer therapy.

Symposium harbored many interesting reports, concerning the choice of the targets for cancer therapy, presented by Ukrainian researchers (see enclosed abstracts). Cancer therapy and the problems of personalized cancer treatment were also in the spotlight. During conference, largely supported by the VACTRAIN of Horizon 2020 program, there were workshops with the training in wet lab on different modern research techniques, such as quantitative real-time PCR (qRT-PCR), Comet assay, Fluorescence activated cell sorting (FACS), and also immunohistochemistry. Many Master and PhD students and also young and experienced researchers took part in the workshops.

This conference was also a platform to popularize the European School of Oncology, represented by Dr. Wojciech Wysocki from Poland. We are sure that this information will increase mobility of our medical students.

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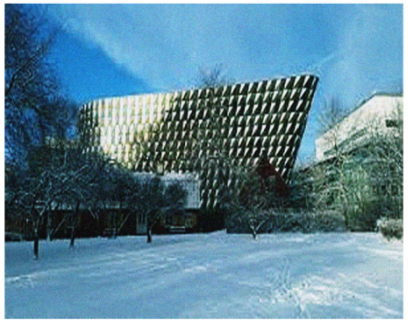
Dissemination of project results - Internal Scientific Events

International Workshop on cancer diseases

"SEARCH FOR TUMOR SPECIFIC TARGETS"

Karolinska institutet, Stockholm, January 16-17, 2017

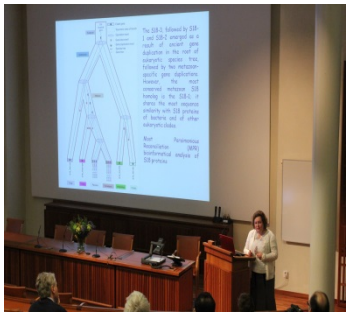
Cancer is an enemy that requires attack from all sides and with all available resources. The meeting was focused on what we have newly learnt about cancer, the current situation, and the important challenges for the future including routes to combat oncological diseases. Two days of the meeting were filled with the lectures by the invited speakers and oral presentations selected in the basis of submitted abstracts from younger researchers.



Main topics of the program

- ✓ Molecular and cellular pathobiology.
- ✓ Tumor metastases and role of cellular microenvironment.
- ✓ Paradigm of personalized treatment in oncology.
- ✓ Detection of potential targets for target therapy and cancer vaccines.
- ✓ Search for new markers for diagnostics of tumors with different genesis.
- ✓ Multidisciplinary cancer research

Workshop brought together 73 participants. Nineteen from Ukraine: KIEPOR, 11; Institute of Molecular Biology and Genetics of NASU, 3; Palladin institute of biochemistry of NASU, 2; Taras Shevchenko State University, 1; Odessa University, 1. One researcher was from Poland, three from Latvia, one from the Netherlands, one from Russia, and 48 from Sweden. This meeting has definitely served to strengthening of the current and establishment of the new research connections cross the boarders. Knowledge knows no boundaries and by working together a progress both within the field of research and in bringing our countries closer could be achieved



All lectures were highly attended, vivid discussions followed after each presentation. Second day was devoted to both lectures and poster sessions filled with active discussions of current and future collaborations. Meetings demonstrated the importance of translation of scientific discoveries into the new treatment approaches, new medicines & medications, and new technologies. http://vactrain.lv/events_3rd_cancer_diseases.html

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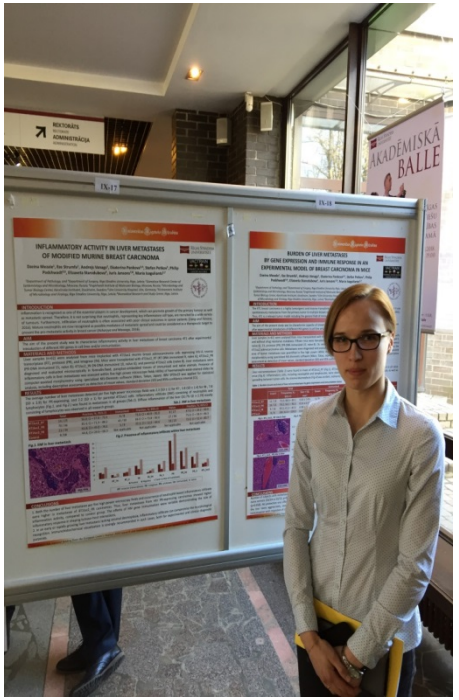
Dissemination of project results - *Internal Scientific Events*

Riga Stradins University Research Conference/ Rīgas Stradiņa universitāte Zinātniskā konference, Riga, Latvia, March 22-23, 2018

VACTRAIN trainees made poster presentations in the section "Anatomy, embryology, histology, structure biology, and pathology"

IX-15	**Differentiating non-small cell lung carcinoma: doublet of immunohistochemistry to be on the safe side** **A. Jukna, I. Štrumfa, A. Vanags, J. Gardovskis**
IX-16	Diagnostic value of cytokeratin expression profile in primary non-small cell lung carcinomas A. Jukna, I. Štrumfa, A. Vanags, J. Gardovskis
IX-17	Inflammatory activity in liver metastases of modified breast carcinoma Dž. Mežale, I. Štrumfa, A. Vanags, J. Pankova, M. Issagouliantis
IX-18	Burden of liver metastases by gene expression and immune response in an experimental model of breast carcinoma Dž. Mežale, I. Štrumfa, A. Vanags, J. Pankova, M. Issagouliantis



VACTRAIN trainee Dzeina Mezale by her poster


RĪGAS STRADIŅA UNIVERSITĀTE
VITA BREVIS ARS LONGA

SERTIFIKĀTS

Rīgas Stradiņa universitātes Zinātniskā konference

Maria Issagouliantis

piedalījās konferencē ar zinātnisku darbu
2018. gada 22. –23. martā
Rīgā, Latvijā

Zinātnes departamenta direktors asoc. profesors Uldis Berķis

Certificate of conference attendance to VACTRAIN members.

This project has received funding from the European Union's Horizon 2020 Research and innovation programme under grant agreement No 692293

Dissemination of project results - *Internal Scientific Events*

Symposium "FUNDAMENTAL PRINCIPLES OF CANCER BIOTHERAPY"

KIEPOR, Kyiv, Ukrain, May 21-22, 2018

Symposium "Fundamental principles of cancer biotherapy" was organized with an aim to bring in the specialists in the field and give an update on the latest developments in oncology and vaccine development. It was an additional activity, aimed to enhance VACTRAIN visibility in Ukraine.

MAIN TOPICS OF THE PROGRAM

- Tumor progression and role of cellular microenvironment;
- Molecular and cellular pathobiology;
- Nanotechnology and systems biology;
- New trends in cancer therapy and diagnostics.

Symposium had 87 registered participants from a broad scope of Ukrainian medical universities and research institutes. Six participants were from VACTRAIN partner teams: two researchers were from Karolinska Institute, Stockholm, Sweden (Galina Selivanova and Andrey Alexeyenko), two – from Lodz' University, Poland (Julita Pietrzak and Agnieszka Robaszkiewicz), two – from RSU, Riga, Latvia (Džeina Mežale and Ilze Fridrihsone). Meeting was covered by Ukrainian SME.

Participants stressed the importance of translation of research into the new treatment approaches, and new technologies. Symposium served for strengthening of the current and establishment of the new research connections cross the boarders.

Information about this event and symposium abstracts are published at the KIEPOR website
http://iepor.org.ua/en/conferences/events-2018-05-21-24-fundamental-principles-of-cancer-biotherapy.html
A digest on the conference is published in an open access journal Experimental Oncology, 2018: 40 (2), 154-176
http://exp-oncology.com.ua/article/11303/materials-of-symposium-and-summer-school-fundamental-principles-of-cancer-biotherapy-may-21-23-2018-kyiv-ukraine.

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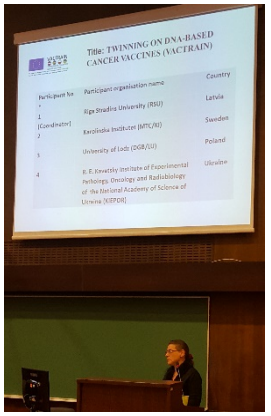
Other internal dissemination activities

Seminar for students of Utrecht University

Rīga Stradiņš University, Riga, April 18, 2017



45 medical students from Utrecht University visited RSU as part of their study trip to Latvia. The main purpose of the seminar to get them acquainted with the VACTRAIN project coordinated by RSU, and promote this type of theoretical and practical postgraduate education.

After a general presentation about the university, a presentations on VACTRAIN project and research methods were given by several researchers involved in the project:

- ✓ Maria Issagouliantis, Project Coordinator - activities and outcomes of the project;
- ✓ Stefan Petkov, Work Package 5 Leader - DNA immunization techniques and perspectives;
- ✓ Juris Jansons, Leading Researcher - methods of assessing of cellular immune responses;
- ✓ Laura Hippe, Trainee - reflections from individual training.

Dutch students highly appreciated the contents of the lecture and recognized it an interesting experience as compared to what has been seen in the Netherlands.

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Other internal dissemination activities

Seminar of the TWINNING projects implemented in Latvia

Rīga Stradiņš University, Riga, May 31, 2017

The representatives of the VACTRAIN project have participated in a seminar with the colleagues from Transport and Telecommunication Institute - Prof. Irina Yatskiv (Jackiva) (coordinator) and Dr.sc.ing. Mihails Savrasovs (Knowledge sharing workpackage leader).

Prof. Irina Yatskiva and Dr Mihails Savrasovs implement the TWINNING project "Enhancing excellence and innovation capacity in sustainable transport interchanges (ALLIANCE)", focused on stimulating and strengthening the scientific and technological capacity of Latvia and the raising of the profile of the research staff and their institution, by providing knowledge in the field of smart interconnecting sustainable transport networks.

The goal of the seminar was to discuss implementation issues of TWINNING projects in Latvia and assess their impact on institutions and the research community of Latvia.

After a brief presentation of each project, a roundtable took place with an interesting discussion regarding challenges in TWINNING projects' implementation, possibilities to support activities of TWINNING projects by implementing national level projects etc.
Given that the projects come from different research areas, the discussion was constructive for both sides.

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External dissemination activities

Participation in organization and attendance of the international conference "TOOLKITS FOR DNA VACCINE DESIGN, AN UPDATE"

Moscow, November 17–20, 2016

http://onlinereg.ru/DNAvaccTOOLS

CONFERENCE FIELD

This conference covered the whole area of DNA vaccine design starting from gene design, defining of transcription and translational signals, packaging, delivery, induction of innate immune response and role of adjuvants, induction and expansion of adaptive immune response, prime and boost strategies, success of DNA vaccines in cancer and infectious diseases, comparison of DNA vaccines to other vaccine modalities. We also invited the representatives of R&D companies and national agencies involved in the development, production and distribution of vaccines to discuss the business models for DNA vaccines, public perception of the vaccines and their perspectives for human and veterinarian use.

Each thematic block was opened with an introductory lecture introducing the basics and the main achievements in the given area. In the end of the introductory key-note lecture, the lectors shared their views on properties of DNA vaccines and their pros and cons connected to the area covered.

Conference sponsors

Российский научный фонд

VACTRAIN

1955 - 2015

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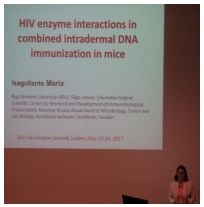
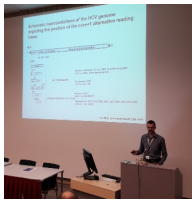
External dissemination activities

The 4th International SKIN VACCINATION SUMMIT

Leiden, the Netherlands, May 22-24, 2017

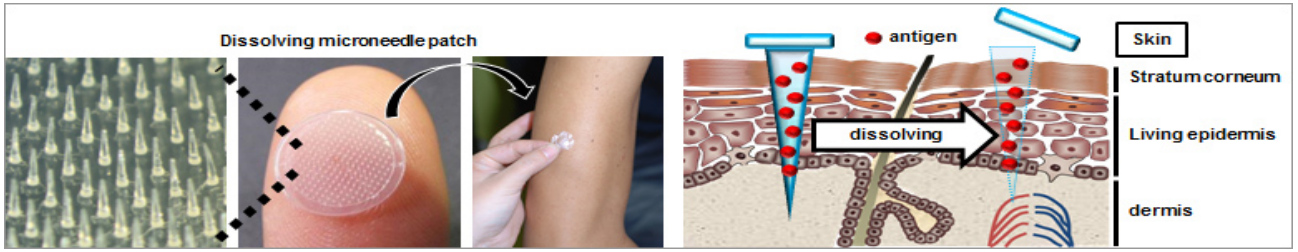
The joint activity of projects VACTRAIN and INNOVIMMUNE (Swedish Institute) was the attendance with presentations of the SKIN VACCINATION SUMMIT (SVS2017). SVS is an international forum for researchers, both academic and industrial, which involves also ethical regulators and skin delivery technologists who meet and discuss current state of the field, technology trends and future directions of development of the dynamically developing field of skin-mediated vaccination.





VACTRAIN members made following presentations:

- ✓ Maria Issagouliantis (RSU) "HIV enzyme interactions in combined intradermal DNA immunization in mice";
- ✓ Juris Jansons (RSU, and BMC) "Role of alternative reading frame protein in the immune response to HCV core, evaluation by intradermal DNA-immunization in mice";
- ✓ Laura Hippe (RSU) "Alternative reporters for monitoring the success of intradermal DNA delivery and expression in a mouse model";
- ✓ Ilia Gordeychuk (MTC/KI, INNOVIMMUNE) "Experimental vaccine protects against hepatitis E virus genotypes 1 and 3 in non-human primate model";
- ✓ Ekaterina Pankova (Gamaleya Research Center, Moscow, INNOVIMMUNE) "Stable expression of HIV reverse transcriptase by murine adenocarcinoma cells enhances growth rate of primary tumors in a mouse model designed to test the therapeutic efficacy of HIV DNA vaccines"



Outcomes: Participants made successful presentations which aroused a fruitful discussion. New research connections with vaccinologists in USA, France, the Netherlands were made. The activity had the synergetic effect for the both of projects in term of dissemination of the scientific results

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External dissemination activities

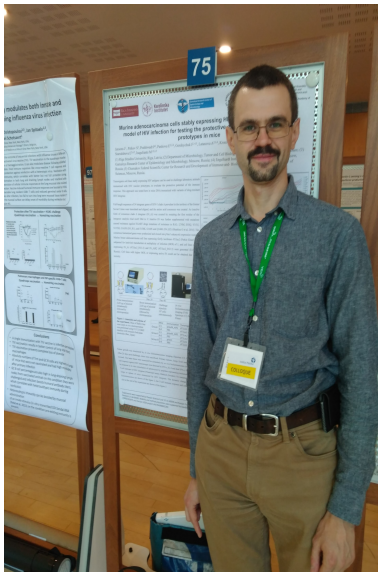
ANNUAL CONGRESS OF THE INTERNATIONAL SOCIETY FOR VACCINES

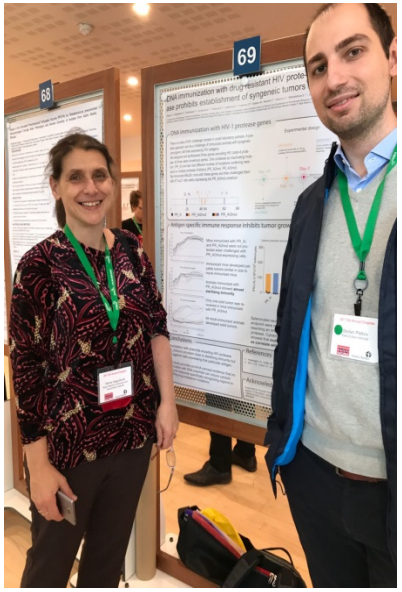
Paris, Institute Pasteur in Paris, October 5th – 7th 2017.

The ISV Annual Congress is the world's largest scientific conference in the field of vaccines and covers a broad range of topics related to vaccines and immunotherapies. ISV2017 Congress brought together individuals from all sectors of the global vaccine community to hear about the latest advances in the field, discuss challenges and opportunities, and network. The Congress featured international leading experts as keynote and symposium speakers as well as a high percentage of oral presentations selected from abstracts submitted by the global vaccine community.

Presentations of VACTRAIN members made on ISV2017 Congress

- Petkov S, Kilpeläinen A, Podshwadt P, Gordeychuk I, Pankova E, Latanova A, Latyshev O, Saleem M, Mezale D, Jansons J, Starodubova E, Isaguliants M. DNA immunization with drug resistant HIV protease gene protects mice from the establishment of syngenic tumors expressing this, but not the homologous HIV proteases (poster)
- Jansons J, Petkov S, Podshwadt P, Pankova E, Gordeychuk I, Latanova A, Krotova O, Mezale D, Saleem M, Latyshev O, Starodubova E, Isaguliants M. Murine adenocarcinoma cells stably expressing HIV antigens as a surrogate model of HIV infection for testing the protective efficacy of DNA vaccine prototypes in mice (poster)
- Starodubova E, Petkov S, Pankova E, Jansons J, Latanova A, Saleem M, Isaguliants M. Gene immunization inhibits mammary carcinoma growth in mice (poster);
- Starodubova E, Petkov S, Latanova A, Kilpeläinen A, Krotova O, Jansons J, Podshwadt P, Sasinovich S, Warholm P, Pankova E, Saleem M, Mezale D, Latyshev O, Tukhvatulina N1, Gorodnicheva T, Logunov DY, Gordeychuk I, Isaguliants M. Efficacy of consensus DNA-immunization against drug resistance in HIV infection evaluated in a murine tumor model (oral).





Poster presentation by S Petkov et al got award of International Society for Vaccines.

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External dissemination activities

International Conference "VACCINES & VACCINATION" together with BAN Network of the Swedish Institute, and Russian Science Fund, Moscow, September 27-October 1, 2017

www.onlinereg.ru/VAC&VAC2017

Presentations of VACTRAIN members made VAC&VAC conference
11 ABSTRACTS;9 ORALS, 2 POSTERS. Four presentations were by VACTRAIN trainees:

- Britta Wahren (Karolinska Institutet, Stockholm, Sweden) Immunotherapeutic targeting of carcinoembryonic antigen (oral)
- Isaguliants M et al Tumor models in experimental testing of efficacy of consensus DNA-vaccines against drug-resistant HIV (oral)
- Isaguliants M. Vaccines against human papilloma viruses, an overview (oral)
- Maxim Ionov & Sylwia Michlewska (Lodz University, Lodz, Poland) Dendrimers in anti-cancer drug delivery
- Petkov S et al (Karolinska Institutet, Stockholm, Sweden) Enhancing cellular immune responses to HIV vaccines using multi-gene, multi-site injections" (oral)
- Podshwadt P et al (Karolinska Institutet, Stockholm, Sweden) Method for rapid in vitro assessment of metastatic potential of primary tumors in mice" (oral)
- **Jansons J et al (RSU, Riga, Latvia) Intradermal DNA immunization with experimental HCV DNA vaccines" (trainee; oral)**
- **Anita Berzina & Maxim Ionov (Riga Stradins University, Riga, Latvia, and Lodz University, Lodz, Poland) Dendrimers in in vitro and in vivo DNA transfection (trainee; oral)**
- **Laura Hippe (A. Kirchenstein Institute of Microbiology and Virology, Riga Stradiņš University, Latvia). Applications of near-infrared reporters to monitor the success of intradermal DNA delivery and expression in a mouse model (trainee; oral)**
- **Jansons J et al ((RSU, Riga, Latvia) Fork-plate electrodes of different configuration demonstrate similar performance in vivo transfection and induction of immune response in DNA-immunized mice" (trainee; poster)**
- Saleem M et al (Karolinska Institutet, Stockholm, Sweden) The effect of plasmid mixing on antibody response of DNA-immunized mice against HIV-1 reverse transcriptase, protease and integrase" (co-authorship; poster).

VACTRAIN
TWINNING ON DNA-BASED CANCER VACCINES
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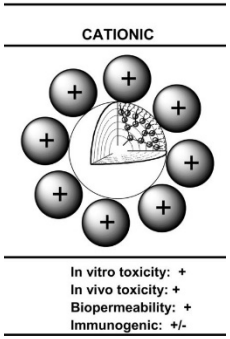
TWINNING ON DNA-BASED CANCER VACCINES

RĪGA STRADIŅŠ UNIVERSITY

Dissemination through teaching

Part I: INSIGHTS IN HEMATOONCOLOGY by Dr. Ida Franiak-Pietryga

- ✓ Gene expression profiling, microarrays - state of the art and their applications in hematooncology.
- ✓ Chronic Lymphocytic Leukemia (CLL)
- ✓ Apoptosis, signaling pathways

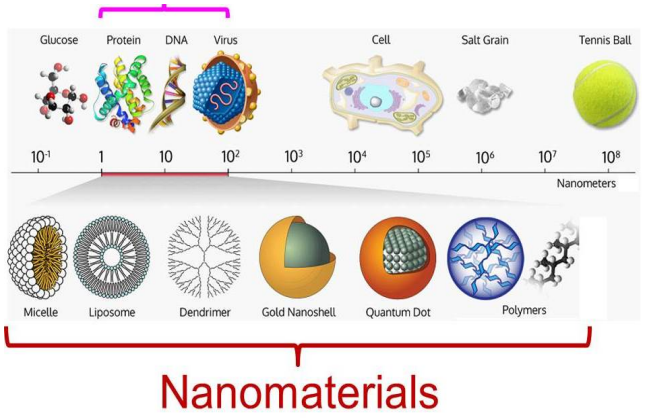


Part II: CHARACTERIZATION OF NANOPARTICLES BY ZETA POTENTIAL AND SIZE MEASUREMENTS by Dr. Elżbieta Pędziwiatr-Werbicka

- ✓ Colloidal stability and DVLO theory.
- ✓ Brownian motion and light scattering theories.
- ✓ Applications of zeta potential and size measurements for nanoparticles characterization.

Lecture course in Applied Biophysics by Lodz University

Rīga Stradiņš University, Riga, May 27, 2016



Part III: BASICS OF FLUORESCENCE AND PHOSPHORESCENCE AND THEIR APPLICATIONS FOR GENE DELIVERY SYSTEMS by Dr. Dzmitry Shcharbin

- ✓ Physical principles of fluorescence and phosphorescence
- ✓ Anisotropy. Quenching. Steady-state fluorescence and phosphorescence.
- ✓ Applications of fluorescence and phosphorescence for gene delivery systems.

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Dissemination through teaching

- ✓ **Joint supervision of Master Thesis February-September 2017 of Erasmus student with Prof Marion Schneider, Ulm University, Germany.**

- ✓ **M. Issagouliantis. Lecturing on DNA vaccines, Science Circle in Molecular Medicine, Ulm University, Germany, October 10, 2017 (invited)**

Philip Podschwadt "DNA vaccination against experimental cancer" Master Defense in Ulm, November 14, 2017.

- ✓ **Lecturing at EAVI2020 course in animal models and vaccination, IDMIT – CEA, Paris, April 8-11, 2018 (invited)**

  - Stefan Petkov (Karolinska Institutet) "In vivo imaging in preclinical trials of genetic vaccines"
  - Maria Issagouliantis (Riga Stradins University) "Tumor model for HIV-1 challenge in mice"

EAVI2020 EUROPEAN AIDS VACCINE INITIATIVE

Fifth course on animal models and vaccination
April 8-11 2018, IDMIT – CEA, Paris

Tumor model for HIV-1 challenge in mice

Maria Isaguliants

Riga Stradins University, and Karolinska Institutet

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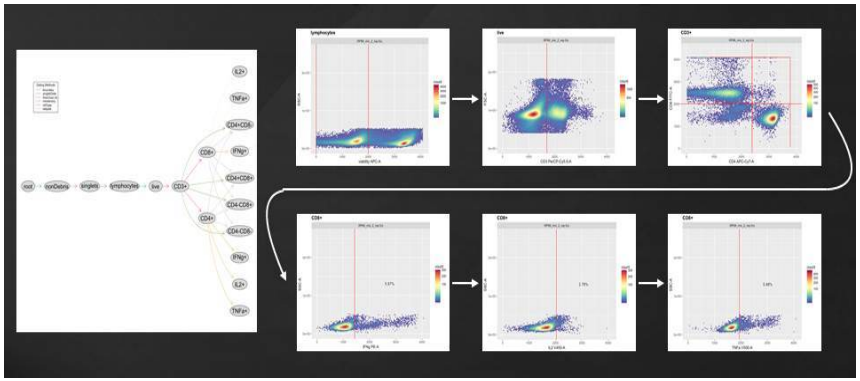
ACTIVITIES FOR AUTUMN 2018:

- **Training of RSU personal in multiparametric flow cytometry, September-October, 2018 using deposits of cells from immunized and tumor challenged mice obtained in the 2nd on-site round of TUMOR TRAINING. Contact person: Juris Jansons, juris.jansons@rsu.lv;**

- **International Conference "Perspective Technologies in Vaccination and Immunotherapy" Moscow, October 5-8, 2018 www.onlinereg.ru/TECHVAC2018**

- **Annual Congress of International Society for Vaccines, Atlanta, USA, October 28-30, 2018;**

- **Workshop "Immunotherapy of Cancer", Riga Stradins University, Riga, Latvia, November 2018, finalizing VACTRAIN project (date to be defined). Contact person: Maria Issagouliantis, maria.issagouliantis@rsu.lv**



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Composed by:

- *Juris Jansons, Riga Stradins University, Riga, Latvia*
- *Maria Issagouliantis, Riga Stradins University, Riga, Latvia*
- *Stefan Petkov, Karolinska Institutet, Stockholm, Sweden*

Photomaterials contributed by:

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- *Elena Kashuba, R.E. Kavetsky Institute of Experimental Pathology, Oncology and radiobiology of National Academy of Sciences of Ukraine, Ukraine*
- *Ilya Gordeychuk, Karolinska Institutet, Stockholm, Sweden*
- *Asja Lunga, Riga Stradins University, Riga, Latvia*
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State of Art
July, 2018