"Cantacuzino" National Institute of Research-Development for Microbiology and Immunology

General presentation

"Cantacuzino" National Institute of Research-Development for Microbiology and Immunology (NIRDMIC) is a national institute of research and development in coordination of Ministry of Public Health. Historically, it was created as a strategic institution to perform research in all domains of microbiology and related sciences in order to develop the production of therapeutic sera and vaccines. Currently, our main fields of activity consist of research in immunology and immune mediated diseases, microbiology, virology, parasitology and other infectious diseases. Other connected fields such as genomics, molecular genetics, transcriptomics, proteomics, metabolomics and bioinformatics are approached in etiological diagnosis and molecular epidemiology of infectious diseases in order to solve problems.

The Cantacuzino Institutes mission, as stated by its founder, Prof. Ioan Cantacuzino is *"to promote public health by high quality and competitive interdisciplinary research, by monitoring, prevention and control of communicable diseases".* Accordingly, the following research directions are focusing on:

- Studies regarding the pathogen circulation and their virulence characteristics;
- Emergence of antimicrobial resistance and research on mechanisms and genetics of resistance;
- New methods for diagnosis, immunology and epidemiology of infectious diseases;
- Biotechnology and vaccine development;
- Mechanisms of infections and immunological response;
- Environment changes and vectors disseminated diseases;

- Genomics and proteomics;
- Immune mediated disease mechanisms and therapies;
- Capacity building for technology transfer in vaccine production.

Strengthening the professional and institutional capacity for an effective participation to the national and international programs for research, control and surveillance of infectious diseases and promotion of national and international co-operation were the main objectives of our activity.

Since 1991, National Institute of Research-Development for Microbiology and Immunology “Cantacuzino” is a member of Institute Pasteur International Network, a partnership of 32 research and public health institutes on five continents.

During 2013 an ECDC (European Center for Diseases Control) appraisal of the activity in the field of Public Health Microbiology was performed and the institute was nominated as EUPHEM site (European Public Health Microbiology) for training the European specialists in this field. This year, 2013, the institute has its first MS-track fellow, for a training period of 2 years.

Appreciating the professional expertise of scientific personnel the specialists from our institute were nominated as disease specific experts for direct collaboration with ECDC. They are participating in studies focusing new methods of diagnosis, harmonizing methods for microbiological surveillance of infectious diseases, in training courses, workshops, and annual meetings organized by ECDC.

Laboratory proficiency is annually evaluated by EQA coordinated by ECDC.

The external scientific evaluation of the institute during 2013 and the SWAT analyze arises the main strengths and weakness and was a good opportunity to highlight the way to continue.

The Department of Public Health Microbiology is grouping the National Reference Laboratories, The Cellular and Microbial Culture Collection, a Unit of Rapid Reaction in epidemiological unusual events and bioterrorism and a BSL3 unit (biosecurity level 3 unit) for high pathogen manipulation.

The main research directions in the Department are focusing on:

- Improving infectious disease diagnostic in order to reduce un-known etiology infection reports;

- Design and proposal of diagnostics algorithms based on classical microbiological and molecular methods;

- Studies on virulence molecular markers used for detection and characterization of highly pathogenic as well as commensal/opportunistic bacteria involved in human infectious diseases;

- Optimization of molecular methods used for tracking infectious diseases (microbial genotyping) and approaches based on the innovative concept of monitoring multiple interfaces: human health / animal health / wildlife / environmental;

- Harmonization of laboratory methods for Public Health Microbiology at the European level for an effective participation in the ECDC programs for surveillance and control of communicable diseases;

- Molecular characterization of emergent, fastidious, un-typable, non-cultivable microorganisms (viruses and bacteria) and evaluation of the impact of their variability on diagnosis and pathogenesis of infections;

- Improve detection of HCV and/or HBV from human patient serum versus hepatic tissue after surgical intervention in order to define the virus's role in carcinogenesis;

- Studies on mechanisms and genetics of antimicrobial resistance (bacterial and viral) and development of new compounds with antimicrobial activity.

Department of Immunology, Biotechnology and Vaccine Development is performing studies in the following directions:

- Studies on innate immunity, inflammation and immune regulation;

- Autoimmunity and therapeutic approaches for autoimmune diseases and allergies;

- Immune mediators profile in human pathology;

- Molecular mechanisms involved in cellular dysfunctions and identification of new therapy for human disorders;

- Diagnosis and identification of new therapeutic targets in communicable and inflammatory diseases;

- Development and implementation of new technologies for cloning and over expression of mammalian and viral proteins in prokaryotic systems;

- Studies on immunogenicity of avian influenza virus in mice and humans in correlation with administration route;

- Development of cell-based vaccines for influenza viruses using modern technology as a replacement for eggs and chicken embryos;

- Bacterial nucleoside monophosphate kinase family as new therapeutic targets for antibacterial agents;

- Development of monoclonal antibodies (mAbs) and immunoassays for detection of highly pathogenic bacteria and viruses;

The institute has a **Laboratory animal facility** where a laboratory for GLP manipulation is in faze of implementation. Elaboration of standard operating procedures and preclinical study models in accordance to national (ANM) and international (EMEA) rules and establishment of regulations for using animals in laboratory experiments are in progress here.

International research collaboration

Cooperation within International Pasteur Network

As a member of the Institute Pasteur International Network (RIIP), the National Institute of Research-Development for Microbiology and Immunology "Cantacuzino" participates to collaborative research projects within the network (Fig.1). Current collaborative research projects aim at improving the capacity to detect and characterize etiological agents involved in different infectious diseases.

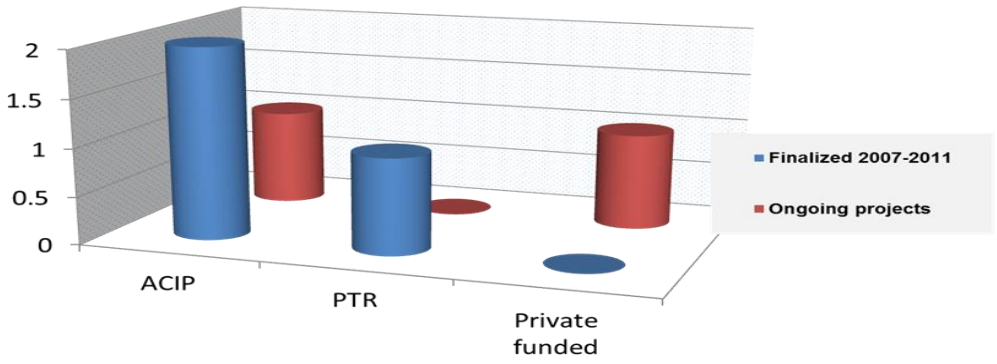

Fig. 1: Research projects within International Pasteur Network

One of the most important (ACIP A-01-2007) project developed in this frame was carried out on molecular diagnosis and typing of *Corynebacterium diphtheriae* strains. These bacteria are able to acquire a bacteriophage harboring genes responsible for diphtheria toxin production. As a consequence in a bacterial population live together pathogenic and non-pathogenic strains. Studies on pathogenicity mechanisms and

genomics of toxigenesis as well as efforts to find and harmonize at the European level the most appropriate method for diagnosis and typing in order to trace the infection, especially in transnational strain circulation were the aims of the international cooperation (ACIP 01 2009).

Collaboration within international projects (Fig. 2)

As the sole national vaccine manufacturer, in an era dominated by the threat of new pandemic influenza emergence, the institute was involved in projects regarding the advancement of capacity for influenza vaccine production (WHO project **Pandemic Preparedness**) according to EMEA conditions, in order not only to cover the national vaccine needs, but also be eligible for distribution to neighboring countries. The DG-SANCO project, **Fast VAC** has as main objective to put into effect a comprehensive set of predictive rules enabling accelerated development, evaluation, production and release of emergency vaccines. “Combating flu in a combined action between the industry and the public sector in order to secure adequate and fast intervention in Europe” was the subject of **Flu Secure**, a DG-SANCO project developed during 2006-2010, a collaborative action between NIRDMIC and NIBSC aimed at technological transfer, immunogenicity evaluation of H5N1 influenza vaccines in animal models and building a reagent bank for the multiplication of seed strains in cell culture.

This grant was complemented by the adjuvanted influenza vaccine development grant with Infectious Disease Research Institute, Columbia Street, Suite 400 Seattle, WA 98103 (F16SUB-2010) subsidiary of the main grant 1 IDSEP100008-01-00 1124 funded by ASPR/BARDA/AMS, „Development and Sustainable Manufacturing of Adjuvanted Pandemic Influenza Vaccines in Developing Countries”. The new adjuvant will reduce the necessary biological material at least to the level identified in the Flu Secure project. After completion, Cantacuzino Institute will not only succeed to develop a modern emergency vaccine but will also end-up with a modern platform for the vaccine development.

Another direction for research is the eco-epidemiology of vector-borne diseases in the hanging European environment which has been developed in the framework of FP6 and FP7 projects: **EDEN** (2005-2009) and **EDENext** (2011-2014).

EDEN project (**FP6, GOCE-CT-2003-010284; Emerging Diseases in a changing European eNvironment**) which included partners from a number of 49 institutions in 24 countries aimed to identify, evaluate an catalogue European ecosystems and environmental conditions linked to global change which are influencing the spatial and temporal distribution

of human pathogenic agents. The project developed and coordinated at the European level a set of generic methods, tools and skills such as predictive emergence and spread models, early warning, surveillance and monitoring tools and scenarios, decision support for intervention and public health policies at both EU and national or regional level. The studies focused on tick-borne diseases, West Nile virus, and malaria.

The project resulted in the gathering of new to science knowledge on the circulation of West Nile virus, ticks as vectors of Lyme disease and Anopheles mosquitoes as potential vectors of malaria. Three doctoral theses were elaborated in the framework of this project.

The same team of scientists participated in the FP7 call on vectors biology and vector-borne diseases (2010), as well as in **„Biology and Control of Vector Borne Infections in Europe", acronyme EDENext, GA 261504"** which is in progress now (2011-2014).

The **EDENext** project addresses biological, ecological and epidemiological components of vector-borne diseases introduction, emergence and spread, and proposes advanced tools for controlling them.

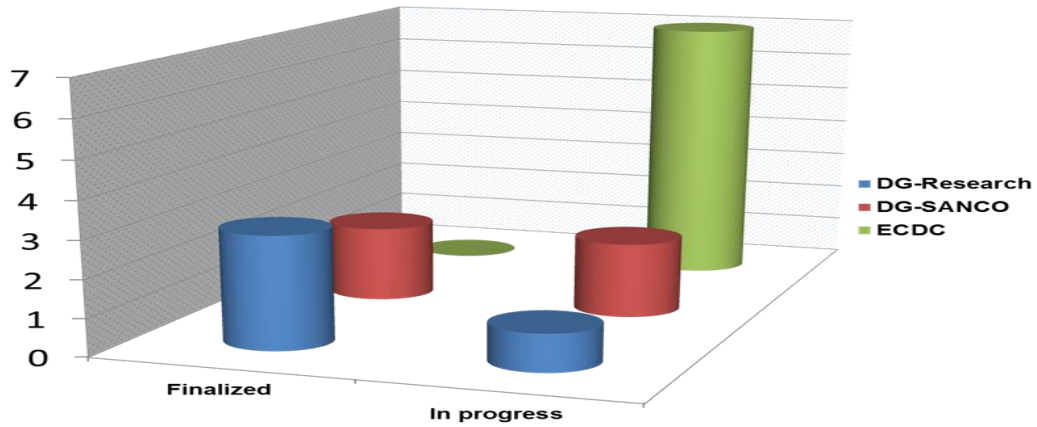

Fig. 2: Projects within international collaboration

The curve of national funded projects is fluctuating and could be partly explained by level of funds (Fig. 3).

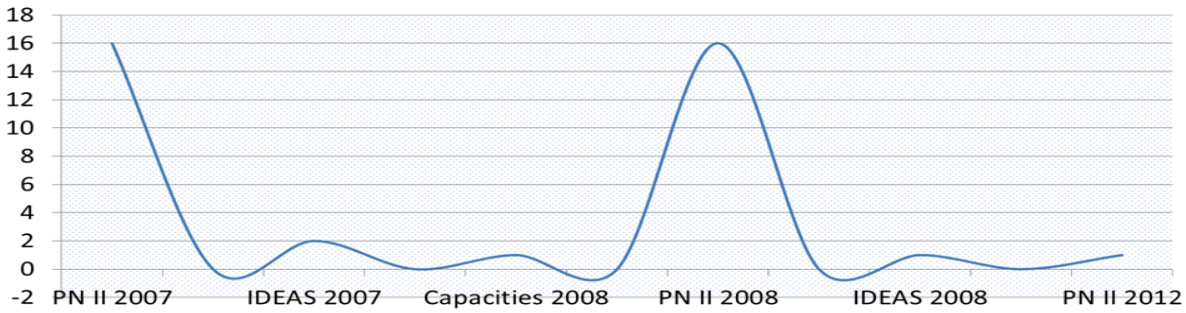

Fig. 3: Projects funded by the national authorites

Project PCCA type 2 no. 88/2012 "Investigation of viral and host related markers correlated with lack of response to anti-viral treatment in chronic hepatitis C virus infection" is the most important ongoing project funded by the Romanian Agency of Scientific Research. The hepatitis C virus (HCV) genotype 1b is associated with higher rates of liver cirrhosis and poorer response to antiviral therapy. Sustained virological response to pegylated interferon/ ribavirin is achieved in only half of genotype 1-infected patients. (prevalent in Romania). Starting with 2011, new treatments are available including specific protease inhibitors in addition to PEG-Interferon and ribavirin. Failure of IFN-based treatments to eradicate HCV infection has been shown to be related to virological (HCV genotype, variability, viral load, on-treatment viral kinetics), host genetic determinants (genetic variation near the IL28B gene) and non-genetic factors (age, sex, fibrosis, etc.). So far, no study characterizing HCV resistance pattern or genetic features among chronic HCV patients from Romania is available. The project aims to define the pre-treatment prediction of response to PEG-IFN/RBV therapy through the integrated analysis of viral factors as well as host factors. This study implies a prospective recruitment of patients with chronic hepatitis C in accordance with the Helsinki Declaration. All serological, biochemical, histo-pathological and genetic assays will be performed on samples routinely taken in regular clinical practice. The presence of the described mutations to the current or the tri-therapy in complete viral protein-coding regions and the variability impact on the resistance phenotype using both the traditional sequencing method and a next generation approach will be evaluated. Selected HCV genomic regions will serve to design primers and to develop specific PCR systems capable to detect resistance mutations. Human genetic markers (IL28B, HLA-B27, ITPA genes polymorphisms) and serum proteins (IP-10) will be tested for

treatment prediction. A computer based algorithm including host and viral factors will be developed to support selection of the optimum treatment in the context of personalized medicine.

Research valorization is mostly represented by ISI and BDI publications (Fig.4)

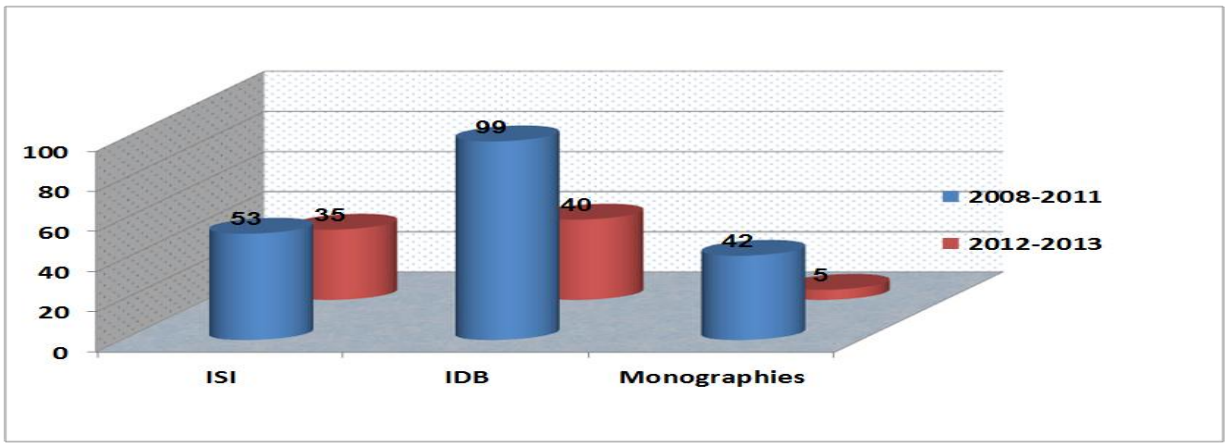

Fig.4: The publications as a research result

Since 1928 Institute is editing a peer-reviewed quarterly journal indexed in Medline/Pubmed, the “Romanian Archives of Microbiology and Immunology”, (Print ISSN: 1222-3891, OCLC: 25545262).