

“Victor Babes” National Institute of Pathology

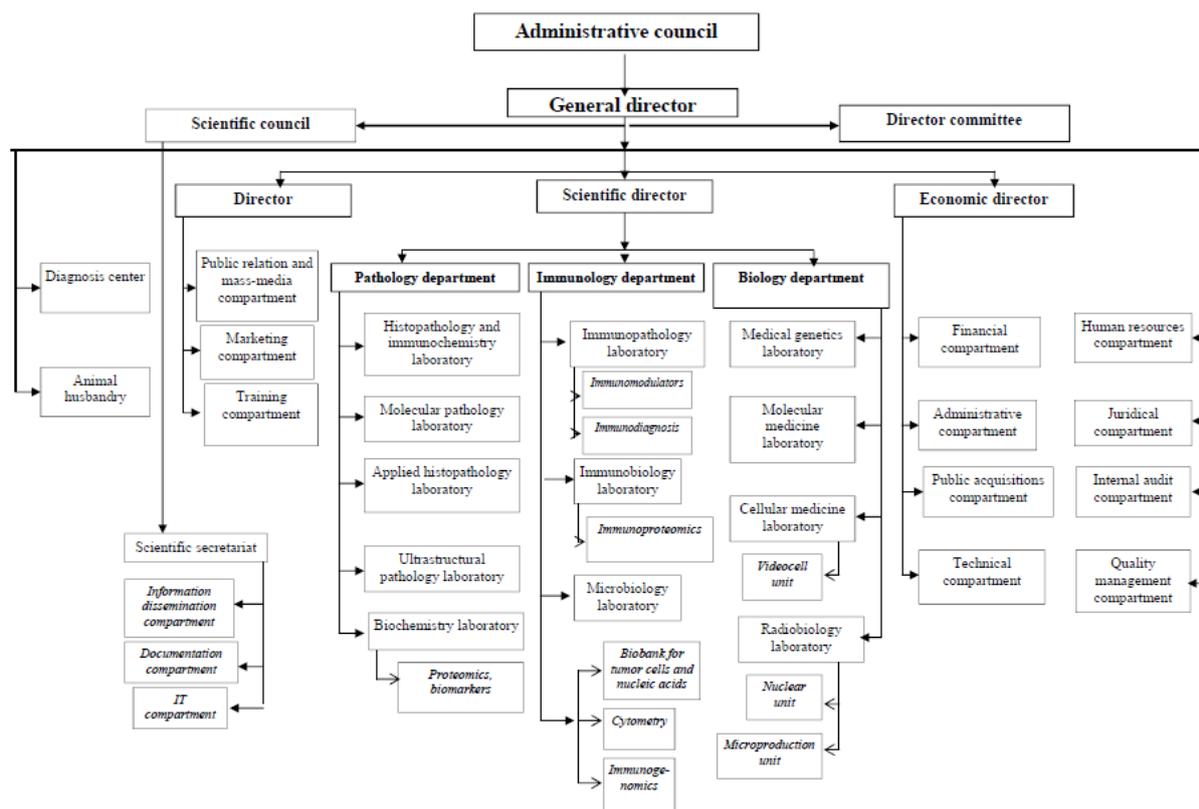
SELF-ASSESSMENT REPORT

2007-2011

Table of Contents

1. Functional administrative structure diagram
2. General activity report
3. Activity report by team
4. Representative project

1. Functional administrative structure diagram



M

Management team:

- General director: Acad. Laurentiu M. POPESCU
- Scientific director: CSI Dr. Mihail Eugen HINESCU
- Director: CSI Dr. Bogdan Ovidiu POPESCU
- Economic director: Ec. Mariana GEORGESCU

Decision-making structures: Administrative Council (9 members), Scientific Council (13 members), Board of directors (4 members).

Departments' organization

PATHOLOGY Dr. Carmen Ardeleanu	IMMUNOLOGY Dr. Cornel Ursaciuc	BIOLOGY Dr. Mihail Hinescu
--	--	--------------------------------------

Histopathology, immunohistochemistry and molecular diagnosis

Prof. Dr. Carmen Ardeleanu

Ultrastructural pathology

Dr. Mihaela Gherghiceanu

Biochemistry

Dr. Cristiana Tanase

Diagnosis center

Georgeta Butur

Immunobiology

Dr. Monica Neagu

Immunopathology

Dan Ciotaru

Animal husbandry

Bogdan Marinescu

Medical genetics

Dr. Aurora Arghir

Cellular medicine

Dr. Mircea Leabu

Molecular medicine

Dr. Bogdan Popescu

Radiobiology

Dr. Gina Manda

Since 2005 the Institute is **certified SR EN ISO/CEI 9001:2008** for research activity and medical services. Several laboratories received national accreditation: Histopathology and immunohistochemistry laboratory (accreditation SR EN ISO/CEI 15189:2007), Biochemistry laboratory (accreditation SR EN ISO/CEI 15189:2007), Animal Husbandry unit (accreditation SR EN ISO/CEI 17 025: 2005), Nuclear unit (national authorization by CNCAN).

2. General activity report

Founded in 1887, “Victor Babes” National Institute of Pathology from Bucharest is the first institute of biomedical research in Romania. Still at the forefront of fundamental research, the Institute is today a center of reference for human disease diagnosis and monitoring.

Mission

Victor Babes Institute of Pathology mission is to conduct cutting edge research in the field of molecular and cellular medicine for the knowledge-based scientific progress in the benefit of society.

We are using all organizational resources to address major societal needs in the area of health and to provide scientifically sound instruments and solutions in the benefit of patients and health professionals. The institute correlates the identified needs at national level with the scientific and health challenges at European level, thus providing the most effective ways of access to state of the art knowledge / solutions and acting as a scientific connection with health and research entities in Europe. The institute provides support for strategic planning and decision at national level for policy makers in the field of biomedical research and healthcare.

The institute’s mission is to expand the knowledge in biomedical and associated sciences by conducting and supporting research, development, education / training and high-quality medical services. The institute’s mission constructively influences the quality of life and healthcare services at national level.

The institute is committed to increase the international visibility of Romanian research in the field of cellular and molecular medicine.

Sharing efforts with partner medical institutions (in an attempt to build a local translational research community), the institute is responding with up-to-date solutions to major human health issues in cancer, (neuro)degenerative diseases, immune disorders, nephropathology and cardiovascular diseases. The institute, organized in three main research departments (pathology, immunology, biology) is using state-of-the-art methodology to develop innovative diagnostic tools and personalized medicine strategies.

The advantage of having infrastructure and experts in different disciplines is that we can adjust our projects along the way, depending on the priorities related to patient needs and in conjunction to the European scientific trends. It is our commitment to be integrated in large scale multidisciplinary projects in biomedical science, aiming at improving health and the quality of life.

Research focus: Multidisciplinary research in the field of cellular and molecular medicine, developed by 10 research teams

1. **Telocytes:** telocytes characterization; telocytes-stem cells tandem; telocytes in regenerative medicine; in vitro and in vivo functional studies on telocytes
2. **Surgical and molecular pathology:** cell signaling pathways in malignant epithelial tumors; molecular bases of therapy modulation in malignant tumors; genotypic profiles variability in cancer
3. **Translational research in cancer:** therapeutic targets in malignant tumors; prognostic and predictive biomarkers in epithelial malignant tumors; molecular identification of etiologic factors in infections and associated tumors
4. **Ultrastructural pathology:** basic research in fundamental mechanisms of cardiac regeneration - from stem cell to heart tissue; 3D electron tomography of the caveolar microdomains in smooth muscle cells; cellular and molecular mechanisms involved in glomerular pathology; cell ultrastructure investigation
5. **Proteomic biomarkers:** proteomics technologies for biomarkers discovery in cancer; proteomics biomarkers in pharmacological research; proteomics in the evaluation of environmental risks for human health
6. **Immunomodulation-immunodiagnosis:** tumor immunology; biomarkers in autoimmune diseases; cytokines and immunomodulation; innovative immunotherapies
7. **Genomics and genetic diagnosis:** genetics of neuropsychiatric disorders; genetic/epigenetic and genomic biomarkers relevant for cancer onset and progression
8. **Neurosciences:** trophic factor receptors expression in central and peripheral nervous system; tight junction proteins in brain and peripheral nerves; neurodegeneration models relevant to Alzheimer and Parkinson diseases; neuromuscular pathology

9. **Drug development and toxicology:** pathologic mechanisms and drug targets in cardiovascular diseases and rheumatoid arthritis; drug development - biological in vitro and in vivo screening; immunotoxicology; radiobiology
10. **Assay development and alternative testing:** immune-based assay development for bacterial/viral infections; cell-based assays development for drug assessment and nanomedicine.

Collaborations

- **National collaborations** with partners having complementary expertise and infrastructure
- **International collaborations** with Max Planck Institute, Graz University, University of Tuebingen, Universite Catholique de Louvain, Ludwig Cancer Institute, University of Goteborg, University of Turin, University of Medicine Florence, University of Athens, Cyprus Institute of Genetics and Neurology, Center of Cardiovascular Research Aachen, Saint George's University of London, Descartes University of Paris, Hospital Cochin, Chinese Academy of Medical Sciences etc.

Project-based research

Research excellence in the field of life sciences is sustained by a broad array of research projects:

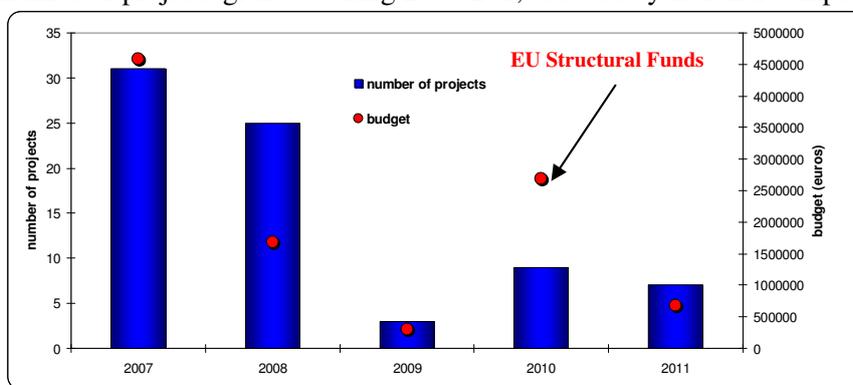
International projects: 4 bilateral projects with France, China and Cyprus, 1 NATO Science for Peace project, 1 MNT-ERA NET project, 1 FP7 – People project, 1 project in the EU Education and Culture-Lifelong Learning Program.

Research structural funds: 2 POSCCE projects with foreign coordinator (Priority axes 2 – Competitivity by research, technological development and innovation)

- o Proteomics technologies for cancer biomarkers discovery (coordinator Prof. S. Constantinescu)
- o Implementation of molecular tissue assays for cancer in Romania. State-of-the-art research focused on personalized oncology (coordinator Prof. G. Bussolati)

Projects financed by the national research programs CEEEX (Health, Biotech, Matnantech, Infosoc), CNCISIS, PNII Partnerships and Capacities = **125**; 21 projects were coordinated by INCD “Victor Babes” and in 104 projects the institute participated as partner

Dynamics of projects granted during 2007-2011, selected by national competition



The significant cut-offs in number of granted projects and budgets are explained by lack of national research competition calls in 2009 and 2010, and a dramatic decrease in research public funding as a result of economic slow-down. The 2010-2011 up-trends reflect accessing of European structural funds. Getting alternative financial support in the frame of Sectorial Operational Program for Increased Competitiveness is an important advantage for research institutes. However, this opportunity was not a long term option, since the current call topics do not fit the institute's expertise/eligibility.

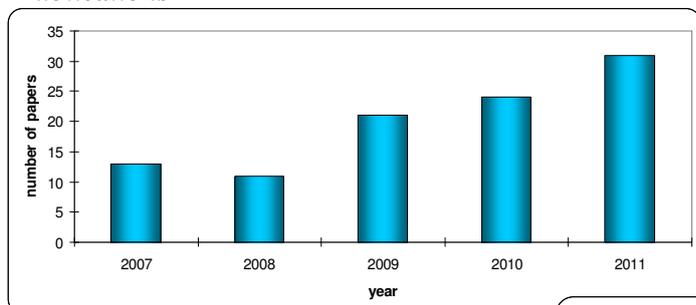
Projects financed by the European Social Fund: 3 projects focused on the training of personnel from the national health system in the field of state-of-the-art biomedical techniques, aiming to implement new diagnosis tools in clinical laboratories.

Infrastructure development projects: **1)** Advanced infrastructure for molecular cytogenetic research; **2)** Upgrading of a biobank for tumor cells and nucleic acids by attaching an immunogenomics laboratory for molecular screening in cancer; **3)** Upgrade of research infrastructure for laboratory animals in INCD "Victor Babes"; **4)** Makeup of the most competitive laboratory in Romania for living cell direct study under microscope in an incubator

Project for defining strategic priorities: “Cell therapy in regenerative medicine development. Strategic priorities” – STRATEC, funded by the National Authority for Scientific Research. Through this project the institute offered its expertise to the main research policy maker in Romania and our researchers gained new insights in the field of cell therapies, paving the way to future research directions.

Major achievements

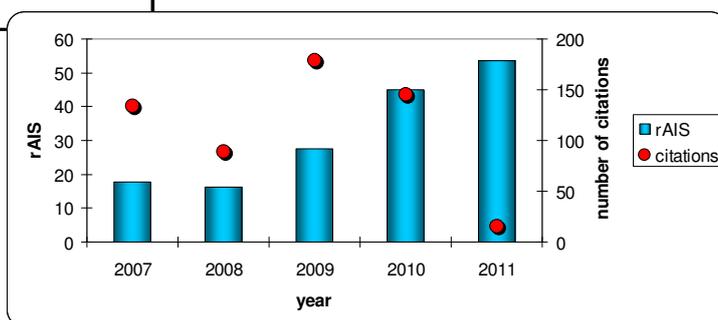
Publications



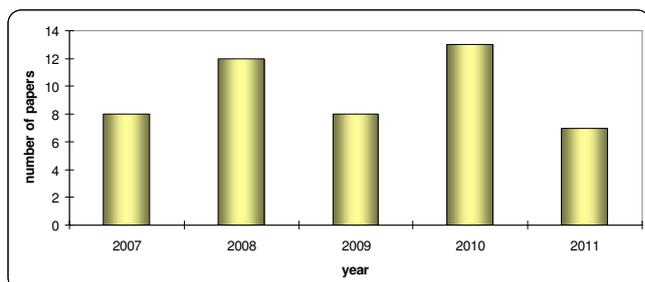
Publications in ISI journals with non-zero rAIS = 99

- total rAIS = 154,15918
- rAIS/researcher = 2,03
- total number of citations = 561
- mean citations number/researcher = 7,38

The institute’s research activity became significantly more visible at international level during 2008-2011, both as publications and citations number in ISI ranked journals. Considering the number of contributing researchers (76), it is obvious that the objective of the institute’s strategy to enhance international visibility was reached.



Publications in ISI journals with zero rAIS = 48



Other publications

77 papers published in *non-ISI journals*

9 *books* (1 published by Elsevier) and 20 *book chapters* (7 published by international publishing houses)

Patents

- **3 registered patents** 1) Tetra-sulphonated porphyrin application for producing a dermatologic therapy – photosensitizer; 2) Tetrapirolic compound asymmetrically substituted – synthesis and biological evaluation; 3) Equipment and procedure for microwave irradiation in in vitro models with concomitant registration of biological behavior in a fluorescence microscope; **3 submitted patents.**

The patent “Tetra-sulphonated porphyrin application for producing a dermatologic therapy – photosensitizer” received Gold medal at Brussels Innova 2008, Special Prize of Rudy Demotte, Minister President of the Walloon Government, Gold medal at The 37th International Exhibition of Inventions of Geneva 2009 and Special Prize of the Ministry of Education of Russia, 2009; Gold medal at The International Fair for Innovation, Moscow, 2009.

The project proposals of the Institute at the 2011 Call “Partnerships – Collaborative projects of applied research” reflect our commitment to develop applied research in consortia with other public and private R&D institutions, resulting in patents and publications.

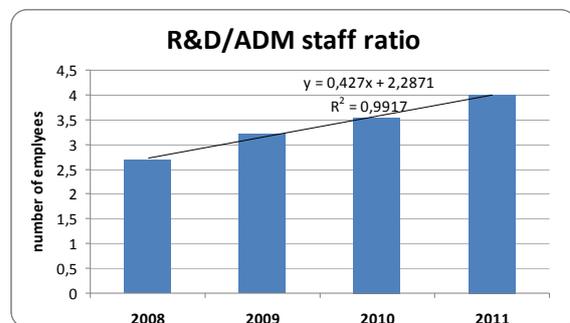
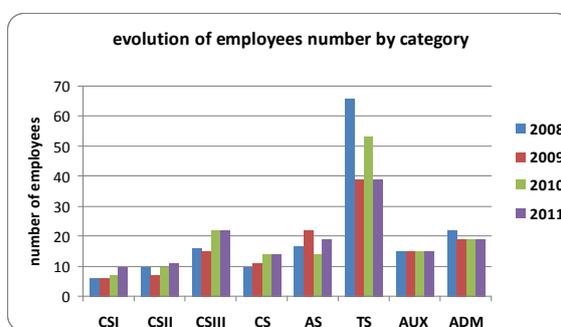
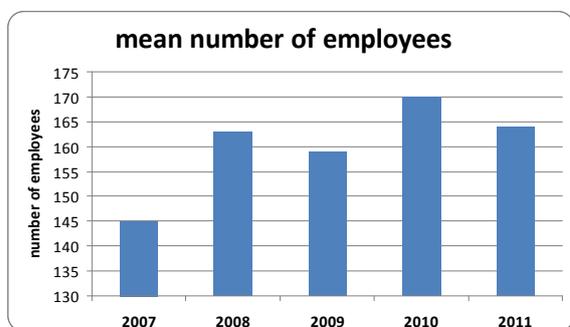
Staff

- 76 researchers, out of which 21 senior researchers and 19 PhD students, along with 39 technicians and 34 NRDS personnel (situation at 15 December 2011), with a mean age of 44 years, represent a critical mass for self-sustaining and further growth in biomedical research. Nevertheless, we are committed to recruit *young scientists* and to offer them adequate support to develop competitive

research. The stability of R&D personnel indicates that the institute assured appropriate conditions for research and career development.

Major achievements of the institute’s human resources policy

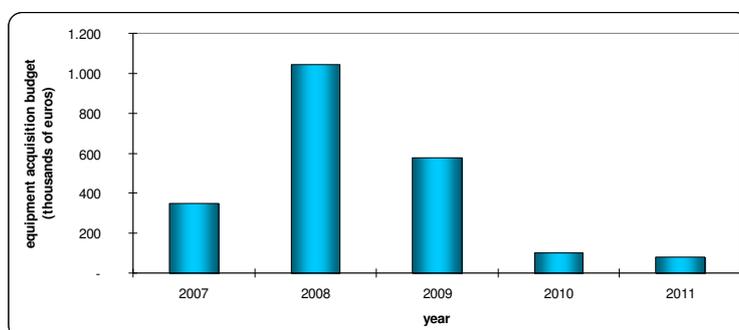
- **Recruitment as project coordinators of foreign scientists with outstanding scientific visibility:** Prof. Dr. Stefan Constantinescu and Prof. Dr. Giovanni Bussolati
- **Recruitment of young researchers with foreign experience in prestigious research institutions and universities:** *Valeriu Cismasiu* – specialization in stem cells: post-doctoral fellow at Lund University, Stem Cell Center, Sweden (2007-2009) and EMBL Mouse Biology Unit, Monterotondo, Italy; visiting scientist at Weatherall Institute of Molecular Medicine, Oxford, UK (2008-2009). *Andreea Tutulan-Cunita* – specialization in molecular biology and genetics; master degree (2000-2002) and PhD (2002-2005) at Hiroshima University, Japan; postdoctoral fellowship (2005-2006) at Manchester University, UK. *Sevinci Pop* – specialization in transcription activation, DNA-protein interaction-in vivo, chromatin structure and function, gene expression, transcription initiation; doctoral fellowship (2000-2002) and postdoctoral fellowship (2003-2008), University of Illinois at Urbana-Champaign, USA, Department of Cell and Developmental Biology. *Georgeta Cardos* – doctoral fellowship (2004-2008) at Hamburg University, Germany
- **Training of our researchers in prestigious research laboratories**



There is an obvious increase trend in the R&D/ADM personnel ratio during the last 4 years, proving an appropriate human resources policy according to the main activity of the institute (research).

The personnel structure reflects the needs of the institute for project development and for reaching excellence in life sciences research.

Infrastructure upgrading was one of the main priorities of our strategic plan for 2007 - 2011. Funds were obtained from 4 specific projects financed by the national Capacities Program and from research projects. Investments were focused on developing cytometry, imaging, genomics and proteomics research units, a biobank for tumor cells and nucleic acids and the animal husbandry.



*

Taking together our achievements in the last 4 years, we conclude that “Victor Babes” Institute of Pathology has a leading position in life science and biomedical research in Romania.

3. Activity reports by team

TEAM 1 - TELOCYTE - A NEW TYPE OF INTERSTITIAL CELL

Team leader: Laurentiu M. **Popescu**

Senior Researchers: Mihail Eugen **Hinescu**; Eugen **Mandache**

PostDoc researchers: Mihaela **Gherghiceanu**; Bogdan Ovidiu **Popescu**; Sanda **Cretoiu**

Laura Cristina **Suciu**; Valeriu Bogdan **Cismasiu**; Bogdan Gabriel **Marinescu**

PhD students: Catalin Gabriel **Manole**; Dragos **Cretoiu**; Mihnea Ioan **Nicolescu**

Technicians: Marin Teodor **Regalia**; Maria **Dumitrescu**; Rodica **Stanca**; Petrica **Musat**

L.M. Popescu, MD, PhD, Dr. h.c.mult., is currently Head of the National Institute of Pathology, Bucharest, Romania. He is member of the National Academy of Sciences and of the Academy of Medical Sciences. Recently, he became President Elect of the Federation of European Academies of Medicine, and of the International Society for Adaptive Medicine. He published over 100 scientific articles in international peer-review journals and is cited more than 1500 times. He has a Hirsch Index of about 30. Professor Popescu is Editor-in-Chief (and founder) of the Journal of Cellular and Molecular Medicine (Wiley/Blackwell), with a 5-year IF of 5. He is credited with the discovery of Telocytes.

Recently, our team discovered a new cell type in humans and mammals. We called recently (2010) these cells **Telocytes**, replacing the term previously used by us – Interstitial Cajal-like Cells (acronym: ICLC). This discovery is cited in more than 100 scientific papers, and very recently we are trying to impose the concept of “Telocytes / Stem-Cells Tandem” existing and working in the so-called Stem Cell Niches. The Telocyte concept is already adopted by many scientists: *e.g.* Eyden *et al.* – UK; Fausone-Pellegrini & Bani – Italy; Gittenberger-de Groot *et al.* – The Netherlands; Klumpp *et al.* – Germany; Kostin – Germany; Polykandriotis – Greece; Zhou *et al.* – China; Cantarero *et al.* – Spain; Gard & Asirvatham – USA; Gevaert *et al.* – Belgium; Marban *et al.* – USA; Limana *et al.* – Italy; Radenkovic – Serbia; Rupp *et al.* – Germany; Russell – USA; Tommila – Finland, etc.

Major research topic is the connective tissue (CT) cellular and molecular biology with emphasis on telocytes involvement in tissue physiology and pathology. CT represents the essential microenvironment to coordinate body structure and function hosting and joining together three major systems (the circulatory, nervous and immune systems) and integrating all others tissues and organs. More than 200,000 publications showed that CT is involved in organ development, renewing, repair, regeneration, and tumour development. Recently, new types of cells (*e.g.* resident mesenchymal stem cells, resident stem cells and telocytes) have been described but specific markers for the majority of the CT cells are still missing. Their detection and discrimination *in situ* is highly biased by their almost exclusive characterisation *in vitro*.

Major questions concerning CT biology must be addressed:

- How many distinct cell types reside in the adult CT?

- Are these cells tissue specific?
- Do stem cells need a specific CT microenvironment to survive and differentiate?
- How does the CT network change in specific pathological conditions?

We plan to perform a systematic inventory of the old and the new cell types of the CT, their molecular profiling, tissue distribution and interactions with emphasis on fibroblasts, fibroblast-like cells, telocytes, and mesenchymal stem cells.

Future research plans are to:

- define specific phenotypic, genetic and functional markers for particular CT cells and to assess their tissue specificity;
- identify novel cellular, molecular and signalling networks involved in CT physiology and remodelling;
- re-evaluate homo/heterocellular and cell-to-matrix communication;
- investigate cellular aspects of tumour-stroma interaction.

Our approach involves structural/ genomic/ transcriptomic/ proteomic/ functional assays using *in situ/ in vitro/ in vivo* models to define the CT 'connectomics'. CONNECT research could offer new ways for regenerative medicine (e.g. poly cellular treatment instead of stem cell approach only) as well as anti-tumour therapies for stromal tumours.

Main expected outcomes of our research:

- 1) Better characterized resident CT cells (e.g. fibroblasts, mesenchymal stem cells, telocytes) and their microenvironment
- 2) Endorsing markers for CT cells recognition *in situ*
- 3) Increasing knowledge about cellular and molecular mechanisms of tissue renewal
- 4) Better characterization of telocyte - the cell described in preliminary studies by our group

Existing equipment and facilities:

- electron microscopy unit (ultrastructural analysis, cellular tomography, array tomography):

Transmission electron microscope Morgagni 268 FEI, 100 kV; Olympus MegaView CCD; Electron microscope Tecnai G2 BioTwin Spirit FEI, 120 kV, single tilt holder; Olympus MegaViewG2 CCD; UV/Cryo-chamber EMS; LKB and RMC XL ultra-microtomes; diamond knives, ovens, etc.

- identification and localization of molecules by immunofluorescence: laser scanning microscope Nikon; microscopes Nikon E 600 with UV, CCD; 2 Nikon 200; Leica cryotom; immunostainer, paraffin embedding station; biobank for tissues, cells and nucleic acids; deep freezing unit; Leica microtome.

- light microscopy facility: motorized AxioZ1 Zeiss microscope with light bright field and epifluorescence, equipped with high resolution monochrome cooled CCD camera and image processing software.

- cell culture facilities: Laminar flow hoods; CO2 Incubators; Centrifuges; Phase contrast microscope, Sterilization unit, Ultra pure water system.

- cytometry unit: fluorescence and confocal microscopes, flow-cytometer Becton-Dickinson FACSCalibur

- **videomicroscopy:** Biostation IM (Nikon Corp., Japan).
- **cell layer impedance measurement:** xCELLigence (Roche Diagnostics, Germany).
- **microarray facility:** Agilent DNA Microarray Scanner (G2565CA) with SureScan High-Resolution Technology with Agilent Scan Control Software and Genomic Workbench; Agilent BioAnalyzer and hybridization oven.
- **molecular genetics facility:** Corbett Palm Cycler PCR, Corbett Real Time PCR;
- **nucleic acid isolation and manipulation facilities:** chemical hoods, water baths, centrifuges, spectrophotometer, transilluminator, freezers, ultrafreezers.
- **microRNA qPCR:** NanoDrop ND-1000 Spectrophotometer; QuantiMir RT -System Biosciences; iCycler system and software- Bio-Rad.
- **proteomics:** SELDI-TOF mass spectrometry system, Luminex-xMAP; 2D and 2D-DIGE; software – PDQuest; protein microarray platform.
- **animal Models Core Unit** (SR EN ISO 17025:2005)
- **image processing:** photographic laboratory; 4 Dell computers, 1 Siemens; Wacom Intuos 3 pen tablet; software for image analysis: Axiovision Zeiss; ImageJ -NIH, iTEM FEICo, Explore3D FEICo, Amira.

International collaboration:

- **Prof. Maria-Simonetta Fausone-Pellegrini** and **Prof. Daniele Bani** from Department of Anatomy, Histology and Forensic Medicine, University of Florence, **Italy**
- **Prof. Ofer Binah** from the Department of Physiology; Ruth and Bruce Rappaport Faculty of Medicine, Technion Israel Institute of Technology, Haifa, **Israel**
- **Prof. Sawa Kostin** from Max-Planck Institute for Heart and Lung Research, Franz Groedel Institute, Bad Nauheim, **Germany**
- **Prof. Shengshou Hu** from the Center for Cardiovascular Regenerative Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, **China**
- **Prof. Changyong Wang** from the Tissue Engineering Research Center, Academy of Military Medical Sciences, Beijing, **China**

TEAM 2 - SURGICAL AND MOLECULAR PATHOLOGY

Team coordinator: CS1. Prof. Dr. Carmen Ardeleanu, Head of Pathology Department

Mission: Obtaining new tissue biomarkers regarding the structure and molecular profiles of pathological processes (tumors, inflammations etc.)

Research focus:

- ***Molecular biology of malignant tumors focusing on inter- and intracellular signaling.:***
exploring signaling pathways molecular factors
 - development and implementation of high throughput methodologies using in vitro assays with particular end-points, for identifying and characterization of new biomarkers of cell signaling in tumors
 - partnership with other research teams from clinical oncology, surgical clinics, for testing and promoting new diagnostic and therapeutic tools potentially efficient in cancer
- ***Identifying new genes implication in tumor progression by means of genotypic and phenotypic profiles***
 - implementation of advanced molecular methods for characterizing the genetic variability of malignant tumors
- ***Developing and extending standardized methods for processing and stock tissues aiming to obtain available results for the telepathological interpretation.***

Research topics:

1. Cellular signaling pathways in malignant epithelial tumors

No	Research area / coordinator	Research contracts / budget (Euro)
1.1	Tumor-microenvironment interactions CS1 Prof. Dr. Carmen Ardeleanu CS3 Biol. Sp. Georgeta Butur CS2.Dr. Dorel Arsene CS3 Dr. Florina Vasilescu CS Dr. Alina Grigore	F20/140.968,04 F70/154586,78
1.2	Intercellular signaling in malignant lymphoma CS1 Prof. Dr. Carmen Ardeleanu CS3 Ass Prof. Dr. Camelia Dobrea Dr. Florina Cionca	F21/ 90.211,59
1.3	Signal transduction anomalies in epithelial malignant tumors CS3 Dr. Cristina Iosif CS3 Dr. Florin Andrei	F40 / 127.272,73

2. Molecular bases of therapy modulation in malignant tumors

No	Research area / coordinator	Research contracts / budget (Euro)
2.1	Breast cancer molecular features Ass.Prof. Dr. Maria Comanescu	CF10 / 68.181,82
2.2	Microsatellite instability of colon carcinoma CS2 Ass.Prof. Camelia Vrabie CS2 Prof. Dr. Maria Sajin	F19 / 159.090,91
2.3	Proteinkinase receptors in lung carcinoma CS3 Dr. Florina Vasilescu	F69 / 45.271,59

3. Genotypic profiles variability in neoplasia

No.	Research area / coordinator	Research contracts / budget (Euro)
3.1	Molecular genetics in epithelial malignant tumors Senior Res Prof. Dr. Gianni Bussolati Prof. Dr. Carmen Ardeleanu	CF8 / 1.231.125,00 F24/150.000,00

4. Phenotypic and genotypic profiles in inflammatory and degenerative diseases

No	Research area / coordinator	Research contracts / budget (Euro)
1.1	Proapoptotic and antiapoptotic factors CS1 Prof. Dr. Carmen Ardeleanu CS3 Biol. Sp. Georgeta Butur CS Dr. Alina Grigore	F22/102.272,73
1.2	Cellular interactions in autoimmune diseases CS1 Prof. Dr. Carmen Ardeleanu CS3 Ass Prof. Dr. Dana Terzea Dr. Florina Cionca	F26/ 93.429,55
1.3	Inflammatory disease of bowel Ass.Prof. dr. Gasbriel Becheanu CS3.Dr. Cristina Iosif	F72/ 45.454, 56 F73/29.021,82 F74/40.590,73

Research team

Name	Laboratory	Name	Laboratory
CS1 Carmen Ardeleanu	Head of pathology Department	Biol. Gaina Gisela	Histopathology Department
CS 3 Biol sp. Georgeta Butur	Head of Diagnostic Center Team coordinator	CSIII. Dana Cristina Terzea	Histopathology Department
CSI Prof. Gianni Bussolati	Histopathology Department	CSII Dorel Eugen Arsene	Histopathology Department
C3 Florina Vasilescu	Histopathology Department	CSII Camelia Vrabie	Histopathology Department
CS 3 Camelia Dobrea	Histopathology Department	TS. Florina Alexandru	Histopathology Department
C3 Cristina Iosif	Histopathology Department	TS. Alina Anghel	Histopathology Department
CS Alina Grigore	Histopathology Department	TS. Valentina Muntean	Histopathology Department
Dr. Gabriel Becheanu	Histopathology Department	TS. Elena Ion	Histopathology Department
Dr. Maria Comanescu	Histopathology Department	TS. Tatiana Nora Petre	Histopathology Department
Res Assist Adina Balan	Histopathology Department	Ts. Florina Manda	Histopathology Department
Cs. Dr. Georgeta Cardos	Histopathology Department	TS. Daniel Anghel	Histopathology Department
CSII. Maria Sajin	Histopathology Department	TS. Monica Haghight	Histopathology Department
Dr. Florina Cionca	Histopathology Department		
ASC Staicu Viorela	Histopathology Department		

- PhD students: molecular biology of nonsmallcell lung carcinoma; breast carcinoma molecular features in young women; genic anomalies in colon carcinomas
- Post doc: Advanced studies in molecular biology and gene profiling by gene microarray in breast triple negative breast carcinoma.

Training: **1.** Systemic pathology , European School of pathology, Craiova: pancreas and liver– 2007 (3 researchers), gastro-intestinal pathology – 2008 (4 researchers), gynaecological pathology – 2009 (3 researchers), male genito-urinary tract pathology – 2010 (3 researchers), thyroid pathology – 2011 (3 researchers); **2.** Agilent Gene microarray training for theory and practical applications (3 researchers). **3.** Stem cell implication in regenerative medicine, London, 06.10.2011 (1 researcher).

Methodological approach

- To *apply* in surgical and molecular medicine the *know-how* of our research team in *human pathology, cellular and molecular biology*

- Histopathology , cytopathology, histochemistry, immunohistochemistry, in situ hybridization (fluorescent and chromogenic), PCR, Real-time PCR, reverse -transcription PCR, PCR array, gene profile microarray (morphological analysis of tumors, immune phenotyping on archived tissue, for cell differentiation, typing secreting cells, identification of intercellular and intracellular signaling factors, identification of diagnostic, prognostic and predictive factors in tumors, cellular activation and proliferation biomarkers, apoptosis, amplification of genome sequences in infections and tumors, genes mutations, gene profiles of tumors), etc.

Infrastructure

Histopathology unit: accreditation according to SR EN ISO 15189 for histopathology and immunohistochemistry (microtome, automatic tissue processor, vacuum automatic tissue processor, scientific microscopes, professional microscopes, routine staining machine); **Laboratory of Immunohistochemistry unit:** (immunostainers, water baths, microwave oven, automatic cover slipping machine); Hybridization compartment (hybridization plate, fluorescence microscope – Nikon 800, water bath); Molecular diagnosis compartment (thermocyclers, real-time PCR automatic system, GEL-Doc, Nanodrop, nucleic acids extractor).

International project proposals

Proposal full title: Molecular workflow for the effective detection of multiplex HPV markers in cervical cancer patients Proposal acronym: CERVIFLEX, Type of funding scheme: Collaborative Project Small and medium-scale focused research Work programme topics addressed: HEALTH.2010.1.2-1 Name of the coordinating person: Prof. Dr. Giorgio Stanta

Bilateral Italian-Romanian project: Impact of immuno- and geno-typing for improving diagnoses and planning treatment of human tumors. **SUPPORT ACTION FOR BILATERAL COOPERATION, 2005-2008**

TASTE: Telepathological assessment of histopathological and cytological techniques (financed) **Education and Culture-Lifelong Learning Programme, 2011-2014, Pr. Nr. 519108-LLP-2011-1-IT-KA3-KA3MP**

Partner institutions

1. University of Medicine and Pharmacy “Carol Davila”, Bucharest; **2.** University of Bucharest; **3.** University of Medicine and Pharmacy Craiova, **4.** “Gr.T.Popa” University of Medicine and Pharmacy, Iassy, **5** “Victor Babes” University of Medicine and Pharmacy, Timisoara, **6** “Ion Cantacuzino” National Institute of Immunology and Microbiology, **7** Università degli Studi di Torino, Italy, **8** Roche Pharma

Publications

6 ISI publications A1,A2,A14,A28, A26,A71

We were involved in national project regarding cellular signaling pathways in malignant epithelial tumors; molecular basis of therapy modulation in malignant tumors; genotypic profiles variability in neoplasia; phenotypic and genotypic profiles in inflammatory and degenerative diseases

Other relevant publications: O2,O6, O10, O11, O16, O31, O40, O53, O61, O65, O95, O33, O43, O54, O62, O66, O109.

Development plan

I. New research areas:

1. Improvement of molecular signature detection in cancer diagnosis, prognosis and therapy.
2. Applied oncology: Development of molecular approach of archived tumor tissue aiming to offer new potential biomarkers of predictive value in malignant tumors.
3. Development and adapting molecular biology technologies for early detection of malignant tumors and improvement of the therapy.

II. Patent submission for new molecular tools in cancer.

III. Validation of new experimental models for evaluation of tumor aggressiveness.

IV. Introduction of new validated investigation methods in medical practice.

TEAM 3 - TRANSLATIONAL MEDICAL RESEARCH IN CANCER

Team coordinator: CS3. Biol. Sp. Georgeta Butur, Head of Diagnostic Center

Mission: translation of high level technology in medical practice to improve survival and life quality of cancer patients

Research focus:

- Development of molecular diagnosis
- Implementation of new prognostic and predictive biomarkers in malignant tumors
- Application of advanced methods for identification of molecular targets in cancer therapy.

Research topics:

1. Molecular identification of etiologic factors in infections and associated tumors

No	Research area / coordinator	Research contracts / budget (Euro)
1.1	Molecular diagnosis of mycobacterial infections CS1 Prof. Dr. Carmen Ardeleanu CS3 Biol. Sp. Georgeta Butur Res. Ass. Biol. Sp. Diana Teletin	F23/22.727,27
1.2	Hepatitis viruses implications in chronic lymphoproliferations CS3 Ass Prof. Dr. Camelia Dobrea CS3 Biol sp. Georgeta Butur	F18/ 39.897,73
1.3	Gene E4 expression of HPV in cervical lesions CS3 Dr. Florin Andrei	F68 / 81.433,86

2. Prognostic and predictive biomarkers in epithelial malignant tumors

No	Research area / coordinator	Research contracts / budget (Euro)
2.1	Biomarkers in malignant tumors CS3 Dr. Florin Andrei CS1 Prof. Dr. Carmen Ardeleanu	F67 / 14.09,91. F24/150.000
2.2	Therapeutic targets in nonsmall cell lung carcinoma, liver disease, melanoma CS3 Dr. Florina Vasilescu CS3 Dr. Camelia Dobrea CS1 Prof. Dr. Carmen Ardeleanu	F70 / 154.586, 78 F17/113.640 F25/45.450
2.3	Prognostic markers in hepatocellular carcinoma, melanoma Asso. Prof. Mariana Costache Asso. Prof Dr. Gabriel Becheanu	F88/121.990 F90/16.540 F71 / 56.818,18
2.4	Biomarkers of renal carcinoma involved in therapy Dr. Mihaela Mihai	F95/ 68.181,82

3. Therapeutic targets in malignant tumors

No.	Research area / coordinator	Research contracts / budget (Euro)
3.1	High-throughput methodologies in personalized oncology) Senior Res Prof. Dr. Gianni Bussolati	CF8 / 1.231.125,00
3.2	Molecular targets in the therapy of malignant tumors CS1 Prof. dr. Carmen Ardeleanu CS3 Biol.sp. Georgeta Butur	Medical services contracts (ROCHE)
3.3	Immunohistochemical biomarkers in malignant tumors therapy CS1 Prof. Dr. Carmen Ardeleanu CS3 Dr. Florina Vasilescu, CS3 Dr. Camelia Dobrea, CS3 Dr. Florin Andrei, CS3 Dr. Cristina Iosif , CS Dr. Simona Enache, CS Dr. Alina Grigore, Ass. Prof. Dr. Maria Comanescu	Medical services contract with Pharma St. Invest., Medical Insurance Agencies (Bucuresti,Dambovita, Pitesti, Prahova, Vrancea, Buzau, Ilfov, Bacau, Giurgiu, Calarasi)

Research team

Name	Laboratory	Name	Laboratory
CS1 Carmen Ardeleanu	Head of pathology Department		Histopathology Department
CS 3 Biol sp. Georgeta Butur	Head of Diagnostic Center Team coordinator	Res Ass. Staicu Viorela	Histopathology Department
	Histopathology Department	Biol. Gaina Gisela	Histopathology Department
C3 Florina Vasilescu	Histopathology Department	TS. Catalina Culda	Histopathology Department
CS 3 Florin Andrei	Histopathology Department	TS. Florina Alexandru	Histopathology Department
CS 3 Camelia Dobrea	Histopathology Department	TS. Alina Anghel	Histopathology Department
C3 Cristina Iosif	Histopathology Department	TS. Valentina Muntean	Histopathology Department
CS Simon Enache	Histopathology Department	TS. Elena Ion	Histopathology Department
CS Alina Grigore	Histopathology Department	TS. Tatiana Nora Petre	Histopathology Department
CS Maria Neagu	Histopathology Department	TS. Daniel Anghel	Histopathology Department
Dr. Mihaela Mihai	Histopathology Department	TS. Monica Haghigat	Histopathology Department
Dr. Mariana Costache	Histopathology Department	TS. Elena Naita	Histopathology Department
Res Assist Adina Balan	Histopathology Department	Dr. Simona Chirlomez	Histopathology Department
Res Ass. Diana Teletin	Histopathology Department	TS. Georgiana Preda	Histopathology Department
Dr. Valentin Enache	Histopathology Department	TS. Georgeta Melinte	Histopathology Department
	Histopathology Department	TS. Ene Eugenia	Histopathology Department

PhD students: breast cancer stem cells, molecular features in GISTs, Parasitic infections in humans, phenotypic and genotypic aspects in renal cell carcinoma, Endometrial carcinoma - immunohistochemical and molecular characteristics.

Training: ESOT 2009, Paris (2 researchers), International course of pathology of digestive tract, Bucharest, 2008 (5 researchers), 2009 (4 researchers), 2010 (6 researchers), 2011 (3 researchers), Diagnostic histopathology of soft tissue tumors, Treviso Italy, 2008 (4 researchers), Breast pathology, Harvard Medical School, Boston, 2007 (2 researchers).

Methodological approach

– To improve the diagnostic in pathology of tumors

Histopathology, cytopathology, histochemistry, immunohistochemistry, in situ hybridization (fluorescent and chromogenic), PCR, Real-time PCR, (morphological analysis of tumors, immune phenotyping on archived tissue, for cell differentiation, typing secreting cells, identification of intercellular and intracellular signaling factors, identification of diagnostic, prognostic and predictive factors in tumors, cellular activation and proliferation biomarkers, amplification of genome sequences in infections and tumors).

- To *collaborate* with medical institutes and clinical hospitals and ambulatories at national and international level, with *complementary expertise* using high throughput methodologies.

Infrastructure

Diagnostic center for translation of know-how and the results of research as services to regional and local health units, aiming to a higher quality of life and to improve the policy of healthcare by personalized therapy.

Histopathology unit: accreditation according to SR EN ISO 15189 for histopathology and immunohistochemistry (microtome, automatic tissue processor, vacuum automatic tissue processor, scientific microscopes, professional microscopes, routine staining machine); ***Laboratory of Immunohistochemistry unit*** (immunostainers, water baths, microwave oven, automatic cover slipping machine); Hybridization compartment (hybridization plate, fluorescence microscope – Nikon 800, water bath); Molecular diagnosis compartment (termocyclers, real-time PCR automatic system, GEL-Doc, Nanodrop, nucleic acids extractor).

International project proposals

Characterization of early disseminating tumorigenic breast cancer cells with CD44⁺CD24^{-/low} phenotype and their microenvironment in the bone marrow and bone of patients with Breast cancer

Proposal acronym: METASTEM; Type of funding scheme: Collaborative Project: Small or medium scale focused research project Work programme topics addressed: HEALTH-2007-2.4.1-6: Understanding and fighting metastasis, Name of the coordinating person: Prof. Dr. T. Bauernhofer,

You have submitted a proposal to the Electronic Proposal Submission System. Your proposal is now stored on the EPSS system with **number 201269** for subsequent evaluation by the Commission. (nonfinanced).

Partner institutions

1. University of Medicine and Pharmacy “Carol Davila”, Bucharest; **2.** Medical services contract with Pharma St. Invest., **3** Medical Insurance Agencies (Bucuresti, Dambovita, Pitesti, Prahova, Vrancea, Buzau, Ilfov, Bacau, Giurgiu, Calarasi, **4.** “Gr.T.Popa” University of Medicine and Pharmacy, Iassy, **5** “Victor Babes” University of Medicine and Pharmacy, Timisoara, **6** “Ion Cantacuzino” National Institute of Immunology and Microbiology, **7.** Universita degli Studi di Torino, Italia, **8** Roche Pharma

Publications

1. Diagnosis of difficult cases: A1,A2,A26

- we were involved in national project regarding biomarkers in malignant tumors (lung, breast, malignant lymphomas) and degenerative disease.
- **Other relevant publication:** O34, O44, O57, O63, O81, O35, O36, O48, O59, O82, O37, O39, O135, O136.

Development plan

I. New research areas:

1. Development and introduction in the **Diagnosis Center** of new molecular tools (RT-PCR, microRNA, PCR-array) applied on archived tumor tissue for a more accurate identification of new molecular targets in personalised therapy.
2. Development of multi-skill teams (pathologists, genetists, biochemists, biologists, oncologists, biophysicists, biostatisticians), for molecular diagnosis of malignant tumors by means of high-throughput technologies.

II. Validation and patent submission for newly developed molecular tools in the diagnosis and prognosis of cancer.

III. Theoretical and practical training of pathologists aiming to improve the diagnosis in cancer for applying novel personalized therapies.

TEAM 4 - ULTRASTRUCTURAL PATHOLOGY LABORATORY

The **Ultrastructural Pathology laboratory (UPL)** is fully equipped laboratory for transmission electron microscopy with modern ancillary equipment and a new FEI Morgagni 100 kV Transmission Electron Microscope (acquired in 2008) for ultrastructural investigation in diagnosis and research. Since 2009, UPL hosts also a Tecnai G2 Spirit BioTWIN Transmission Electron Microscope with single tilt holder which undoubtedly will attract great scientific projects focused on data collection for electron tomography. This technique allows 3D reconstruction of large molecules, organelles and small cells.

The UPL is headed by Mihaela Gherghiceanu MD, PhD who has extensive experience in both diagnostic and research applications of electron microscopy and electron tomography. Additional expertise is provided by Eugen Mandache MD, PhD who has over 40 years of experience in the ultrastructural evaluation and diagnosis. He was head of the laboratory until 2010. Technical support is provided by three highly trained electron microscopy technicians and more than 400 samples are processed for ultrastructural investigation for diagnosis or research.

UPL TEAM AND ASSOCIATE RESEARCHERS

Mihaela Gherghiceanu, MD, PhD (48 ISI papers; 557 ISI citations, h-index 15)

Eugen Mandache, MD, PhD (25 ISI papers; 169 ISI citations, h-index 6)

Mihail Hinescu MD, PhD (30 ISI papers; 421 ISI citations, h-index 11)

Elena Moldoveanu, PhD (28 ISI papers; 82 ISI citations, h-index 5)

PostDoc: Laura Suciu MD, PhD; Marta Daciana PhD

PhD students: Gabriela Catalin Biol; Catalin Manole MD

EQUIPMENT

- Ultrastructural analysis _ electron microscopy and tomography.

Transmission electron microscopes: Morgagni 268 FEI, 100 kV with CCD; Tecnai G2 BioTwin Spirit FEI, 120 kV, single tilt holder with CCD.

Ancillary electron microscopy equipment: RMC XL ultra-microtomes; UV/Cry-chamber EMS; Leica cryo-substitution system, diamond knives, ovens, etc.

- Identification and localization of molecules by immunofluorescence: microscopes Nikon E 600 with UV, ZEISS CCD 11Mp; Nikon 200; Leica cryotom.

- Image processing: photographic laboratory; 4 Dell and 1 Siemens computers; scanners; software for image analysis (iTEM Olympus, FEI Explore3D, Amira Visage Imaging).

DIAGNOSIS

The UPL is a nationally recognized, reference center for diagnostic renal pathology and electron microscopy. Diagnostic services are available and include diagnostic evaluation of kidney, muscle, nerve, skin, liver and other biopsy material (~200 specimens/year). In addition to routine ultrastructural techniques, we offer specialized immunofluorescence analysis of biopsy specimens (e.g., kidney, skin).

RESEARCH

Research projects carried out in the last 5 years by senior researches of UPL were financed with more than 1.000.000 euro. Based on the results obtained from the ultrastructural studies performed in UPL, 36 articles (245 ISI citations) have been published in international journals in the last 5 years. Ultrastructural analysis for "Telocytes" project has been performed in UPL.

COLLABORATIVE PROJECTS (preliminary studies)

- **Ultrastructure of hESC and iPSC derived cardiomyocytes** - collaborative project with **Prof. Ofer Binah** from the Department of Physiology; Ruth and Bruce Rappaport Faculty of Medicine, Technion Israel Institute of Technology, Haifa, Israel
- **Zebra fish heart regeneration** -collaborative project with **Christopher Antos, Ph.D.** laboratory Center for Regenerative Therapies; Technische Universitaet Dresden; Germany
- **Engineered heart tissue** - collaborative project with **Prof. Changyong Wang** from the Tissue Engineering Research Center, Academy of Military Medical Sciences, Beijing, China
- **Telocytes in human heart pathology** - collaborative project with **Prof. Shengshou Hu** from the Center for Cardiovascular Regenerative Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China

FUTURE DIRECTIONS

Infrastructure investment plan and strategy

Correlative Microscopy at cryo-temperatures

Integrated or correlative microscopy attempts to combine multidimensional information from complementary techniques to bridge the various resolution gaps and thus to be able to integrate structural information gathered from multiple levels of the biological hierarchy into one common framework. Cryo-fluorescence microscopy for example can be exploited to navigate the cellular landscapes for features of interest before zooming in on these areas by cryo-electron tomography. It offers an independent and unambiguous confirmation of the identity of the investigated features. For correlative studies we aim to acquire a cryo-holder and cryo-ultramicrotome. We also envisage the acquisition of a state-of-art confocal laser scanning microscope with 3-5 channels for simultaneous detection of multiple markers in multi-labelling protocols.

Research focus

Cellular and molecular determinants of heart regeneration

Stem cell therapy for cardiac diseases has been started before an intrinsic regenerative capacity of heart to be proved and accepted. The dogma that mammalian heart is terminally differentiated organ has been challenged by the reports of few types of resident cardiac stem or progenitor cells. Moreover, a new type of interstitial cell – telocyte - has been described in the adult heart and one important role seems to be nursing stem cells and progenitors in the cardiac stem cell niches. We plan to study the cellular and developmental biology of cardiac stem niches and their involvement in cardiac renewal considering that basic mechanisms governing its physiology are still unknown. By extensive ultrastructural investigation (electron tomography included), confocal microscopy and miRNAs detection we plan a basic research of telocytes and cardiac stem cell niches in normal, ageing and diseased mammalian heart. We also will run a comparative study of regeneration in mammalian and zebrafish (known to have high regenerative capacity) injured hearts. We will try to answer major question: there are one or more types of cardiac stem cell; which cells are mandatory for cardiac renewal; which factors are most important in stem cell differentiation; how newly formed cardiomyocytes are integrated in contractile myocardium; how all these are challenged in diseased heart? All these questions must be answered before an effective cell therapy could be envisaged.

ULTRASTRUCTURAL PATHOLOGY LABORATORY DEVELOPMENT PLAN

I. RESEARCH AREAS

1. Basic research in fundamental mechanisms of cardiac regeneration - from stem cell to heart tissue

- The identification and classification of somatic stem and progenitor cells that reside in the adult heart.
- The definition of heterocellular networks that direct stem cells toward a cardiac fate.
- Comparison of specific cardiac and hematopoietic stem/ progenitor cells by correlative microscopy (fluorescent and ultrastructural analyses).
- The establishment of fate-mapping strategies to define the contribution of telocytes and selected stem/progenitor cell populations to the cardiac lineage during development and after myocardial injury.

2. Structural and molecular characterization of intercellular communication in the interstitial space

- Spatial structure in intercellular interactions - electron tomography of classical junctions
- Molecular and structural characterization of 'atypical' hetero-cellular junctions in the interstitial space by correlative microscopy

2. Collaborative projects

- Specialized assistance for the research projects involving electron microscopy / tomography (research design and technical expertise): virus ultrastructure and virus -cell interaction; nanoparticles interaction with human cells; induced pluripotent stem cells characterization and tissue integration, etc.
- development of ongoing international collaborative projects

II. HUMAN RESOURCES DEVELOPMENT

- Researcher (junior or senior post-doc) for data collection, computer vision & image processing for electron tomography of cells and macromolecules
- Electron Microscopy Research Technician to oversee the laboratory's facilities; to assist and train visitor researchers

III. INFRASTRUCTURE DEVELOPMENT FOR CORRELATIVE MICROSCOPY

- confocal laser scanning microscope
- cryo-holder for TECNAI BioTwin
- cryo-ultramicrotome

TEAM 5 - PROTEOMIC-BIOMARKERS

Team coordinator: CS2 Dr. Cristiana Tanase,

I. The quality of the results of the research activity

Mission:

Research focus: Advanced proteomics: in discovery and application of proteomics biomarkers.

“Core concept”: application of multiple proteomics technologies/platforms in “biomarker driven” research in diagnostics and pharmacology. Research areas:

- biomarker discovery and application as powerful diagnostics instruments in cancer,
- proteomics biomarkers in pharmacological research
- proteomics in the evaluation of environmental risks for human health,
- integration in European Research Area and medical services for biochemistry analyses.

Approach:

Multidisciplinary approach, based on integrating proteomics research with other key fields, generating specific investigation platforms, addressing biomarker research in diagnostics, pharmacology and toxicology. **To apply** in clinical diagnostics, pharmacotoxicology the know-how of our research team in proteomics, pathology, immunology, cellular and molecular biology, bioinformatics. Key technologies integrated: SELDI-TOF-MS, 2D-DIGE electrophoresis, multiplex-xMAP analysis, protein microarrays, ELISA, cell-cultures.

Goals:

- **To provide and translate research** results in the field of biomarkers discovery (mainly in cancer) into clinical applications; transferring towards hospitals, Ministry of Health and Health Insurance House of technologies/protocols and/or services for the determination of biomarkers.
- **To expand and integrate biomarkers research** to other disease areas that may benefit from the use of biomarkers in diagnostics, patient stratification, monitoring, etc.
- To **collaborate** within **consortia** joining institutions with **complementary expertise** and **multidisciplinary teams** to apply individual and/or panels of biomarkers in the fields of molecular diagnostics, pharmacology and risk assessment.
- Preparedness for the **integration in European Research Area**

Expertise: proteomics techniques (mass spectrometry, electrophoresis, multiplex-analysis, immunoassays, cell cultures, (immuno)-toxicology, implementation of techniques for biomarker detection (cytokines, chemokines, growth factors, signal transduction molecules, protein profiling, nucleic acids), bioinformatics. The state-of-the-art technology available in these laboratories and the expertise of the team allow complex, interdisciplinary studies

Research projects:

The Proteomics team is involved in **3** international ongoing **projects** in cancer research and drug testing: CF14 - POS CCE 2.1.2, 152 “Proteomics technologies for cancer biomarkers discovery”; CF11 - bilateral cooperation Romania- China; “Biomarker discovery in digestive tract cancer and skin melanoma using proteomic approaches” and CF16 - FP7- PIRSES-GA-2008-230816 “Natural Antidiabetic and Antihypertensive Drugs. The team was involved in 19 national projects, as follows: cancer 7, pharmacotoxicology 6, risk assessment 6, networking – 3.

Scientific outcomes:

The results materialized in 15 articles, out of which 10 are published in journals with non-zero relative Article Influence Score (cumulative score: 17,70719) and 6 books/book-chapters at international publishers, and over 30 presentations at prestigious international conferences with ISI indexed abstract books, of which 7 as invited speakers.

Relevant results:

- (1) The role of Caveolin-1 in cancer progression: we contributed to the annotation of this gene in cancer pathology
- (2) Evaluation of specific markers for proliferation, apoptosis and angiogenesis in cancers
- (3) Key signalling molecules and the main microRNAs in pituitary and, digestive tumours respectively.
- (4) Importance of immune markers in the diagnosis, prognosis and therapy monitoring of cutaneous melanoma.

Integration of proteomic biomarkers with in vitro models for the assessment of safety and efficacy of new potential drugs

Strategic scientific objectives and directions

The major target of the group is to enhance its performance in biomarkers studies, by continuing to develop its output in proteomics biomarkers research. As established priorities for the medium and long term, the team identifies:

- a) Diagnostic “omics” – development of complex, high performance biomarker based tools with application in the fields of diagnostics, monitoring, therapy optimization and personalization for cancer, to foster the transfer the approach in other major diseases that will benefit of similar instruments.
 - Application of “Omics” technologies for high-throughput biomarker studies
 - Signal transduction and other regulatory mechanisms in cancer and cancer stem cells
 - Systems medicine approaches in biomarker discovery (integration of proteomics, miRNomics, interactomics)
 - Biomarker panels for early diagnostics, optimized and personalized therapies; integration of proteomic, miRNA and other biomarkers.
 - Development of “customized” detection instruments
 - Translational research: transfer of protocols in clinical units
- b) Pharmaco”omics” – profiling induced modulation of expression and activation key regulatory molecules (signal transducers, miRNAs, cytokines) addressing oriented and personalized therapies. Creating a “biomarker driven platform” and validated experimental models for the safety and efficacy assessment in pharmacology and toxicology

II. Quality of human resources

The group comprises 8 senior scientists, 5 young scientists (including 3 Ph.D. students), and 3 lab technicians, with and involvement of 6,05 (scientists) and 2,8 (technicians) full time equivalents. Due to the complementarities of basic and advanced trainings, the group is inter-disciplinary, covering expertizes in medicine, molecular biology, proteomics, bioinformatics, toxicology, immunology. The group has an average age of 37.7 years.

Name	Title	Involvement*	Name	Role	Involvement*
Cristiana Tanase	MD, PhD, CS2	0.60	Mihail Hinescu	MD, PHD, CS1	0.1
Radu Albulescu	Biochemist, PhD, CS1	0.25	Monica Neagu	Biochem, PhD, CS2	0.25
Stefan Constantinescu	MD, PhD, Prof.	0.50	Mircea Leabu	Chem., Ph.D., CS2	0.30
Ionela Daniela Popescu	Chemist, PhD student, CS	0.70	Carolina Constantin	Biochem., PhD, CS3	0.25
Elena Codrici	Biologist, PhD student, CS3	0.80	Irina Radu	Technician	0.80
Lucian Albulescu	Biochemist, PhD student	0.70	Nicoleta Constantin	Technician	1.00
Simona Mihai	MD, RA	1.00	Nicuta Lopazan	Technician	1.00
Alina-Ionela Nita	Economist, RA	0.80			

*see the structure of Research Teams

Continuous education and training: An estimated 6 persons/month spent in the last years for training, covering advanced laboratory techniques in proteomics, bioinformatics etc. Examples of training courses: International course Advanced Proteomics, St.George’s Medical Biomics Centre, University of London, Protein Protein Interaction, European Institute of Bioinformatics, Cambridge, England (2011), SELDI-TOF-Advanced Training Course, Biorad-Paris (2010), ProteinChip SELDI-Basic Training Course, USA (2008), Luminex IS 2.3 Basic Training and Luminex Fundamental Assay Techniques, Oosterhout, The Netherlands, 2D electrophoresis training, Prague, Protein Arrays for Biomarker Discovery and Protein Expression Profiling and Profiling Kinases for Disease Biomarker and Drug Target Discovery, Amsterdam (2007). **Bursaries:** FEBS 2010 Gothenburg, Sweden (2 persons), FEBS 2011, Turin, Italy (2 persons)

Exchanges of Personnel: PIRSES-GA-2008-230816 (CF16 Ongoing)

Mobilities senior scientists: Invited speakers & Chairmen – Molecular Diagnostics Europe, 2011, London; Cancer Proteomics, 2010, Berlin; Molecular Diagnostics Europe, 2010, Hannover; European biomarkers Congress, 2010, Florence; Proteomics Europe Congress, 2009, Barcelona; BIT Life Sciences' and Molecular Diagnostics, 2009, Beijing

III. Quality of Infrastructure and degree of exploitation

Based on a coherent policy, the group established and maintained a relevant rate of development of research infrastructure, that parallels the progression of its scientific outcome. Thus, the group established capabilities of research in proteomics that allows multiple, complementary investigations, such as de novo discovery of novel biomarkers (supported by Mass spectrometric, bidimensional electrophoresis and DIGE platforms), multiplex quantitative assays by fluorescence (Luminex xMAP and Luminex xTAG) for cytokines, growth factors, hormones, signal transducers, miRNA, etc). Also, other techniques such as ELISA assays, Western blot, on chip electrophoresis and cell culture assays are available and applied by the group.

Domain	Equipment	Level of exploitation	Year of purchase
Proteomics	2D electrophoresis	25%	2007
	2D-DIGE (Typhoon 9000)	<25%	2011
	SELDI-ToF-MS	75%	2008
	Western blotting	25-50%	2007
	Multiplex xMAP® technology	100%	2007
	Protein microarray	< 25%	2010
	Complete ELISA lines	100%	2007
	MiniVIDAS	100%	2007
	HITACHI 912	100%	2005
In vitro assays	xCELLigence	25%	2010
	Complete unit for cell culture	75%	2007-2009
	Microplate Multimode Detector, Anthos Zenyth 3100	75%	2009

Most of the existing equipment is purchased and installed after 2007, and based on functionally proven “state of the art” technologies for both hardware and software components. Validation of the results obtained using the key equipments (thus, of whole “operating chains” for each specific application) is a standard procedure of the group. Interlaboratory comparisons (where such schemes are available) or staff exchange/visits in other laboratories (such as at Biorad labs in Malverne, US, or in “Biomics Center” at St. George’s University, London, UK

While mostly used (60% of the functioning time) for the projects coordinated by the senior members of the group, the research infrastructure was also used in projects conducted by other groups, in which individual members of the groups were involved (ca. 30%), while a quota was reserved also for activities involving visiting scientists (e.g. in the NAAN project, during 2010-2011, when 5 scientists visited and worked for 1 month each in the laboratory.

International collaboration – Biomics Medical Centre, Saint George’s University of London; Beijing Institute of Genomics, Chinese Academy of Science and BioRad Proteomic Research Center, Malvern, USA and in the FP7 project NAAN – Graz University, University of Lecce, Sekem Egypt..

Recognition at national and international level:

The group members are presently involved in international and national activities, as follows:

- National Expert in The member State Group of Innovative Medicines Initiative – Joint Undertaking (IMI-JU), Expert in the Program Committee FP7 Health of the EC, Expert evaluators for the FP7 (themes Health - 2010, 2011 calls, NMP - 2011 call), Expert evaluators for the programme “EuroNanoMed”, (2010, 2011 calls), Expert-evaluators for National Plan 2 and for CNCSIS competitions.
- Board member of journals: Recent Patents on Biomarkers; Bentham Science Publisher Ltd.), Journal of Immunoassay and Immunochemistry
- Peer reviewers for ISI indexed journals: International Journal of Cancer; Future Oncology; Acta Endocrinologica; Recent Patents on Biomarkers; Journal of Immunoassay and Immunochemistry; Medical Principles and Practice Toxicology in Vitro, Materials Science & Engineering B, JMPR Medicinal Plants Research, Roumanian Biotechnology Letters.

TEAM 6 - IMMUNOMODULATION-IMMUNODIAGNOSIS

The “Immunomodulation-Immunodiagnosis” team is composed by 11 graduated members with different specialties, and 4 auxiliaries, as follows:

1. Ursaciuc Cornel - MD, PhD, senior researcher 2, chief of Immunology Department, head of the team – 80%
2. Neagu Monica - biochemist, PhD, senior researcher 2, chief of Immunobiology Laboratory – 30%
3. Manda Gina - biophysicist, PhD, senior researcher 2, chief of Radiobiology Laboratory – 30%
4. Ciotaru Dan - biologist, senior researcher 3, chief of Immunopathology Laboratory – 50%
5. Constantin Carolina - biochemist, PhD, senior researcher 3 – 30%
6. Surcel Mihaela - chemist, senior researcher 3 – 70%
7. Huica Radu - MD, Drd, researcher – 50%
8. Dobre Maria - biologist, researcher – 60%
9. Munteanu Adriana - biochemist, researcher – 100%
10. Neagoe Ionela - biologist, researcher – 30%
11. Pirvu Ioana - chemist, assistant researcher – 100%
12. Sorca Silvia - nurse – 100%
13. Caralicea Mariana - nurse – 30%
14. Pisica Mariana - technician – 30%
15. Dumitrascu Georgiana - medicine student, technician – 30%

Previous activity:

The main directions of research activity during last 5 years implied diverse pathology, addressing directly, or participating in the development of topics such as:

- Immunomodulatory factors of tumor development
- Immune markers in melanoma
- Tumor immunogenomics
- Predictive biomarkers in inflammatory rheumatic diseases
- Therapeutic potential of environmental factors from salines and caves
- Innovative immunotherapies in tumors and autoimmune diseases
- Markers for diagnosis and prognosis of non-viral hepatitis
- Pannels of markers applicable in personalized medicine
- Cytokines and immunomodulation

This activity consisted in 5 original projects (developed as project coordinator) and 9 partner projects (developed as partner project responsive) with the above themes, funded by CEEEX and PN2 national research programs, as follows: F29-F37, F45-F47, F80, F114, CF5 (see Annex 1). Besides, the team co-operated with other teams in “Victor Babeş” institute (Cellular pathology, Molecular diagnosis technology transfer, Genetics, Drug development & toxicology, Assay development) and other research groups in “Carol Davila” University of Medicine and Pharmacy, as supplier of testing services.

The results of scientific activity can be measured, besides the projects applications, in a list of 22 publications, like this: 5 ISI articles, 4 books or book chapters and 13 other relevant publications (see Annex 2).

The team is also the contact associated partner in an infrastructure FP7 project:

- FP7 Capacities/2007 – “Biobanking and Biomolecular Resources Research Infrastructure” (BBMRI) – European Commission Grant Agreement Number 212111 - Associate Partner Project responsive Cornel Ursaciuc – 2008-2012

The team also was/is involved in several scientific service contracts:

- Immunofluorescence evaluation of test slides prepared for diagnosis of different serum autoantibodies categories. "Tody Laboratories" SRL Bucharest, 2006-2009
- Experimental model and serum determinations in order to quantify the Simvastatin effects on systemic inflammation, subclinical heart modifications, and early atherosclerosis in rheumatoid arthritis. "Cantacuzino" Clinical Hospital Bucharest, 2007-2008
- Evaluation of cellular immune status by lymphocyte immunophenotyping and fagoburst test in children with humoral immunodeficiency. IOMC Bucharest, 2007-2011, in progress
- Flow cytometry evaluation of irradiation and flavonoid effects on cell cycle and apoptosis – data acquisition. „Carol Davila” University of medicine and Pharmacy Bucharest, 2010-2011, in progress

A constant activity and tradition of this group was performing of humoral and cellular immunodiagnostic tests and hematology tests as currently medical services practised in the "Victor Babeş" Institute's Diagnosis Center.

Members of the group also participate in sustaining the Cytometry Unit, Immunogenomics Unit, Biobank and Microbiology Laboratory.

At a present, the team is involved as part of the work group in 5 "Victor Babeş" institute's project proposals and as partner project responsive in other 5 applications by other institutions at the 2011 partner projects call of PN2 national research programme.

Personnel:

The group includes various specialists covering the whole area of the biomedical domains (2 medical doctors, 3 biologists, 3 biochemists, 2 chemists, 1 biophysicist). The mean age of the graduated personnel is ~45 years, therefore a partial team rejuvenation is necessary for the next period. We have in view physicians and biochemists, but this is dependent on the financial support of the group during the future time. All the actual staff are good professionals both in laboratory methods and theoretical knowledge and in the same time they are able to perform teaching activity for learning students in immunology and immuno-detection. Part of the staff are involved in formation activities developed through immunology courses organised by Romanian Society of Immunology or human resources projects.

The team personnel policy is focused on stimulation of research creativity in order to obtain competitive scientific results. Otherwise the members of the team have attended training courses with the aim of updating their level of scientific information:

- Workshop of Fundamental Immunology , Bucharest, Romania, April 2007
- Immune system: genes, receptors and regulation, Hvar, Croatia, 2007
- Molecular biology in diagnosis and epidemiology of infectious diseases. INCDMI "Cantacuzino" Bucharest, Romania, 2007
- The Course Molecular Diagnostics. The Erasmus Postgraduate School Molecular Medicine, Rotterdam, 2007.
- Training in real time PCR at TATAA Biocenter, Prague, 2008
- The Advanced BD FACSCanto™ II and BD FACSDiva™ 6 Training, "Victor Babeş" Institute, Bucharest, Romania, June 15-18 2009
- Epigenetics and new therapies in cancer. ESO-CNIO, Madrid, Spain, May 2007
- Cell culture Seminar, Bucharest, Romania, 2009
- Operator Training on BD FACSCanto II and BD FACSDiva 6.1.2, Heidelberg, Germany, June 2009
- 6th European Course on Clinical Cytometry, Valencia, Spain, September 2010
- 1st EFIS-EJI Intensive Educational Course in Clinical Immunology, Centre de Recherche des Cordeliers, Paris, France, December 1-4 2010.
- Autumn Days of Cytometry, Bucharest, Romania, October 2011

Equipments:

The team has performance equipment, and is able to perform a lot of top methodologies in view of accomplishing the diverse scientific directions mentioned above. These equipments are located in several units which the team are responsible with: Immunobiology, Immunopathology, Immunoproteomics, Cytometry, Immunogenomics, Immunomodulators. A list of purchased equipments are presented in Annex 3.

One of the main intension is to upgrade the Immunogenomics unit endowment with a hybridization unit for the gene microarray platform, a new bioanalyser for DNA/RNA evaluation and a supplementary laminar flow hood for nucleic acid extraction. Besides, a refrigerated centrifuge and a small autoclave unit are necessary for Immunopathology.

Perspectives:

Short- and medium term projects will most likely include acknowledged investigative techniques applied in research or clinical immunological studies: flow cytometry, immunofluorescence, serology, cell culture. Besides, additional investigative techniques such as PCR-array and next-generation sequencing are considered to be developed as an enlargement of immunogenomics branch of laboratory activity.

The team will continue several tools of the actual research and also will activate in the diagnosis activity, formation area and biobanking, as:

- new scientific projects funded by national programs
 - co-participants as team work in institute's proposals
 - partner institution in consortium projects
- service contracts in research or clinical immunology – every kind of immunological investigation performed for beneficiaries from scientific or clinical area.
- continued diagnostic work pay activity in institute's Diagnostic Center
- endowment possibilities reached by structural European projects (POS)

As constant preoccupations in the research activity will remain:

- autoimmune diseases and their diagnosis
- tumor immunogenomics
- cytokine modulation of cultured cells
- serum biomarkers in melanoma and other malignant tumors

Other directions will be approached in connection to the results that will be obtained at the partner projects applied to the PN2 national program, whose outcome will appear in spring 2012.

Besides, our team will encourage internal and international collaborations (FP projects, bilateral co-operations, service contracts, clinical trials) which implies the laboratory as a data supplier and scientific consulting collaborator. These will represent a supplementary funds sources for scientific work and researchers mobilities.

The above mentioned options will serve also as data sources used for meeting presentations, scientific articles or books.

TEAM 7 - GENOMICS AND GENETIC DIAGNOSIS

Research interests

- ✚ **genetics of neuropsychiatric disorders** associated with intellectual disabilities (ID). Multiple genetic anomalies are associated with ID phenotypes, and new data are reported at a high rate. However, the underlying genetic mechanisms are seldom clear. Currently, **we focus on the identification of genetic aberrations associated with ID** at genomic and cytogenetic level with the aim to further the knowledge regarding the **etiopathogeny of complex neuropsychiatric disorders**.
- ✚ characterization of genetic and epigenetic alterations underlying the initiation and progression of cancer (**hematologic neoplasms and solid tumors**); cancer immunogenomics. The genetic abnormalities associated with cancer bear a well-known clinical significance and are of outmost interest in the diagnosis, risk assessment, and management of the disease; therefore, their characterization and understanding brings multiple benefits for the medical science, and ultimately for the patients. Epigenetic studies, due to the reversible nature of epigenetic modifications, have a great potential not only for biomarkers discovery relevant for cancer detection and prognosis, but most importantly for identifying new targets for epigenetic drugs. Presently, we focus on the **detection and characterization of these abnormalities** at gene, chromosome and genome level, aiming to identify new aberrations and their **contribution to the malignant phenotype**. We also started to investigate the epigenetic changes in breast cancer cell lines, aiming to extend these studies to breast cancer diagnosis and prognosis.

Our experimental approaches cover molecular cytogenetics, molecular genetics, cell and molecular biology, and genomics (immunogenomics included). The team uses state-of-the art infrastructure for several applications, including: optical microscopy (transmitted light and epifluorescence), molecular cytogenetic techniques (*e.g.* FISH, mBAND), „in house” preparation of FISH probes using bacterial artificial chromosomes, PCR based techniques, and high-resolution genome wide approaches (DNA microarray). The research strategy is based on using unique patient material obtained through close collaboration with Clinical Departments from major tertiary hospitals. Additionally, our team makes constant efforts to preserve the valuable biological material that it is not used in ongoing studies, for future in depth or large scale approaches.

Research grants

Since 2007, the team participated in 6 national projects and 2 bilateral cooperation projects (Romania-France). The projects were granted within national peer-reviewed competitions.

No.	Research area	Research grant ID no/ funding(Euro)
1	Genetics research of neuropsychiatric disorders	F1, F10/ 386,237
2	Hematologic neoplasms genetic characterization	F6, F96/ 213,635
3	Human foetal hepatic stem cells characterization	F3/68,181
4	Infrastructure project for genetic research	F4/425,452
5	Bilateral Cooperation projects – knowledge and expertise sharing in the field of genetic/genomic defects of complex pediatric neuropsychiatric disorders	F2, CF1/ Funding for bilateral visits

Infrastructure

Up-grading the research infrastructure with state-of-the-art technologies has been the mainstay of our team strategy. A major step forward was made with a grant dedicated to enhancing research capabilities (2007-2009). Consequently, microarray (Agilent platform) and molecular genetics facilities were developed; the light microscopy facility was greatly improved by the acquisition of a motorized optical microscope. Presently, the team uses the following research equipments/facilities:

Microarray facility: Agilent DNA Microarray C Scanner with Surescan, Agilent Scan Control, Feature extraction and Genomic Workbench Software; Agilent BioAnalyzer 2100;

Molecular genetics facility: Corbett Real Time PCR, Corbett Palm Cycler PCR;

Nucleic acid isolation and manipulation facility: chemical hoods, water baths, cooling centrifuges, spectrophotometer, BioDoc transilluminator, freezers, ultrafreezers;

Cell culture facility: safety cabinets, incubators;

Light microscopy facility: motorized Axio Z1 Zeiss microscope with examination in transmitted light bright field and epifluorescence, equipped with high resolution monochrome cooled CCD camera; Metafer and

Ikaros software for scanning, automatic detection of metaphases, capture, karyotyping, FISH (standard, M-FISH, m-BAND, Q-FISH, CGH).

Research team

Name	Laboratory
Aurora Arghir, CS3	Medical Genetics Laboratory
Andreea Tutulan-Cunita, CS ^{1)*} **	Medical Genetics Laboratory
Sorina Papuc, CS*	Medical Genetics Laboratory
Magdalena Budisteanu, MD	Medical Genetics Laboratory
Cornel Ursaciuc, CS2	Immunopathology Laboratory
Monica Dobre, CS	Immunopathology Laboratory
Radu Huica, CS3*	Immunopathology Laboratory
Sevinci Pop, CS3 ^{2)*} **	Cell Biology Laboratory
Valeriu Cismasiu, CS ^{3)*} **	Cell Biology Laboratory
Georgeta Cardos, CS ^{4)*} **	Pathology Department
Gisela Gaina CS3	Pathology Department
Maria Neagu*, CS	Pathology Department
Angela Petrescu, CS 3	IT Department
Agripina Lungeanu, CS1***	Medical Genetics Laboratory
Ioana Borcan, Technician	Medical Genetics Laboratory
Marioara Cristea, Technician	Medical Genetics Laboratory

* PhD students

**¹⁾ Specialization in molecular biology and genetics (Master degree 2000-2002/ PhD 2002-2005 - Hiroshima University, Japan; Postdoctoral fellowship 2005-2006 - Manchester University, UK); ²⁾ Doctoral fellowship: 2000-2002, Postdoctoral fellowship: 2003-2008 University of Illinois at Urbana-Champaign, USA, Department of Cell and Developmental Biology; ³⁾ Specialization in molecular biology, protein biochemistry and cell biology within several postdoctoral fellowships (Albany Medical Center, NY, USA; Stem Cell Center, Lund, Sweden; Wetherall Institute of Molecular Medicine, Oxford, UK); ⁴⁾ Doctoral fellowship: 2004-2008, Hamburg University, Germany.

*** Retired - 2010.04

Research / clinical collaboration

The research activities, focused on detecting clinically relevant genetic lesions in neuropsychiatric disorders and cancer, add data to the body of knowledge and ultimately contribute to the improvement of clinical management of the patients. This is achieved through close **collaboration with groups from clinical hospitals** such as: “Prof. Dr Alexandru Obregia” Clinical Hospital of Psychiatry, Coltea Clinical Hospital (Hematology Department), Emergency University Hospital Bucharest (Hematology Department), other oncological departments.

In order to address the technological challenge of analyzing large amount of data generated by genome-wide high through-put technologies (microarrays) our team recently established collaboration with a **bioinformatics/biostatistics group from “Al. I Cuza” University, Faculty of Computer Sciences**.

Several **international collaboration** were established by our team with research groups from **France (Professor Jean-Michel Dupont team, Cochin Hospital, Paris)**, **Germany (Professor Evelin Schrock team, Carl Gustav Carus Faculty of Medicine, Dresden)** and **United States (Professor Kenneth Kosik team, University of California, Santa Barbara)**. A long term collaboration has been developed with Cochin Hospital through two bilateral cooperation (Romania-France) projects focused on genetic defects underlying intellectual disabilities and autistic spectrum disorders, respectively. This collaboration materialized in mutual visits and short-term staff trainings, exchange of technologies, and joint publications. The cooperation with professor Kosik team focus on understanding the pathogenetic mechanisms of neuropsychiatric disorders, such as microdeletion syndromes; it involves the generation of induced pluripotent stem cells (obtained in professor Kosik lab from fibroblasts) with subsequent differentiation into neuronal tissue in order to obtain a more accurate disease model. A recent collaboration was established with **Yolanda de Diego** team from **Carlos Haya Hospital, Malaga, Spain** on the topic of fragile X etiopathogeny and the perspective of antioxidative therapeutic strategy.

Publications: A49, A72, A93, A94, A95.

Training: **1.** Advanced training in aCGH (Institute of Clinical Genetics, Carl Gustav Carus Faculty of Medicine, Dresden, Germany) – 1 researcher; **2.** Oligonucleotide Microarray training (Institute G. Roussy, Paris, France) – 3 researchers; **3.** Clinical Cytogenetics Course (European Cytogeneticists Association, South Tyrol, Italy) - 1 researcher; **4.** Courses of Dymorphology 2006, 2008, 2009, 2011

(European Society of Human Genetics, Rome, Italy) – 1 researcher; **5.** European Course on Genetics of Mental Retardation (European Society of Human Genetics, Braga, Portugal) - 1 researcher; **6.** Workshops on laboratory accreditation, internal quality control and external quality assessment, (EuroGentest, Nice, France and Berlin, Germany) – 2 researchers; **7.** Project Management Course (AFPA, Bucharest, Romania) – 2 researchers.

International project proposals: FP7 – ERC Starting grants, 2010 Call “Integrative analysis of DNA methylation, miRNA and gene expression profiles in idiopathic autism” (not financed); **FP7 – ERC Starting grants, 2011 Call** “Genome-wide analysis of drug responsiveness in attention deficit hyperactive disorder” (under evaluation); **FP7 - HEALTH-2012- INNOVATION-1** „A phase III randomized double blind multicenter clinical trial to investigate the efficacy of the combination of ascorbic acid and tocopherol versus placebo for the treatment of cognitive deficit and behavioral problems in the fragile X syndrome. Functional relevance of the RAC1-GTPase as molecular target” (under evaluation).

Perspectives - Since 2007, our team advanced from molecular cytogenetic and molecular genetic studies of individual genes/ chromosomal regions towards genome-wide molecular strategies.

The envisaged strategy of our team consist of identification and characterization of constitutional and acquired genomic abnormalities, by genome-wide molecular approaches and use of the resources generated by the Human Genome Project.

✚ Understanding the biological bases of **complex neuropsychiatric disorders is one of the most important medical challenges** of the present time and most probably of the next years. Expanding usage of whole-genome molecular strategies has given a new quality to the analysis of these disorders. Thus, array-CGH can detect discrete copy-number changes and allows the definition of new clinical syndromes. Additionally, the possibility to combine CNVs data with genotyping information (SNP arrays) provides the advantage of adding high resolution and investigation of copy-neutral events. Our team is involved in searching for clinically relevant genes/genomic regions through array-CGH studies, with further interest in **extending our investigations at transcriptomic, epigenetic/epigenomic as well as model organism level**. The collaboration with professor Kosik lab will allow us to participate in the development of more accurate disease models for complex neuropsychiatric disorders such as microdeletion syndromes.

✚ **Cancer is caused by genetic and epigenetic anomalies** that alter the balance among cell proliferation, survival, and differentiation. However, **only a fraction of the cancer-associated genetic aberrations have been identified. In this context, our team aims at identifying novel oncogenic lesions relevant for pathogenesis as well as studying the cooperation between genetic events**. On short and medium term, our team intends to expand the area of interest toward the **investigation of acquired uniparental disomies, alterations of gene expression and epigenetic changes** in relation with disease onset and progression in cancer (hematologic malignancies, breast cancer, other solid tumors). Identifying and characterizing immunogenomic markers and assessing their diagnostic and prognostic impact is yet another objective of our team. **A long term goal** is to bring new evidence on the role of **microenvironment in tumorigenesis**, with special emphasis on **mesenchymal stem cells**. By analyzing the genetic/genomic abnormalities of tumor-associated cell populations, new factors at interplay in the oncogenic process can be identified and new potential treatment targets be found.

✚ While array-CGH is already in place and functioning in our institute, the expansion towards the genome-wide investigation of copy-neutral alterations (e.g. uniparental disomy, loss of heterozygosity) demands **an upgrade of the existing technology** (Agilent High Resolution Scanner Upgrade License - License for C Scanner upgrade – allows CGH+SNP array as well as scanning of high definition platforms).

✚ For **adding higher resolution – up to nucleotide level** - to the genome-wide investigations, our team foresees **developing partnerships with neighbouring institutions** (e.g. Carol Davila University of Medicine and Pharmacy and Institute of Cellular Biology and Pathology “N. Simionescu”), as well as foreign institution. By joining expertise and sharing resources (**next-generation sequencing technologies** available in the above mentioned institutions) our team intend to expand its whole genome molecular strategies towards hypothesis independent mutation screening in cancer and complex neuropsychiatric disorders.

✚ In order to **exploit** both the research and **diagnostic potential of array-CGH**, its introduction in clinical laboratory as a diagnostic tool is one of our goals. In close collaboration with a bioinformatics team as well as with clinical groups, we intend to device a reliable and sensitive method for the investigation of genomic structural variation in several human pathologies.

TEAM 8 - NEUROSCIENCES

Evolution of the human resources

Neuroscience Team composition between 2007 and 2011

Period	Name	Team position
2007-2011	Bogdan O. Popescu , MD, PhD, senior researcher, neurologist	Head of laboratory
2008-2011	Emilia Manole , PhD, senior researcher, biologist	Member
2007-2011	Paula Gratiela Chelu , MD	Member
2007-2008	Raluca Colesniuc , researcher, biologist	Member
2007-2011	Catalin Manole , MD, research assistant, PhD student	Member
2007-2011	Dragos Cretoiu , MD, research assistant, PhD student	Member
2009-2011	Mihnea Nicolescu , MD, research assistant, PhD student	Member
2008-2010	Oana Romanitan , MD, PhD	Member
2011	Laura Suciu , MD, PhD	Member
2007-2011	Mariana Nicolae , technician	Member

In 2007-2009, two students were carried out their research work for the undergraduate thesis: Radu Stoica and Maria Tuineag (on effects of antiepileptic drugs on neuronal apoptosis and neuroplasticity).

In 2009-2011, Ana Maria Enciu, MD, PhD student, worked in the laboratory for her PhD thesis (Molecular mechanisms in neurodegeneration).

Our team we have a balanced distribution of age (mean age of 38, 6 years) and sex (6 women, 4 men). Members of the team have different complementary expertise (both medicine and biology). Dr. Bogdan O. Popescu has graduated a PhD in Neuroscience in Karolinska Institute, Stockholm and works as well as a senior neurologist.

The dynamic of the research subjects and directions

Models and techniques currently used in laboratory are: cell cultures, animal models of neurodegeneration, light microscopy (phase contrast, fluorescence, confocal), histology, histochemistry, immunohistochemistry, Western blot, ELISA, PCR.

The main research directions of the laboratory during this period were: studies of the trophic factor receptors expression in the central and peripheral nervous system, the distribution and expression of the tight junction proteins in the brain, new neurodegeneration models relevant to Alzheimer disease and Parkinson disease. Since 2008, we performed as well studies of distribution and expression of different proteins involved in skeletal muscle and peripheral nerve pathology.

The most important achievements

Grants

For the period 2007-2010 we obtained two research grants (PN II - Partnerships, 2007):

1. PN II 41-013 /2007: Expression and function of tight junction proteins – a study in experimental models and in patients with dementia. Project Director (National Institute of Pathology “Victor Babes”): Bogdan O. Popescu, MD, PhD; Partners: International Center of Biodynamics, Bucharest and Bucharest Emergency University Hospital.
2. PN II 61-019/2007: Implementation and optimization of the technological process of obtaining active therapeutic serum F (ab ') 2 against highly bacterial and viral pathogenic agents. Project Director (National Institute for Microbiology and Immunology “Dr. I. Cantacuzino”): Nadia Bucurenci, PhD; Project Director of the Partner 1 (National Institute of Pathology “Victor Babes”): Bogdan O. Popescu, MD, PhD; Partner 2: University of Medicine and Pharmacy “Carol Davila”, Bucharest.

For the period 2008-2011 we obtained two research grants (PN II - Partnerships, 2008):

1. PN II 42-124/2008: Molecular analysis of the proteins implicated in the main types of peripheral neuropathies with a demyelinating component. Project Director (National

Institute of Pathology “Victor Babes”): Bogdan O. Popescu, MD, PhD; Partners: University of Bucharest and Bucharest Emergency University Hospital.

2. PN II 42-133/2008: Cellular and molecular bases of muscle ageing. Project Director (University of Bucharest): Emilia Manole, PhD; Project Director of the Partner 1 (National Institute of Pathology “Victor Babes”): Bogdan O. Popescu, MD, PhD; Partner 2: Colentina University Hospital.

Education

Starting with 2011, we contribute to the TDM project (Education of medical personnel - new technologies for health system/molecular diagnosis-POSDRU/81/3.2/S/58819, coordinated by our Institute. The Western blot expert from the Proteomics Section is Dr. Emilia Manole and the Direct Immunofluorescence expert from the Molecular Imaging Section is Dr. Laura Suci, both from our laboratory.

Dr. Bogdan O. Popescu authored one textbook for medical students and two book chapters for clinical neuroscience specialists. He is the Executive Editor of Romanian Journal of Neurology (CNCSIS B+) and Secretary General of the Romanian Society of Neurology as well. Dr. Bogdan O. Popescu served during this period as reviewer for several ISI journals and international grant evaluations (European Science Foundation and American Alzheimer Association).

Publications

Since 2007, the members of our laboratory published 20 ISI full-text articles and 8 full-text articles indexed in other international data bases and contributed with more than 50 lectures and posters in international and national scientific meetings.

National scientific awards

- Dr. Bogdan O. Popescu - ‘Victor Babeş’ award of the Romanian Academy for medical research activity – 2007.
- Dr. Bogdan O. Popescu - ‘Science and Art National Foundation Award of Excellence for research in the field of Neuroscience and Neuropathology – 2010.

List of ISI publications (2007-2011)

1. Negreanu L, **Popescu BO**, Babiuc RD, Ene A, Bajenaru OA, Smarandache GC., Duodopa infusion treatment: a point of view from the gastroenterologist., *J Gastrointest Liver Dis.* 2011 Sep; 20 (3): 325-7.
2. Enciu AM, Constantinescu SN, Popescu LM, Mureşanu DF, **Popescu BO.**, Neurobiology of vascular dementia., *J Aging Res.* 2011; 2011: 401604. *Epub* 2011 Aug 17.
3. Enciu AM, **Nicolescu MI**, **Manole CG**, Mureşanu DF, Popescu LM, **Popescu BO**, Neuroregeneration in neurodegenerative disorders, *BMC Neurol.* 2011 Jun 23; 11:75.
4. Enciu AM, **Popescu BO**, Gheorghisan-Galateanu A., MicroRNAs in brain development and degeneration, *Mol Biol Rep.* 2011 Jun 5. [*Epub ahead of print*]
5. Popescu LM, **Manole E**, Serboiu CS, **Manole CG**, **Suciu LC**, Gherghiceanu M, **Popescu BO**, Identification of telocytes in skeletal muscle interstitium: implication for muscle regeneration., *J Cell Mol Med.* 2011 Jun; 15(6): 1379-92.
6. Popescu LM, Gherghiceanu M, **Suciu LC**, **Manole CG**, Hinescu ME., Telocytes and putative stem cells in the lungs: electron microscopy, electron tomography and laser scanning microscopy, *Cell Tissue Res.* 2011 Sep; 345(3): 391-403.
7. Hinescu ME, Gherghiceanu M, **Suciu L**, Popescu LM., Telocytes in pleura: two- and three-dimensional imaging by transmission electron microscopy, *Cell Tissue Res.* 2011 Feb; 343(2): 389-97.
8. Nicolae L, Iacob G, Poparda M, **Popescu BO**, Case report. Gelastic seizures in a patient with right gyrus cinguli astrocytoma, *J Med Life.* 2010 Oct-Dec; 3(4): 433-6.
9. Negreanu LM, **Popescu BO**, Babiuc RD, Ene A, Andronescu D, Băjenaru OA., Cutting the Gordian knot: the blockage of the jejunal tube, a rare complication of Duodopa infusion treatment, *J Med Life.* 2010 Apr-Jun; 3(2): 191-2. *Erratum in: J Med Life.* 2011 Jan-Mar; 4(1): 7 p following 123.

10. Bajenaru O, Tiu C, Moessler H, Antochi F, Muresanu D, **Popescu BO**, Novak P., Efficacy and safety of Cerebrolysin in patients with hemorrhagic stroke, *J Med Life*. 2010 Apr-Jun; 3(2): 137-43.
11. Dumitriu A, **Popescu BO**., Placebo effects in neurological diseases, *J Med Life*. 2010 Apr-Jun; 3(2): 114-21. Review.
12. Jianu DC, Muresanu DF, Bajenaru O, **Popescu BO**, Deme SM, Moessler H, Meinzingen SZ, Petrica L., Cerebrolysin adjuvant treatment in Broca's aphasics following first acute ischemic stroke of the left middle cerebral artery., *J Med Life*. 2010 Jul-Sep; 3(3): 297-307. Erratum in: *J Med Life*. 2011 Jan-Mar; 4(1): 7 p following 123.
13. Muresanu DF, Alvarez XA, Moessler H, Novak PH, Stan A, Buzoianu A, Bajenaru O, **Popescu BO**., Persistence of the effects of Cerebrolysin on cognition and qEEG slowing in vascular dementia patients: results of a 3-month extension study, *J Neurol Sci*. 2010 Dec 15; 299(1-2): 179-83.
14. Hort J, O'Brien JT, Gainotti G, Pirttila T, **Popescu BO**, Rektorova I, Sorbi S, Scheltens P; EFNS Scientist Panel on Dementia, EFNS guidelines for the diagnosis and management of Alzheimer's disease, *Eur J Neurol*. 2010 Oct; 17(10): 1236-48.
15. Negreanu L, **Popescu BO**., Evaluation of successful treatment in achalasia with timed barium esophagogram: revisiting an old friend, *J Med Life*. 2010 Jan-Mar; 3(1): 64-6.
16. Ghitoiu A, Rusu EC, Slăvoacă D, Aigyul E, **Popescu BO**., A hypertensive patient with multiple intracerebral hemorrhages due to brain metastases., *J Med Life*. 2009 Oct-Dec; 2(4): 437-9.
17. **Romanitan MO**, **Popescu BO**, Spulber S, Băjenaru O, Popescu LM, Winblad B, Bogdanovic N., Altered expression of claudin family proteins in Alzheimer's disease and vascular dementia brains, *J Cell Mol Med*. 2010 May; 14(5):1088-100.
18. **Suciu L**, **Nicolescu MI**, Popescu LM., Cardiac telocytes: serial dynamic images in cell culture, *J Cell Mol Med*. 2010 Nov; 14(11): 2687-92
19. **Suciu L**, Popescu LM, Gherghiceanu M, Regalia T, **Nicolescu MI**, Hinescu ME, Fausson-Pellegrini MS., Telocytes in human term placenta: morphology and phenotype, *Cells Tissues Organs*. 2010; 192(5): 325-39.
20. **Popescu BO**, Toescu EC, Popescu LM, Bajenaru O, Muresanu DF, Schultzberg M, Bogdanovic N., Blood-brain barrier alterations in ageing and dementia, *J Neurol Sci*. 2009 Aug 15; 283(1-2): 99-106. Review.
21. Cretoiu SM, **Cretoiu D**, **Suciu L**, Popescu LM., Interstitial Cajal-like cells of human Fallopian tube express estrogen and progesterone receptors., *J Mol Histol*. 2009 Oct; 40(5-6): 387-94.
22. **Suciu L**, Popescu LM, Regalia T, Ardelean A, **Manole CG**., Epicardium: interstitial Cajal-like cells (ICLC) highlighted by immunofluorescence, *J Cell Mol Med*. 2009 Apr; 13(4): 771-7.
23. Muresanu DF, Alvarez XA, Moessler H, Buia M, Stan A, Pinteau D, Moldovan F, **Popescu BO**., A pilot study to evaluate the effects of Cerebrolysin on cognition and qEEG in vascular dementia: cognitive improvement correlates with qEEG acceleration, *J Neurol Sci*. 2008 Apr 15; 267(1-2): 112-9
24. **Popescu BO**., Still debating a cause and diagnostic criteria for Alzheimer's disease, *J Cell Mol Med*. 2007 Nov-Dec; 11(6): 1225-6
25. Oprica M, Hjorth E, Spulber S, **Popescu BO**, Ankarcrona M, Winblad B, Schultzberg M., Studies on brain volume, Alzheimer-related proteins and cytokines in mice with chronic overexpression of IL-1 receptor antagonist., *J Cell Mol Med*. 2007 Jul-Aug; 11(4): 810-25.
26. **Romanitan MO**, **Popescu BO**, Winblad B, Bajenaru OA, Bogdanovic N., Occludin is overexpressed in Alzheimer's disease and vascular dementia., *J Cell Mol Med*. 2007 May-Jun; 11(3): 569-79.
27. Cowburn RF, **Popescu BO**, Ankarcrona M, Dehvari N, Cedazo-Minguez A., Presenilin-mediated signal transduction., *Physiol Behav*. 2007 Sep 10; 92(1-2): 93-7 Review.
28. **Suciu L**, Popescu LM, Gherghiceanu M., Human placenta: de visu demonstration of interstitial Cajal-like cells, *J Cell Mol Med*. 2007 May-Jun; 11(3): 590-7.

TEAM 9 - DRUG DEVELOPMENT AND TOXICOLOGY

Team coordinator: CS2 Dr. Gina Manda, Head of Radiobiology Laboratory

Mission: applied biomedical research in drug development, medicinal chemistry and (immuno)toxicology

Research focus:

- Identification of *pathology-relevant drug targets and action mechanism*
- *medicinal chemistry*: screening & hits to leads (biological activity assessment)
 - development and implementation of in vitro assays with particular end-points, for selecting candidate drugs, for defining their action mechanism and safety profile
 - assistance of partner research teams (organic and analytic chemists, pharmacists) in designing and producing new compounds (libraries of synthetic or natural compounds), potentially efficient in cancer, autoimmune, cardiovascular diseases etc.
 - preclinical studies for these new compounds, both in vitro and in vivo in animal models (selection of candidate compounds by in vitro studies, in vivo proof-of-action studies using relevant animal models, in vitro and in vivo mechanistic studies and toxicological screening)
- *immunotoxicology*
 - implementation of advanced screening methods for characterizing the biological impact of xenobiotics on human health (medicines, drugs of abuse, heavy metals, mycotoxins, ionizing radiation etc)

Research topics:

1. Pathologic mechanisms and drug targets

No	Research area / coordinator	Research contracts / budget (Euro)
1.1	Biomarkers and molecular targets in cardiovascular diseases Prof. Dr. Elena MOLDOVEANU and CS3 Dr. Daciana MARTA	F49, F50, F51 / 336.381
1.2	Immune targets in the treatment of rheumatoid arthritis CS2 Dr. Gina MANDA	F77 / 73.070
1.3	Integrin – extracellular matrix interactions in cell motility and metastasis CS2 Dr. Mircea LEABU	F108 / 100.676

2. Drug development

No	Research area / coordinator	Research contracts / budget (Euro)
2.1	Novel nucleoside analogs for cancer therapy - CS2 Dr. Gina MANDA	F81, F82 / 188.653
2.2	Complexes of physiological, divalent transitional metals for cancer therapy - CS2 Dr. Mircea LEABU	F106 / 92.053
2.3	Functionalized nutrients - CS2 Cristiana TANASE	F41 / 125.000
2.4	Alternative medicines: bioactive phytochemicals CS2 Dr. Cristiana TANASE, CS2 Dr. Mircea LEABU	F38, F52, F53, F105 / 347.780

3. (Immuno)Toxicology

No	Research area / coordinator	Research contracts / budget (Euro)
3.1	Immunotoxicology of mycotoxins: impact on the food chain and development of counteracting agents (study in porcine model) CS2 Dr. Gina MANDA	F76, F79 / 48.646
3.2	Heavy metals profiling in biological samples and food CS3 Vasile PREOTEASA	Nucleu Programme
3.3	Radiation-induced genotoxicity CS3 Vasile PREOTEASA	Contract with Cernavoda nuclear power plant
3.4	Geno- and hepatotoxicity of xenobiotics CS3 Bogdan Marinescu	Contract with S.C. ALCEDO S.R.L.

Publications

2. Pathologic mechanisms and drug targets: 7 ISI publications A13, A17, A20, A24, A36, A83, A96, O15, 096, 098, 099 (cumulated rAIS **12,32** citations **18**).

- biomarkers in vascular inflammatory pathology (pulmonary hypertension, heart failure and antiphospholipid syndrome). In heart failure we add contribution to the recognition of LpPLA2 as a marker of oxidative stress and vascular inflammation.
- in rheumatoid arthritis we highlighted particular networks of adaptive and innate immunity (B lymphocytes-NK cells) and a key role of peripheral monocytes in mirroring disease outcome; we showed that low doses of immunosuppressive agents (leflunomide) may exert a pro-inflammatory action on pathologic monocytes.

3. Drug development: 12 ISI publications A12, A31, A41, A43, A57, A65, A67, A84, A88, A89, A90, O28 (cumulated rAIS **5.69**, citations **12**), 1 book chapter (O28).

22 new nucleoside analogs from 3 structural classes were investigated in vitro and 3 lead compounds with anti-cancer activity were identified. A preliminary in vivo study on laboratory animals proved their activity and showed a convenient toxicological profile for 2 of the lead compounds. Compounds will be further developed, including by preliminary results-guided structural changes. We will investigate their therapeutic application as radiosensitizers in cancer therapy and we will submit at least 1 national patent.

4. Toxicology: 3 ISI publications A71, A82, A92 (cumulated rAIS **2.43**), 1 book chapter (O19), 071.

Accreditation of immunotoxicology assays according to SR EN ISO 15189 (Biochemistry Laboratory) - F56/181.512 Euro

Research team

Name	Laboratory	Name	Laboratory
CS2 Gina Manda ^{1)**}	Team coordinator , Head of Radiobiology Laboratory. Scientific secretary	CS3 Ciotaru Dan	Head of Immunopathology Laboratory
CS Ionela Victoria Neagoe	Radiobiology Laboratory	CS3 Mihaela Surcel	Immunopathology Laboratory
CS3 Vasile Preoteasa	Head of Nuclear Unit	CS3 Radu Huica*	Immunopathology Laboratory
CS2 Mircea Leabu ^{2)**}	Head of Cell Biology Laboratory	CS2 Cristiana Tanase	Head of Biochemistry Laboratory
CS3 Sevinci Pop ^{3)**}	Cell Biology Laboratory	CS1 Radu Albuiescu	Biochemistry Laboratory
CS Andreea Oana Urs*	Cell Biology Laboratory	CS Daniela Popescu*	Biochemistry Laboratory
Cristina Mariana Niculite*	Cell Biology Laboratory	AS Lucian Albuiescu*	Biochemistry Laboratory
Mihaela Andreea Mocanu	Cell Biology Laboratory		
Catalin Filipescu	Cell Biology Laboratory	CS Alina Grigore	Pathology Department
CS3 Daciana Marta	Ultrastructural Pathology Laboratory	CS Bogdan Marinescu	Head of Animal Care Unit, Laboratory of experimental models
CS3 Gabriela Catalin*	Ultrastructural Pathology Laboratory	CS Gheorghita Isvoranu *	Animal Care Unit, Laboratory of experimental models

Technicians: Daniela Geogia, Sanda Sima, Cristina Vlad, Maria Paraschiv

* 7 PhD students

** ¹⁾ Specialization in rheumatoid arthritis (idiotype/anti-idiotype network), INSERM, Paris, France; ²⁾ Post-doctoral fellow and visiting researcher, 2000-2005, University of Western Ontario, London, ON, Canada; ³⁾ Doctoral fellowship: 2000-2002, Postdoctoral fellowship: 2003-2008 University of Illinois at Urbana-Champaign, USA, Department of Cell and Developmental Biology.

Methodologic approach

- To **apply** in medicinal chemistry and toxicology the **know-how** of our research team in **human pathology, immunology, cellular and molecular biology**
 - cell cultures, flow cytometry (immune phenotyping, cellular activation and proliferation, generation of reactive oxygen species, apoptosis), protein multiplexing (soluble factors profile, signal transduction pathways), fluorescence and confocal microscopy, transmission electron microscopy, impedance measurements for cell adhesion, radio-assays (biodistribution, cell functions), heavy metals profiling in biological samples by ICP-MS etc.
- To **collaborate** within **consortia** joining institutions with **complementary expertise** and **multidisciplinary teams** (biologists, biochemists, medical doctors, chemists, pharmacists, biophysicists etc) for developing **innovative compounds designed and produced by Romanian R&D teams** and for implementing new investigation methodologies for Drug development and Immunotoxicology.

Available infrastructure

Cell culture facilities - class II flow hoods, CO₂ incubators, deep freezing unit; **Cytometry unit** - 2 flow cytometers, fluorescence microscopes, confocal microscope; **VIDEOCELL unit** - xCELLigence system, BioStation IM, fluorescence microscope, electrophoresis and western blot equipment, gas chromatograph; **Proteomics unit** (Luminex platform); **Histology unit**; **Animal Care Unit**: 2008-2011 accreditation according to SR EN ISO 17025 for B12 and B39 assays; **Nuclear unit** (certified by CNCAN): Canberra Packard beta-counter, Sorcerer imager; **ICP-MS unit**: ICP-MS ULTRAMASS 700.

Infrastructure upgrading in the Animal Care Unit and in the Cellular Biology Laboratory (877.466 Euro from INFRAS and Capacities Programmes)

Collaborations

National collaborations: **1.** University of Medicine and Pharmacy “Carol Davila”, Bucharest; **2.** University of Bucharest; **3.** R&D Institute for Chemical-Pharmaceutical Research, Bucharest; **3.** Institute for Nuclear Physics and Engineering “Horia Hulubei”, Magurele; **4.** Institute of Biology and Animal Nutrition, Balotesti (IBNA); **Private companies**: Mecro Systems and SC Biotehnos SA; **International collaborations**: **1.** Universite Libre de Bruxelles, Laboratory of Pharmaceutical Chemistry, Brussels, Belgium; **2.** University of Thessaly, School of Health Sciences, Biochemistry Department, Greece.

International project proposals

1. FP7-NMP-2009-SMALL-3: Nanotechnologies for medical implants (not financed); **2.** IMI_Call_2009_5: “Aberrant Immunity in Chronic Immune-mediated Diseases” (not financed); **3.** FP7-Low Dose Research towards Multidisciplinary Integration (DoReMi): Epidemiologic study - systemic effects of low dose exposure to uranium on the immune status (not financed); **4.** FP7 call Health-2013 Phosphodiesterases, inflammation endothelium dysfunction: a route to cardiovascular disease. Coordinator: Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, Universite Libre de Bruxelles, , Brussels, Belgium (in preparation)

Development plan

I. New research areas: **1.** Theranostics – Development of an integrated platform with fully 3D X-ray tomographic and microbeam fluorescence / luminescence guidance capabilities and of particular nanoparticles for simultaneous imaging and radiation therapy of tumors. Collaboration with the R&D Institute of for Laser, Plasma and Radiation Physics

2. Implementation of „omics” and high-throughput technologies for drug development and immunotoxicology (in collaboration with other teams from the institute)

3. Systemic effects of low dose exposure to ionizing radiation on the immune response; integration of the Radiobiology Laboratory in the European research network DoReMi (Integrating Low Dose Research)

II. Patent submission for newly developed therapeutic compounds and design/screening of new ones

III. Accreditation of new experimental methods relevant for drug development and toxicology

IV. Introduction of new investigations in the **Diagnosis Center** (trace microelement profiling)

V. Enlargement of **national and international collaborations** within IMI, FP7 etc - Focus on private companies

VI. Increased exploitation of existent infrastructure, to be translated in an increased number of publications in ISI ranked journals. Development of Radiobiology laboratory’s specific infrastructure.

11 project proposals at the national 2011 Call “Partnerships – Collaborative projects of applied research” (under evaluation)

Pathologic mechanisms and drug targets: **1.** Fibro-inflammatory biomarkers of early myocardial remodeling in heart failure patients with preserved ventricular ejection fraction–Elena MOLDOVEANU;

2. Optimization of autologous bone marrow stem cell therapy in patients with ischemic heart failure–Elena MOLDOVEANU **3.** The importance of phosphodiesterase and thrombin in pulmonary fibrotic diseases- possible future therapeutic targets – Daciana MARTA. **Drug development**: **1.** New chemicals and natural compounds for improving radiotherapy in cancer – radiosensitization, internal irradiation and radioprotection-Gina MANDA;

2. Translation of coordinative chemistry in biology and therapy. Complex compounds of physiological cations with thiosemicarbazones and tiocarbohyrdazones as antitumoral and/or antibacterial drugs–Mircea LEABU; **3.** New epigenetic drug – DHA – in breast cancer therapy and biomarkers discovery–Sevinci POP; **4.** Magnetic Nanostructures for Targeted Therapy – Sevinci POP; **5.** Tool design for pharmacovigilance. Prediction of life-threatening angioedema for patients treated with renin-angiotensin system inhibitors and/or glyptins–Mircea LEABU. **Toxicology**: **1.** Impact of feed co-contamination and mitigating solutions to increase feed safety, animal health and food quality – Gina MANDA.

TEAM 10 - Assay Development and Alternative Testing

Team members

First and Last Name	Role	Involvement
Monica Neagu	Team Leader	0,50
Carolina Constantin	Member	0,50
Dan Ciotaru	Member	0,3
Mihaela Surcel	Member	0,3
Cristiana Tanase	Member	0,30
Radu Albulescu	Member	0,15
Elena Codrici	Member	0,2
Daniela Popescu	Member	0,25
Lucian Albulescu	Member	0,2
Emilia Manole	Member	0,3
Gheorghita Izvoranu	Member	0,3
Alina Nita	Member	0,2
Angela Petrescu	Member	0,5
TOTAL		4,00

Technical personnel

First and Last Name	Role	Involvement
Mariana Caralicea	Technician	0.5
Georgiana Dumitrascu	Technician	1
Mariana Pisica	Technician	0.5
Irina Radu	Technician	0,2
Laurentiu Anghelache	Technician	0.5
TOTAL		2.7

In the last 4 years our team was involved in the following research directions: **Immune-based** assay development and **Cell-based** assays.

In the domain **Immune-based assay development** we are currently involved in *innovative immune-detection in infectious diseases* through the cooperation with University of Tübingen and University of Athens (NATO SfP 982838/2007). We have established a complete new testing approach for early bacterial infection testing. The innovative assay development consists in designing both new antibodies specific for C-terminal prothymosine peptide and fluorescence-immunoassay detection methods. In the last 4 years we have published the relation between the in vitro and in vivo bacterial infection and the release of a C-terminal prothymosine peptide. In biological fluids the developed immune-assay can detect in experimental mouse models the mentioned peptide as early as 2 hours post-infection. The possibility to detect as early as 2 hours post-infection a marker by means of chemiluminometric testing is a major breakthrough in this domain. The future of these results will be the development of an original international patent and the technological transfer for developing an easy-to-perform, sensitive and quick test for bio-terrorist attack. The research funds obtained in the framework of this NATO SfP 982838/2007 project are 300,000 Euros for Romania.

In the domain of **Cell-based assays development** we are developing efficient technology/workflow for drug potency identification with emphasis on nano-drugs for controlled delivery. In the last 4 years through a long lasting collaboration with the National Institute for

Chemistry Bucharest we have developed several classes of photosensitizing compounds with anti-tumoral effect. Target potency, cytotoxicity, and metabolic liabilities were evaluated and the selected compounds entered the animal models testing. In animal models vivo efficacy was tested when reliable models were available. The hit-to-lead process induced a close collaboration between *Assay Development Team* and other teams, such as *Proteomic and Biomarkers* and *Drug Development*. When a promising lead series has been identified, besides publications, several composition-of-matter patents were accomplished and the internationally recognized value was recognized by awards. The patent OSIM Mr. 00489/25.06.2008 entitled “Tetra-sulphonated porphyrin application for producing a dermatologic therapy – photosensitizer” received Gold medal at Brussels Innova 2008, Special Prize of Rudy Demotte, Minister President of the Walloon Government, Gold medal at The 37th International Exhibition of Inventions of Geneva 2009 and Special Prize of the Ministry of Education of Russia, 2009; Gold medal at The International Fair for Innovation, Moscow, 2009. Recognition of the last 10 years long-standing work performed by us in this domain, resides in our affiliation to the international networks: COST D39 *Metallo-Drug Design & Action* (2006-2010); COST TD1002 *European network on applications ...in NanoMedicine and Life Sciences* (2011-2015). In the cell-based assay development we have developed specific equipments for cell imaging that were subject for patent OSIM A/00351/2019 / 21.04.2010 entitled “Equipment and procedure for microwave irradiation in *in vitro* models with concomitant registration of biological behaviour in a fluorescence microscope”. Developing several nationally granted projects the funds obtained by the research team in this domain heaves up to 850,000 Euros. Through cooperation with the University of Lisbon and Technical Institute of Portalegre, we have developed several classes of nano-compounds intended to be intracellular trackers in tumor cells and indicators for minimum residual disease in blood circulation. The project has already a patent OSIM Nr. RO-BOPI2/2009 entitled “Tetrapirolic compound asymmetrically substituted – synthesis and biological evaluation” and until 2012 it will develop several others in the cell-based assay for efficient drug delivery. The future of this research direction lays in increasing the nano-drug specificity with emphasis in targeting tumour receptors and tissue markers for not only a controlled delivery in time but as well in space. Through this MNT-ERA –NET 7050/2010 project the team was financed with 115,000 Euros. The involved laboratories are affiliated to the National Platform for Nanomedicine.

Related to the domain of **cell-based** assay the team leader is an active member of the **Commission for Advanced Therapies** – European Medicine Agency and one team member is evaluator for **EuroNanoMed** Projects.

Members of the Assay Development team are actively **peer-reviewing** for the following scientific journals: *Pigments and Dyes*, *Photochemical and Photobiological Sciences*, *Patents in Biomarkers*, *Archives of Gerontology and Geriatrics*, *International Journal of Photoenergy*, *Romanian Biotechnological Letters*, *Romanian Archives of Microbiology and Immunology*, *Materials Science and Engineering B*, *Journal of Clinical Laboratory Analysis*, *International Journal of Nanomedicine*. Two team members are also **members of the editorial boards** and **invited editors** for hot scientific topics in scientific journals such as *Journal of Immunoassay&Immunochemistry* and *Recent Patents on Biomarkers*.

The personnel dynamics in our team is remarkable, namely we have a mean age of 40.2 years and equilibrated between young researchers and more experienced senior researchers and have hosted through the international collaborations several PhD students that fulfilled their

thesis in subject developed by the research team. The knowledge up-grading of our team is continuous such as, each of the team members attends international courses on up-to-date technologies. In 2007 the following courses and training stages were attended - Profiling Kinases and Phospho-Sites with Antibody-Based Methods for Disease Biomarker; Drug Target Discovery and Protein Arrays for Biomarker Discovery and Protein Expression Profiling, Amsterdam, Holland; In 2008: Principles and applications of microfluidics in the life sciences, Microfabrication technologies, Barcelona, Spain; ProteinChip SELDI-ToF MS Training Course, Malvern, USA; In vivo confocal microscopy training, Mavig, Bucharest, Romania; In 2009 - Flow Cytometer FACS CANTO II Training Course, Heildelberg, Germany; In 2010-2011 xCELLigence Users Meeting, Munchen, Germany, Intensive Educational Course in Clinical Immunology, Centre de Recherche des Cordeliers, Paris; 2011 Course “Characterizing and applying physiologically-based pharmacokinetic models in risk assessment” – WHO Paris.

We have constant young Bachelor of Science degree personnel that perform their diplomas in the framework of the mentioned domains. The Laboratories involved are constantly hosting in PhD students or post-doctoral fellows in the framework of the mentioned international collaborations.

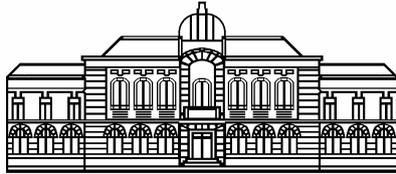
The laboratories involved in the Assay development team have **high-throughput technology** with specialized software. Although the equipment is recently purchased (2007-2011) de level of exploitation by the described team is over 75%.

Domain	Equipment
Proteomics	2D electrophoresis
	2D-DIGE (Typhoon 9000)
	SELDI-ToF-MS
	Western blotting
	Multiplex xMAP® technology
	Protein microarray
	Complete ELISA lines
Cellular physiology testing	xCELLigence impedance measurement equipment
	Confocal microscopy
	Varioskan Multimode Reader for time-resolved fluorescence, chemiluminescence
	Complete unit for cell culture manipulation and storage

Appended publications to the team: A7, A27, A29, A31, A41, A49, A51, A55, A84, A91, A98 with a cumulated rAIS of 8,864 and 20 ISI citations

Projects that funded the mentioned research directions CF3, CF4, CF12, CF13 have a total budget of 600,000 Euro and F27, F28, F29, F35, F43, F45, F46, F109, F110, F112, F113 850,000 Euro

4. Representative project



'Victor Babeș' National Institute of Pathology, Bucharest, Romania

TELOCYTE - A NEW TYPE OF INTERSTITIAL CELL

<http://www.telocytes.com>

KEY PERSONS INVOLVED IN TEOCYTE PROJECT

Team leader: Acad. Laurentiu M. POPESCU

Senior researchers: Hinescu ME, Ardeleanu C, Mandache E

PostDoc: Gherghiceanu M, Suciu LC, Popescu BO, Cismașiu V, Cretoius S

PhD students: Manole CG, Nicolescu MI, Cretoiu D

INTERNATIONAL COLLABORATION

- Prof. **Maria-Simonetta Fausone-Pellegrini** and Prof. **Daniele Bani** from Department of Anatomy, Histology and Forensic Medicine, University of Florence, Italy
- Prof. **Sawa Kostin** from Max-Planck Institute for Heart and Lung Research, Franz Groedel Institute, Bad Nauheim, Germany
- Prof. **Shengshou Hu** from the Center for Cardiovascular Regenerative Medicine, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China
- Prof. **Changyong Wang** from the Tissue Engineering Research Center, Academy of Military Medical Sciences, Beijing, China

TELOCYTES - A CASE OF SERENDIPITY: the winding way from Interstitial Cells of Cajal (ICC), *via* Interstitial Cajal-Like Cells (ICLC) to TEOCYTES

The history of TC is recent since these cells have been discovered only a few years ago. However, the growth of knowledge on TC has been exponential from the beginning and we already have much information. TC were discovered in **2005** when L.M. Popescu's group from Bucharest, Romania, described a new type of cell that resides in the stroma of several organs, which became known as interstitial Cajal-like cells (ICLC). This group named these cells ICLC because of their apparent similarity with the canonical gastrointestinal interstitial cells of Cajal (ICC), the gut pacemaker cells. A few years later, in **2008**, M.S. Fausone-Pellegrini and her team from Florence, Italy, described ICLC in the muscle coat of the human gut and noticed they consistently differed from the ICC in both ultrastructure and immunophenotype. In **2010**, after confirming the presence of this peculiar cell type in the stroma of many organs and characterized it by immunohistochemistry and electron microscopy, the two groups agreed they were describing a 'novel' cell type and that the name ICLC had to be changed with a more appropriate one. From then on, this novel cell type became known as the telocyte.

TELOCYTES (TC) - NEW TYPE OF INTERSTITIAL CELLS WITH LONG PROLONGATIONS NAMED TELOPODES (Tp).



MORPHOLOGY OF TELOCYTES

To characterize these cells, many different techniques have been used: *in vitro*, isolated cells in culture; *in situ*, observation on fixed specimens; light and fluorescence microscopy; transmission electron microscopy; scanning electron microscopy; electron tomography

All these techniques have shown that TC are cells with a small body and a variable number of Tp. The shape of the cell body depends on the number of Tp and can be piriform/spindle/triangular/ stellate. The nucleus is oval, with a moderately dense chromatin, and has no obvious nucleolus. The cytoplasm surrounding the nucleus is scarce and contains a small Golgi apparatus, some mitochondria, and few cisternae of rough and smooth endoplasmic reticulum. Telocytes are certainly defined by their ultrastructural features. Usually, a TC has extremely long Tp with moniliform aspect generated by alternating podoms and podomers.

Telopodes have particular characteristics:

1. Number : 1–5/cell, usually 1-3
2. Branching: dichotomous pattern
3. Length: tens up to hundred micrometres
4. Aspect moniliform - podomeres alternating with podoms
5. Podomers - 50-100 nm thin segments; usually < 0.2 μm , below the resolving power of light microscopy
6. Podoms - dilated segments accommodating mitochondria, ER and caveolae ('Ca²⁺ release units')
7. Connected each other by junctions form an interstitial network

Immunohistochemistry

To know the chemical code of TC is of fundamental importance since it allows their unequivocal identification and also helps evaluate their size, shape, number, and, eventually, movements, migration, and pathological changes. Although we made many reliable attempts testing an enormous variety of antibodies, a single marker that can be considered specific for this cell type or, at least, specific for the TC of a given organ has not been found. TC might show different immunohistochemical profiles among organs and even in the same organ examined. However, at present, **CD34** labeling remains the best available choice for TC identification, possibly in combination with **c-kit** and **vimentin** labeling. Due to these important differences in TC immunolabelling and since none of the markers tested are 'specific', we need to solve this issue and perform further immunohistochemical techniques, including immunoelectron microscopy.

Distribution

TC have been found in a large variety of **cavitary organs**: [heart (endo-, myo-, and pericardium); stomach and intestine, with mesentery; gallbladder; uterus and Fallopian tube] and **non-cavitary organs** [lungs and pleura; pancreas (exocrine); mammary gland; placenta].

All the cells identified as TC were:

- organized in a 3D network, dispersed in the extracellular substance, and intermingled with resident (fibroblasts, mast cells, adipocytes) and nonresident (macrophages, immune cells, granulocytes) cells
- localized at the connective border of various tissues (epithelial, muscular, and nerve tissues) lining them and around blood vessels.

ROLES OF TELOCYTES

Several roles have been suggested for TC, most of which are believable and not mutually:

- key players in organ specific renewing (heart, lung, striated muscle) and could act as stromal support cells for stem cells. Ultrastructural analysis proved that TCs cardiac network could integrate the overall 'information' from vascular system (endothelial cells and pericytes), nervous system (Schwann cells), immune system (macrophages, mast cells), interstitium (fibroblasts, extracellular matrix), stem cells, progenitors and working cardiomyocytes. Generally, heterocellular contacts occur by means of minute junctions (point contacts, nanocontacts and plane contacts) and the mean intermembrane distance is often within the range of tens of nm (10-30 nm) which fits in the molecular interactions domain. Our study showed that homotropic and heterotropic ultrastructural interactions of TCs in adult heart form an integrative interstitial system. Possibly, TCs network assure physiological coordination of multicellular signals, essential for stem cells (resident or circulating) decision to proliferate, differentiate and mature into new cardiomyocytes or other cardiac cell types.
- sustain myocardial tissue organization in developing and adult heart
- in immune surveillance (stromal synapse with mononuclear cells, granulocytes, mast cells, macrophages)
- TC could be mesenchymal stem cells (MSC); *in vivo* identity of MSC is still unknown (TC and MSC are CD34+ cells)
- tensional integration of the tissue, considering their characteristic ultrastructure (extremely long and contorted processes with intermediate filaments and microtubules parallel to the long axis of the cell, attachment plaques connecting it to the extracellular matrix)
- in neurotransmission in the gut, possibly contributing to spread the slow waves generated by the ICC.

Even the TCs are not fully characterized and their roles are speculative, recent studies showed that TC may be involved in a few important pathologies:

- isolated atrial amyloidosis and atrial fibrillation;
- neoangiogenesis in cardiac recovery after experimental myocardial infarction;
- PEComas -perivascular epithelioid cell tumours;
- GISTs-gastro-intestinal and extra-gastrointestinal stromal tumours.

Funding from research grants: NUCLEU PN 06.26/2005-2008; CEEX 112/2006-2008; NUCLEU PN PN09_33/2009-2011

FUTURE RESEARCH DIRECTIONS

- **Telocytes - specific markers**
- **Telocytes - origin and lineage tracing**
- **Role of telocytes in physiology and pathology**

ANNEX 1 - Reprint of "Faussone-Pellegrini MS, Popescu LM (2011) TELOCYTES. *BioMolecular Concepts* DOI: 10.1515/BMC.2011.039"

ANNEX 2 - Reprint of "Popescu LM, Faussone-Pellegrini MS (2010) TELOCYTES – a case of serendipity: the winding way from Interstitial Cells of Cajal (ICC), via Interstitial Cajal-Like Cells (ICLC) to TELOCYTES. *J Cell Mol Med* 14: 729–740 (24 ISI citations)

ONGOING PROJECT:

'TELOCYTES IN HEART RENEWAL'

project coordinated by **Acad. L.M. Popescu (348.837 euro)**

IDEI-PCE 2011 competition for 3 years (2011-2014)

Stem cell therapy for cardiac diseases has been started before an intrinsic regenerative capacity of heart to be proved and accepted. The dogma that mammalian heart is terminally differentiated organ has been challenged by the reports of few types of resident cardiac stem or progenitor cells. Moreover, a new type of interstitial cell – telocyte - has been described in the adult heart and one important role seems to be nursing stem cells and progenitors in the cardiac stem cell niches. We plan to study the cellular and developmental biology of cardiac stem niches and their involvement in cardiac renewal considering that basic mechanisms governing its physiology are still unknown. By extensive ultrastructural investigation (electron tomography included), confocal microscopy and miRNAs detection we plan a basic research of telocytes and cardiac stem cell niches in normal, ageing and diseased mammalian heart. We also will run a comparative study of regeneration in mammalian and zebrafish (known to have high regenerative capacity) injured hearts. We will try to answer major question: there are one or more types of cardiac stem cell; which cells are mandatory for cardiac renewal; which factors are most important in stem cell differentiation; how newly formed cardiomyocytes are integrated in contractile myocardium; how all these are challenged in diseased heart? All these questions must be answered before an effective cell therapy could be envisaged.

Visibility

All Databases [Select a Database](#) [Web of Science](#) [Additional Resources](#)
[Search](#) [Search History](#) Compound Marked List (0)

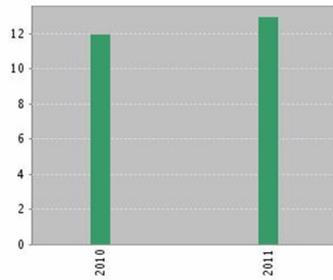
All Databases

[<< Back to previous results list](#)

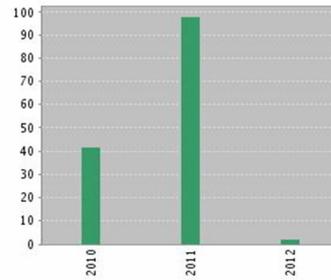
Citation Report Topic=(telocytes)
Timespan=2005-2011.

This report reflects citations to source items indexed within All Databases.

Published Items in Each Year



Citations in Each Year



Results found: 25
Sum of the Times Cited [?]: 142
Sum of Times Cited without self-citations [?]: 12
Citing Articles[?]: 27
[View Citing Articles](#)
[View without self-citations](#)
Average Citations per Item [?]: 5.68
h-index [?]: 8

All Databases [Select a Database](#) [Web of Science](#) [Additional Resources](#)
[Search](#) [Search History](#) Compound Marked List (0)

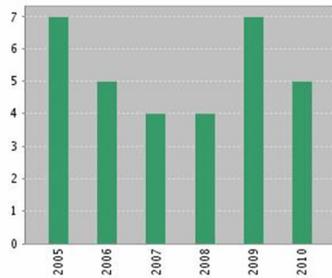
All Databases

[<< Back to previous results list](#)

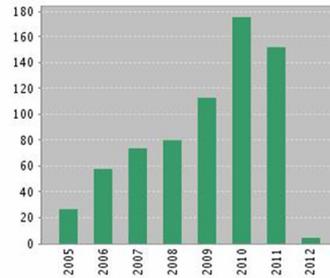
Citation Report Topic=(Cajal like) AND Author=(Popescu LM)
Timespan=2005-2010.

This report reflects citations to source items indexed within All Databases.

Published Items in Each Year



Citations in Each Year



Results found: 32
Sum of the Times Cited [?]: 685
Sum of Times Cited without self-citations [?]: 486
Citing Articles[?]: 241
[View Citing Articles](#)
[View without self-citations](#)
Average Citations per Item [?]: 21.41
h-index [?]: 17