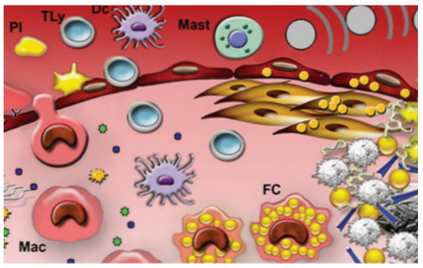
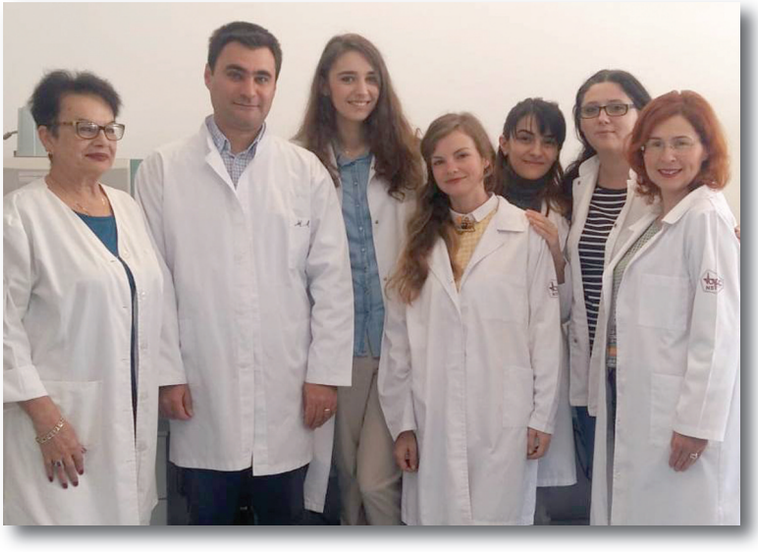


MOLECULAR AND CELLULAR PHARMACOLOGY - FUNCTIONAL GENOMICS LABORATORY

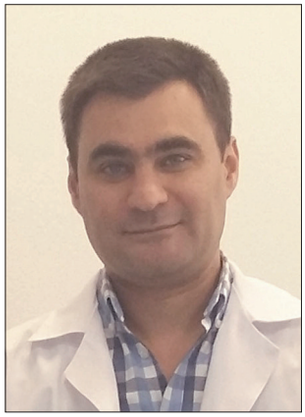
***Adrian Manea**, PhD*
HEAD OF LABORATORY

STAFF
***Monica Raicu,** PhD*
***Simona-Adriana Manea,** PhD*
***Mihaela-Loredana Vlad,** PhD Student*
***Alexandra-Gela Lazăr,** PhD Student*
***Monica-Teodora Cosac,** Master Student*

FORMER RESEARCH STAFF:
Rozalia Mora, Şerban Tasca, Nicolae Ghinea, Anton Fixman, Lucian Ghiţescu, Luminiţa Pojoga, Stela Florea.



MOLECULAR AND CELLULAR PHARMACOLOGY-FUNCTIONAL GENOMICS LABORATORY



Adrian Manea, *PhD*

Head of Laboratory
E-mail: *adrian.manea@icbp.ro*

Major position /appointments

- Principal investigator grade I
- Member of the Scientific Council

PUBLICATIONS

Over 35 original articles (> 1.400 citations) were published in Web of Sciences Core Collection journals and 3 book chapters (Intech, Springer) between 2004-2019 by researchers of the Laboratory.

MAJOR RESEARCH INTERESTS

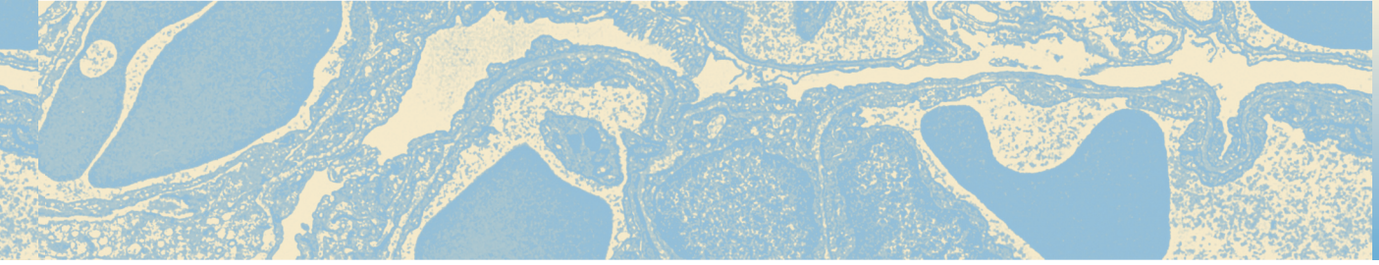
- **Reactive oxygen species and redox signaling in vascular physiology and pathology.**
- **Investigation of epigenetic control mechanisms (DNA methylation, post-translational modifications of nucleosomal histones, non-coding RNA) underlying oxidative stress and vascular inflammation in atherosclerosis and diabetes.**
- **Development of innovative in vivo imaging techniques employing nanocarriers for targeted delivery of redox and/or inflammation sensitive contrast agents to better estimate the burden of atherosclerotic lesions in individuals at risk for cardiovascular disorders (in collaboration with Medical and Pharmaceutical BioNanoTechnologies Laboratory).**
- **Development and testing of novel ultrasound-based pharmacological approaches for targeted delivery of drugs in experimental atherosclerosis and diabetes.**

SELECTED NEW FINDINGS

Pericytes express functional NADPH oxidase (Nox) complex.

- Pericytes are able to produce reactive oxygen species (ROS) in response to several diabetic factors such as high concentration of glucose and advanced glycation end products. These findings prompted us to investigate the sources of ROS that may contribute to these effects.
- Pericytes constitutively express functional Nox that is up-regulated by high glucose and angiotensin II. These are the first studies indicating that pericytes express Nox, an enzymatic complex whose unique biological function is the production of ROS.
- Pharmacological targeting of Nox (currently under clinical development) could be an important therapeutic strategy in the treatment of microvascular disorders such as diabetic retinopathy.

In diabetes, the mechanisms of endothelin-1 regulation imply activation of mitogen-activated protein kinase (MAPK), protein tyrosine kinase (c-Src) and pro-inflammatory transcription factors (STAT, C/EBP).



● JAK/STAT pro-inflammatory signalling pathway plays a key role in the up-regulation of ET-1 in high glucose-exposed human ECs. Since ET-1 also activates JAK/STAT, the results of this study anticipate a novel auto-regulation mechanism whereby JAK/STAT may contribute to the sustain release of ET-1 in diabetes.

● C/EBPα, C/EBPβ, and C/EBPδ pro-inflammatory transcription factors are activated by high glucose *via* mitogen-activated protein kinase (MAPK) signalling, and that C/EBP isoforms are co-ordinately implicated in the upregulation of ET-1 in high glucose-exposed human ECs.

● Protein tyrosine kinase c-Src plays a key role in mediating the up-regulation of ET-1 and pro-inflammatory molecules (MCP-1, ICAM-1, VCAM-1) in diabetic mice.

● Pharmacological targeting of JAK/STAT, MAPK-C/EBP axis, and c-Src may represent important therapeutic strategies to reduce vascular inflammation and remodelling in diabetes.

Gene variants of the nitric oxide synthase (NOS), endothelin-1 (ET-1) and renin-angiotensin system (RAS) as predictive biomarkers of chronic vascular complications in diabetes.

● Genetic biomarkers are critical predictors of diabetes-associated cardiovascular disorders. In previous population studies we have identified novel gene variants of the nitric oxide synthase

● NOS (NOS3 VNTR, NOS3 G894T), ET-1 (EDN1 C8002T), and RAS (ACE I/D, AGT M235T and AGTR1 A1166C) genetic variants confer risk or protection against microangiopathies in type 2 diabetic obese subjects.

● Polymorphism in exon 7 of the endothelial NOS gene is associated with low incidence of microvascular damage in type 1 diabetes.

● In a population study, we have demonstrated the existence of a correlation of low molecular weight advanced glycation end products and NO metabolites with chronic complications in type 1 diabetic patients.

NF-kB, AP-1, STAT1/3, and C/EBP pro-inflammatory transcription factors are implicated in the regulation of NADPH oxidase expression and function in vascular cells.

● The pro-inflammatory transcription factor AP-1 regulates NADPH oxidase expression in human cultured human aortic smooth muscle cells: role of p22phox subunit

● Jak/STAT signalling pathway regulates Nox1 and Nox4-based NADPH oxidase in human aortic smooth muscle cells.

● NADPH oxidase 5 expression is positively regulated by proinflammatory-related mechanisms in human aortic smooth muscle cells.

● C/EBP transcription factors regulate NADPH oxidase in human aortic smooth muscle cells in vitro.

● High-glucose-increased expression and activation of NADPH oxidase in human vascular smooth muscle cells is mediated by 4-hydroxynonenal-activated PPARα and PPARβ/δ.

Expression and regulation of NADPH oxidase 5 in human monocytes and macrophages.

● Human monocytes and macrophages express different Nox5 variants that are up-regulated in human atherosclerotic plaques.

Histone acetyltransferase (HAT) and histone deacetylase (HDAC)-related epigenetic mechanisms underlay vascular oxidative stress and inflammation in experimental atherosclerosis and diabetes.

● Activation of histone deacetylase-dependent epigenetic pathways induces vascular NADPH oxidase expression and reactive oxygen species overproduction in experimental diabetes.

● Histone acetyltransferase-dependent pathways mediate up-regulation of NADPH oxidase 5 in human macrophages under inflammatory conditions: a potential mechanism of reactive oxygen species overproduction in atherosclerosis.

CURRENT PROJECTS

1. UNCOVERING OF NOVEL EPIGENETIC PATHWAYS THAT INDUCE FORMATION OF ANTI-INFLAMMATORY MACROPHAGES AS POTENTIAL THERAPEUTIC TARGETS IN ATHEROSCLEROSIS

Despite the progresses in primary and secondary prevention, cardiovascular diseases (CVD) remain the leading cause of morbidity and mortality worldwide. Thus, new approaches are essential to implement novel anti-atherosclerotic therapies.

Recent data (including ours) demonstrated that monocyte-derived macrophages (Mac) actively orchestrate important inflammatory and oxidative reactions in atherosclerotic plaque formation. Two major Mac populations with distinct phenotypes have been described: the pro-inflammatory Mac (M1-Mac) and the anti-inflammatory Mac (M2-Mac). Consequently, the active induction of M2-Mac may be therapeutically relevant for the outcome of atherosclerosis.

Emerging evidence indicates that epigenetic mechanisms, such as histone acetyltransferases (HAT) and histone deacetylases (HDAC)-regulated gene expression, play a role in the pathoetiology of CVD.

The goal of this project is to find approaches to stimulate the generation of M2-Mac by modulating novel HAT and HDAC-dependent pathways. Subsequently, the specific HAT/HDAC isoforms could become novel pharmacological targets in atherosclerosis.

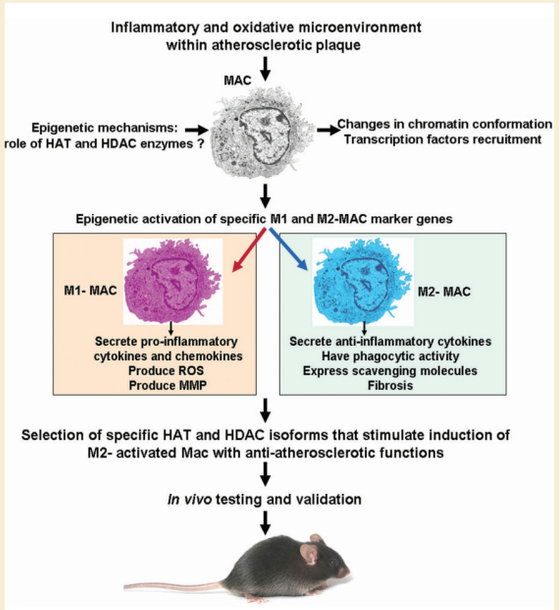
The specific objectives are:

(1) Analysis of HAT and HDAC-dependent epigenetic mechanisms implicated in Mac polarization – in vitro studies on mouse and human Mac.

(2) Testing the selected HAT and HDAC isoforms implicated in M2-Mac polarization on ApoE-/- mice as potential therapeutic targets in atherosclerosis - preclinical in vivo studies.

The expected outcomes are:

(a) to provide new knowledge on the mechanisms of Mac polarization within the atherosclerotic plaque; **(b)** to find the HAT/HDAC isoforms that promote the generation of M2-Mac in vivo; and **(c)** to create an experimental platform for testing isoform-specific HAT/HDAC inhibitors to stimulate the induction of M2-Mac, with anti-atherosclerotic functions.



Schematic representation of the general concept and the goal of the project. *In response to inflammatory and oxidative micro-environment within atherosclerotic plaque several epigenetic mechanisms (HAT and HDAC enzymes) are activated in monocyte-derived Mac. This triggers changes in chromatin conformation that affect transcription factor-DNA interactions in the genome leading to M1 or M2-Mac polarization. M1-Mac secrete pro-inflammatory cytokines and chemokines, reactive oxygen species (ROS), and matrix metalloproteinases (MMP) that contribute to plaque development and vulnerability. M2-Mac produce anti-inflammatory cytokines*

and play a role in tissue repair. Induction of M1 and impaired M2 differentiation lead to inflammation and oxidative stress. We plan to uncover the epigenetic pathways of Mac polarization in atherosclerosis involving HAT and HDAC-dependent mechanisms and to select specific HAT/HDAC isoforms implicated in M2-Mac polarization for preclinical validation in atherosclerotic ApoE-/- mice. Understanding the fine-tuning mechanisms of Mac polarization will lead to the development of innovative drugs and new anti-atherosclerotic therapeutic strategies.

2. PRECLINICAL STRATEGY TO REDUCE VASCULAR INFLAMMATION AND OXIDATIVE STRESS BY TARGETING NOVEL NON-CODING RNA PATHWAYS IN ATHEROSCLEROSIS

Recent data (including ours) demonstrated that inflammation and oxidative stress are critically involved in the atheroma formation and destabilization of vulnerable plaque. Left untreated, atherosclerosis predisposes to major cardiovascular events such as stroke, myocardial infarction, heart failure, and sudden death. Thus, there is a stringent need for the development of specific atherosclerotic plaque-oriented therapies to significantly reduce the incidence of major cardiovascular events. Evidence exists that microRNAs may be effective therapeutic targets in atherosclerosis. Yet, the role of miRNAs in vascular pathobiology represents an open issue. Mimicking of anti-atherogenic miRNAs and/or blocking the pro-atherogenic miRNAs could become a reliable intervention in cardiovascular clinical practice.

The goal of this project is to identify and functionally characterize miRNAs underlying vascular inflammation and oxidative stress in order to develop novel nanotechnology-based miRNA therapeutics in atherosclerosis.

The specific objectives:

(1) Identification of miRNAs underlying vascular inflammation and oxidative stress in atherosclerosis - in vitro and in vivo studies.

(2) Molecular targeting of selected inflammation and oxidative stress-related miRNAs to reduce atherosclerotic plaque development - preclinical in vitro and in vivo studies.

The expected outcomes: (a) A new panel of in vivo validated miRNAs that may serve as biomarkers, therapeutic targets and/or therapeutic agents in atherosclerosis; **(b)** A novel preclinical nanotechnology-based strategy to reduce the development of atheroma by modulating key miRNA-related pathways.

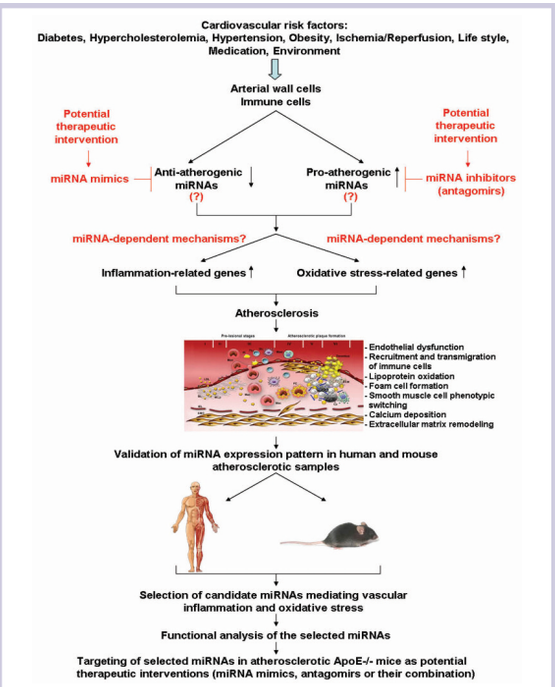


Diagram depicting the general concept and the goal of the project. Arterial wall cells (e.g., endothelial cells - EC, smooth muscle cells - SMC, fibroblasts) and immune cells (e.g., monocytes, lymphocytes, mast cells, and macrophages) are activated in response to cardiovascular risk factors, and following of a series of complex mechanisms different miRNAs are up- or down-regulated. The imbalance between anti- and pro-atherogenic miRNAs leads to the activation of inflammation- and oxidative stress-related genes thus contributing to atherosclerotic

plaque development. The diagram shows by question marks and red text that we plan to identify novel miRNA-dependent pathways underlying inflammation and oxidative stress, key pathological processes in atherogenesis. The newly identified miRNAs will be validated in both human and mouse experimental settings. The selected miRNAs will be targeted employing a nanocarrier for systemic delivery of miRNA mimics, antagomirs or their combination as potential therapeutic strategy to reduce atheroma formation.

3. TARGETING INNATE IMMUNE MECHANISMS TO IMPROVE RISK STRATIFICATION AND TO IDENTIFY FUTURE THERAPEUTIC OPTIONS IN MYOCARDIAL INFARCTION (INNATE-MI)

a multi-laboratories project coordinated by Maya Simionescu

Project goal:

To identify central molecules that mediate the crosstalk between sub-populations of PMN and MAC, and determine their involvement in MI. Additionally, we will test the ability of specific therapies to regulate myocardial inflammation and to improve cardiac function in-vivo.

Objectives of Molecular and Cellular Pharmacology – Functional Genomics Laboratory:

(1) To identify key mediators that determine post-MI myocardial remodeling and prognosis – We will investigate the relationships between the soluble PMN/MAC mediators, post-MI cardiac function and prognosis in MI patients.

(2) Investigation of the crosstalk between PMN and MAC in MI – We will use in-vitro studies, genomics and proteomics to identify mediators that govern the PMN-induced MAC polarization into sub-populations that promote repair. The role of the identified molecules will be tested in-vivo.

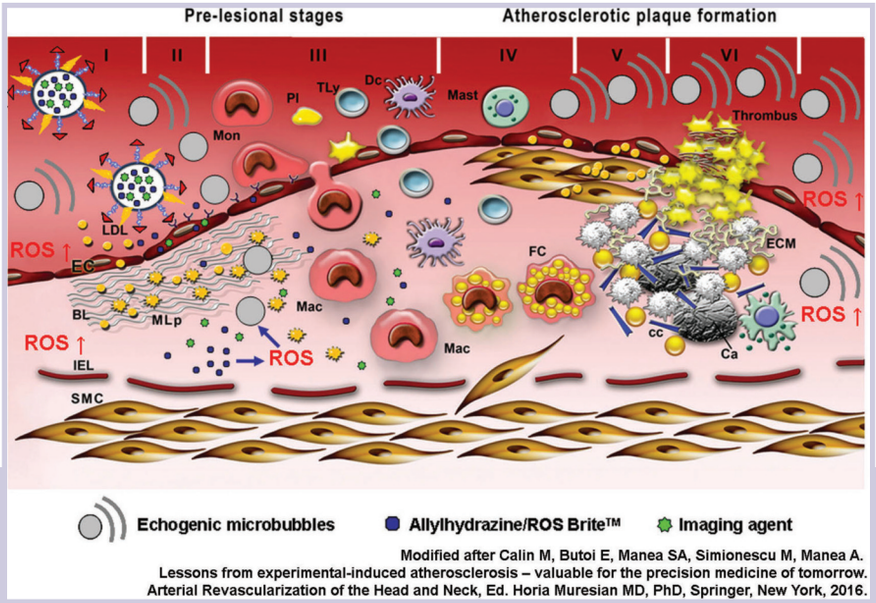
4. NANOTECHNOLOGY-BASED METHOD FOR NON-INVASIVE MOLECULAR IMAGING OF OXIDATIVE STRESS IN CARDIO-VASCULAR DISORDERS

Cardiovascular diseases (CVD) represent the main cause of morbidity and mortality worldwide. Atherosclerosis is the most common CVD that affects the function and structure of the arterial walls. Atherosclerotic plaque destabilization and rupture leads to major cardiovascular events such myocardial infraction, stroke, and sudden death. Since atherosclerosis remains asymptomatic for a long period of time, finding straightforward, non-invasive, sensitive, and cost-efficient imaging procedures to detect early atherosclerosis is needed. Our previous studies demonstrated that reactive oxygen species (ROS) production is enhanced in all the stages of atherosclerosis and is directly correlated with the severity of atheroma in humans and mice. The scope of this project is to develop and validate at preclinical level an innovative ultrasound-based imaging method employing nanocarriers for targeted delivery of redox-sensitive ultrasound contrast agents to assess oxidative stress in atherosclerosis. The proposed strategy entails a systematic set of experiments employing scattered and highly synergistic methods and in vitro an in vivo models.

Our hypothesis is that acoustic detection of ROS via chemically-generated gas microbubbles may be an efficient strategy to routinely diagnose early atherosclerosis and a valuable tool to set-up and optimize the treatment patients at high risk for CVD.

To demonstrate the feasibility of this hypothesis we plan to develop an alternative and efficient nanotechnology-based method to better estimate early atherosclerotic lesions employing a redox-sensitive ultrasound contrast agent (allylhydrazine) and sterically stabilized targeted liposomes designed by our group.

The project end-product will consist in an innovative and preclinical validated method for non-invasive imaging of oxidative stress in CVD. This method may be used for diagnosis of other maladies marked by oxidative stress (cancer, neurodegeneration, diabetes, etc.).



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Concept diagram depicting the strategy of detecting ROS in different stages of atherosclerosis by ultrasound imaging. Polyethylene glycol (PEG) stabilized targeted Lp will be used to increase the transport efficiency and to reduce the concentration of allylhydrazine at non-target vascular sites. ROS-sensitive probes (ROS Brite™), coupled with high resolution in vivo fluorescence imaging will be employed as validating strategy. Imaging agents will be incorporated into the Lp to monitor their biodistribution. Changes in acoustic impedance triggered by ROS-induced microbubbles will be detected by scanning vascular territories prone to atherosclerosis (aorta, carotid, coronary, and femoral arteries).

GRANTS AWARDED BY COMPETITION (2003-2019)

INTERNATIONAL

European Foundation for the Study of Diabetes - New Horizons Collaborative Research Initiative, (Project coordinator Adrian Manea), 2010-2012. Partner: Prof. Shlomo Sasson, The Hebrew University of Jerusalem, Israel. **Project title:** *Investigation of the role of PPARs in mediating oxidative stress signals in vascular cells in diabetes*.

European Cooperation in Science and Technology (COST): Biomedicine and Molecular Biosciences COST Action BM1203**.** 2012-2016. **Project title:** *The European Network on Oxidative Stress and Redox Biology Research*. **Acronym**: EU-ROS. **Member of the Management Committee:** Adrian Manea (RO).

NATIONAL

Romanian CNCS-UEFISCDI, Project number TE 51/2018, (Project coordinator Adrian Manea), 2018-2020. Project title: *Preclinical strategy to reduce vascular inflammation and oxidative stress by targeting novel non-coding RNA pathways in atherosclerosis***.**

Romanian CNCS-UEFISCDI, Project number PCE 69/2017, (Project coordinator Maya Simionescu), 2017-2019. Project title: *Novel epigenetic pathways to induce anti-inflammatory macrophages as potential therapeutic targets in atherosclerosis.*

Romanian CNCS-UEFISCDI, Project number PED 137/2017, (Project coordinator Adrian Manea), 2017-2018. Project title: *Nanotechnology-based method for non-invasive molecular imaging of oxidative stress in cardiovascular disorders*.

Romanian CNCS-UEFISCDI, Project number TE 26/2011, (Project coordinator Simona-Adriana Manea), 2011-2014. Project title: *Investigation of molecular mechanisms of endothelin system in diabetes; development of new pharmacological strategies to improve vascular function*.

Romanian CNCS-UEFISCDI, Project number TE 65/2010, (Project coordinator Adrian Manea), 2010-2013. Project title: *Novel molecular pharmacology strategies for reduction of oxidative stress and inflammation in the vascular wall cells in diabetes*".

Romanian CNCS-UEFISCDI, Project number IDEI 1005/2009, (Project coordinator Adrian Manea), 2009-2011. Project title: *Inflammation and oxidative stress in atherosclerosis: cellular and molecular mechanisms, identification and characterization of novel biomarkers of vascular dysfunction*.

Romanian Academy, Project number 73/2007, (Project coordinator Adrian Manea), 2007-2008. Project title: *Study of molecular mechanisms implicated in the regulation of NADPH oxidases transcription in vascular smooth muscle cells*.

Romanian Academy, Project number 65/2007, (Project coordinator Simona-Adriana Manea), 2007-2008. Project title: *Impact of genetic polymorphisms of vasoactive mediators on vascular dysfunction associated with metabolic syndrome*.

Romanian Academy, Project number 109/2005, (Project coordinator Simona-Adriana Manea), 2005-2006. Project title: *Impact of genetic polymorphisms of vasoactive mediators in myocardial infarction*.

Romanian Academy, Project number 62/2003, (Project coordinator Simona-Adriana Manea), 2003-2004. Project title: *Polymorphisms of nitric oxide synthase in diabetic nephropathy*.

AWARDS

2014-2019 – ***7 poster presentation awards at international congress*** (Adrian Manea, Simona-Adriana Manea, Mihaela-Loredana Vlad, Alexandra-Gela Lazăr)

2012 – ***"Nicolae Simionescu Award"***, conferred by the Romanian Academy (Adrian Manea).

2010 – ***"In Hoc Signo Vinces Award"***, conferred by Romanian Ministry of Education and Research and the Executive Unit for Financing Higher Education, Research Development (Adrian Manea).

2008 – ***"George Emil Palade Award"***, conferred by the Romanian Academy and National Science and Art Foundation (Adrian Manea).

2008 – ***"Constantin Velican Award"***, conferred by Romanian Society for Cell Biology (Adrian Manea).

1998 – ***"Sanofi Award"*** (Monica Raicu).

1998 – ***"Nicolae Simionescu Award "***, conferred by the Romanian Academy (Monica Raicu).

1995 – ***"Falcon Prize"*** (Monica Raicu).