Annual Report | 2009

Experimental Biology and Biomedicine | Research Programmes

Biology | Nanoscience | Health and Disease | Biotechnology

Center for Neurosciences and Cell Biology | Associate Laboratory

A new culture through Scientific Research

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Introduction | General Objectives

CNC major mission is to foster fundamental and translational research and training in biomedical science with a particular focus on neurosciences.

The current aims at CNC are: 1) Fundamental and Translational research in Neuroscience, Cell Biology and Molecular Biotechnology, 2) Advanced training; 3) Technology transfer and specialized services to the community; 4) Outreach Programme (science and society).

The core scientific activity of CNC is the study of the molecular basis of neurodegenerative processes common to aging, neurodegenerative disorders, cerebral ischemia and epilepsy. In parallel, several groups explore mechanisms of neuroprotection and regeneration, which may be future candidates for the development of potential therapeutic strategies to manage these disorders. This core activity is complemented by supporting areas which also develop their own research activity, opening the scope of intervention of CNC in the biomedical field, while providing novel lines of research applicable to Neuroscience, namely: A) Molecular Biotechnology, with expertise in genetic screening of diseases, structure-function relation of proteins with biomedical or biotechnological interest and development of new vectors for delivery of drugs and genetic material and biomaterials for stem cell- based therapeutics;

B) Molecular and Cellular Toxicology, focused on the study of drug and disease-induced cell dysfunction, aiming to understanding the molecular basis for clinical drug toxicity, with particular expertise in processes involving mitochondrial dysfunction and free radicals; C) Biomedical NMR and Metabolomics with a strong focus on the development of inorganic compounds for medical diagnosis (eg MRI contrast agents), intermediate metabolism and diabetes; D) Cellular and Developmental Biology, whose programs focused on human infertility, disruption of human cell function in cancer, contact dermatitis, osteoarthritis, auto-immune disease, obesity and pathogens biology, involve close partnerships with clinicians at HUC and IPO; E) Microbiology with emphasis on the strategies for adaptation of microorganisms to extreme environments, the screening and development of new anti-mycobacterial drugs and the susceptibility to legionella and fungal infection.

Post-graduate education is a major goal at CNC. Its Doctoral Programme in Experimental Biology and Biomedicine and the participation in the MIT/Portugal Protocol Doctoral Programme provides Master and PhD students with a multi-faceted education in molecular life sciences related to disease and contributes to international scientific networking.

Development of new technologies routed on solid fundamental research, and stimulated by the growing interest in translational research, led to reorganization of the services sector and to the creation of a technology transfer unit at Biocant. Thus specialized services and technology transfer became a current aim of CNC.

Outreach programme, the fourth current aim of CNC, aims at society scientific education and at a public perception of the importance of science for human health. To reach this goal, specific scientific programmes will continue to be implemented in collaboration with schools and with several social and cultured associations.

Facts & Figures (2009)

RESEARCH STAFF

Members holding Ph.D.	109
Ph.D.Students	174
MSc Students	63
Grant Technicians	11

PUBLICATIONS IN 2009	**155**

THESIS CONCLUDED – 2009

Ph.D. thesis	14
MSc thesis	42

Organization

The Center for Neuroscience and Cell Biology (CNC) is a non-profit biomedical research center of public utility at the University of Coimbra. CNC brings together scientists from the Faculties of Science and Technology, Medicine and Pharmacy and from the University Hospital. The CNC is a "Laboratório Associado".

Associate Members of CNC are: Universidade de Coimbra (principal associate – 50%), Hospitais da Universidade de Coimbra, Fundação para a Ciência e Tecnologia, AIBILI, Fundação Bissaya Barreto and two comercial firms – Reagente 5 and ILC.

1- Governing Body

President:	*Catarina Resende de Oliveira*
Vice Presidents:	*Euclides Pires*
	Carlos Faro
	Leonor Almeida
Honorary President:	*Arsélio Pato de Carvalho*
Executive Council	Directors of the Departments
Research Council	CNC members holding PhD
"Conselho Fiscal"	T. Macedo, A. Rodrigues, Leal e Carreira
"Revisor Oficial de Contas"	Leal e Carreira, Sociedade Revisora de Contas

External Advisory Commitee Enrique Cadenas (USA); Roberta Brinton (USA); George Perry (USA); Mark Smith (USA); Helmut Sies (Germany); Stephen Zinder (USA).

2- Scientific Areas and Research Groups

At present, research programmes and projects are organized in 6 scientific areas, each coordinated by a senior scientist. The programme for each area is implemented by small research groups each headed by a research leader in his field of study. In 2009, the research groups for each area can be identified, according to the following organization:

Neuroscience and Disease | *Catarina Oliveira*

Neuromodulation Group (*Head: Rodrigo Cunha*)

Glutamatergic Synapses Group (*Head: Ana Luísa Carvalho*)

Neuroprotection and Neurogenesis in Brain Repair Group (*Head: João Malva*)

Neuronal Cell Death and Neuroprotection Group *(Head: Carlos B. Duarte*)

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group (*Head: A. Cristina Rego*)

Molecular Mechanisms of Disease Group (*Head: Claudia Pereira*)

Neuroendocrinology and Neurogenesis Group (*Head: Claudia Cavadas*)

Molecular Biotechnology and Health | *Euclides Pires*

Molecular Biotechnology Group (*Head: Carlos Faro)*

Molecular Systems Biology Group (*Head: Armindo Salvador)*

Structural and Computational Biology Group (*Head: Rui Brito*)

Vectors and Gene Therapy Group (*Head: M. Conceição Pedroso Lima*)

Biomaterials and Stem Cell-Based Therapeutics Group (*Head: Lino Ferreira*)

Cell and Molecular Toxicology | *Leonor Almeida*

Mitochondrial Toxicology and Disease Group (*Head: Paulo Oliveira*)

Redox Biology in Health and DiseaseGroup (*Head: João Laranjinha*)

Membrane Toxicity Group (*Head: Amália Jurado*)

Pharmacometrics Group (*Head: Amilcar Falcão*)

Microbiology | *Milton Costa*

Microbiology of Extreme Environments Group (*Head: Milton Costa*)

Medical Mycology - Yeast Research Group (*Head: Teresa Gonçalves*)

Biophysics and Biomedical NMR | *Carlos Geraldes*

Inorganic Biochemistry and Molecular Imaging Group *(Head: Carlos Geraldes)*

Intermediate Metabolism Group *(Head: John Grifith Jones)*

Cell and Development Biology | *Celeste Lopes and João Ramalho Santos*

Cellular Immunology and Oncobiology Group (*Head: Celeste Lopes*)

Biology of Reproduction and Human Fertility Group (*Head: João Ramalho Santos)*

Infection, Phagocytosis and Pathogens Group (*Head: Otilia Vieira)*

Molecular and Translational Medicine Group (*Head: Eugénia Carvalho)*

Emerging Group

Chronic Inflammation Group (*Head: Margarida Carneiro)*

Neuroscience and Disease Area

Coordinator: Catarina Resende Oliveira

This Area pursues its research interests on clarification of molecular mechanisms of synaptic activity modulation and its involvement in neurodegenerative disorders with the ultimate goal to develop new strategies of neuroprotection and brain repair. These objectives are accomplished by the seven groups in this Area: *Neuromodulation*: effect of synaptic activity modulators that affect brain metabolism, purines and cannabinoids; *Glutamatergic Synapses*: regulation of excitatory glutamatergic synapses; *Neuronal Cell Death and Neuroprotection*: excitotoxic cell damage and neuroprotection by neurotrophic factors; *Neuroprotection and Neurogenesis in Brain Repair*: identification of inflammatory mediator's and neuropeptides pro-neurogenic effect; *Molecular Mechanisms of Disease*: mechanisms of neurodegeneration associated to peptide aggregation; *Mitochondrial Dysfunction and Cell Death*: mitochondrial-driven neuronal death and transcription deregulation in Huntington's disease; *Neuroendocrinology and Neurogenesis*: adrenal-hypothalamic axis and adipose tissue negative regulation of neuronal protection.

The main achievements of the groups in this Research Area are described in detail in the respective individual reports. In brief:

1. Adenosine A2A receptors were identified as the target for caffeine-mediated neuroprotection, preventing memory impairment in Alzheimer's Disease and diabetic encephalopathy models. Endocannabinoids control brain glucose metabolism.

2. The regulation of AMPA receptors function is critical for the long-lasting synaptic changes underlying learning and memory. A proteomic screening identified novel proteins related to RNA regulation. BDNF promotes the acetylation of cytoskeleton proteins, being suggested to regulate mRNA and dendritic protein stability. Under excitotoxicity, deregulation of proteasomal activity and glutamic decarboxylase isoforms were identified as the target of proteasome for the first time.

3. NO, IL1b and NPY modulate proliferation and differentiation of endogenous neural progenitor cells and mediate the antiproliferative effect of inflammation in neural stem cells. NPY also induced adipogenesis and the overexpressing of hypothalamic endogenous NPY led to an increase of food intake and obesity.

4. Mitochondria-dependent apoptosis was demonstrated to be involved in human Huntington's disease, and BDNF prevented detrimental changes in transcription and apoptotic neuronal death. In mice postnatal neurosphere-derived cells, this neurotrophin increased neuronal differentiation.

5. Mitochondria dysfunction and ER-mitochondria crosstalk were shown to be key players in Ab induced neuronal death in Alzheimer's disease and the crosstalk between mitochondrial ROS and HIF-1 is a link between AD and Type 2 diabetes. Mitochondria dysfunction was also associated with microtubule depolymerisation, alteration of autophagic-lysosomal pathway and a-synuclein aggregation in Parkinson's disease.

6. The development of new imaging platforms useful to functionally identify new oligodendrocytes differentiating from SVZ-derived neural stem cells led to an impressive advance in the identification of inflammatory mediators, angiopoietin-1, galanin and somatostatin as biomolecules involved in glia-neuron communication and in the modulation of the neurogenic niche.

Future plans of Neuroscience of Disease Research Line include the reinforcement and expansion of the ongoing competitive basic research focused on the molecular mechanisms of neurodegeneration, neuroprotection, neurogenesis and brain repair, from the cellular level to in vivo animal models, as specified in each group research plan. Perform high quality research, with international impact in fundamental cellular and molecular neuroscience and mechanisms of brain disease, is a common goal of all the groups of this research area. Some research groups in this area are currently working in the

borderline between basic and applied research. Pushing forward some translational research approach to boost the development of high quality translational research in Neuroscience is one of the aims in a near future. Promoting internal collaborations between groups at Neuroscience and Disease and other research groups working in different areas at CNC, namely Biotechnology and Health, Cellular and Molecular Toxicology will allow to use biocompatible carriers for drug and gene delivery, such as viral vectors, molecular biology and proteomics approaches and the use of new sensors and electrodes to study brain function. These interdisciplinary approaches will lead to innovation and increased quality research projects. Recruitment of new research leaders with expertise in RNA biology and cutting-edge competence in brain imaging and electrophysiology, including multi-electrode recording of neuronal network activity is crucial to develop competitive research in neuroscience.

Neuromodulation Group

Rodrigo A. Cunha	PhD – *Head of group*
Ângelo José Ribeiro Tomé	PhD
Attila Köfalvi	PhD
Geanne M. Cunha	PhD
Henrique Bernardo Silva	PhD
Jean-Pierre Oses	PhD
Lisiane O. Porciúncula	PhD
Paula G. Agostinho	PhD
Daniela Pochmann	Post-Doctoral Fellow
Manuella P. Kaster	Post-Doctoral Fellow
Paula M. Canas	PhD Student
Alexandre S. Rodrigues	PhD Student
Ana Patrícia Simões	PhD Student
Ana Paula Ardais	PhD Student
Carla Sofia G. Silva	PhD Student
Elisabete O. Augusto	PhD Student
Gianna de Paula Cognato	PhD Student
Marco António P. Matos	PhD Student
Pablo Pandolfo	PhD Student
Pedro Manuel V. Garção	PhD Student
Rui Sanches	PhD Student
Samira C. Ferreira	PhD Student
Sílvia Viana da Silva	PhD Student
Tiago Manuel P. Alfaro	PhD Student
Diana Isabel Q. Rodrigues	Grant Technician
Nuno Miguel J. Machado	Grant Rechnician

Glutamatergic Synapses Group

Ana Luísa Carvalho	PhD – *Head of group*
Ramiro Almeida	PhD
Tatiana Catarino	PhD Student
Joana Ferreira	PhD Student
Luís Ribeiro	PhD Student
Carlos Adriano A. Matos	PhD Student
Maria Joana Pinto	MSc Student

Neuroprotection and Neurogenesis in Brain Repair Group

João O. Malva	PhD – *Head of group*
Armando Cristóvão	PhD
Fabienne Agasse	PhD
Ricardo Reis	PhD
Liliana Bernardino	Post-Doctoral Fellow

Sara Xapelli	Post-Doctoral Fellow
Alexandra Rosa	PhD Student
Ana Sofia Baptista	PhD Student
Clarissa S. Schitine	PhD Student
Helena Sofia Domingues	PhD Student
Joana Barbosa	PhD Student
Pedro G. Réu Carvalho	PhD Student
Raquel Ferreira	PhD Student
Sofia Grade	PhD Student
Ana Rita Bento	MSc Student
Tiago Alexandre Santos	MSc Student
Sandrine Pontes Machado	MSc Student

Neuronal Cell Death and Neuroprotection Group

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Emília P. Duarte	PhD
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Andrea Lobo	PhD Student
Graciano Leal	PhD Student
Joana F. C. Fernandes	PhD Student
João R. Gomes	PhD Student
João T. Costa	PhD Student
Marta Dias M. Vieira	PhD Student
Diogo O. Comprido	MSc Student
Patrícia Rebelo	MSc Student

Mitochondrial Dysfunction and Signaling in Neurodegeneration Group

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Mª Teresa Cunha Oliveira	Post-Doctoral Fellow
Tatiana R. Rosenstock	Post-Doctoral Fellow
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Ana Cristina Silva	PhD Student
Márcio Ribeiro	PhD Student
Mário Laço	PhD Student
Rita Perfeito	PhD Student
Sandra Mota	PhD Student
Carla Maria Nunes Lopes	MSc Student
Carolina Noronha	MSc Student
Luana Carvalho Naia	MSc Student
Luis M. Bajouco	MSc Student
Mª João Rodrigues Ribeiro	MSc Student

Molecular Mechanisms of Disease Group

Cláudia M. F. Pereira	PhD – *Head of group*
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Mª Isabel J. Santana	MD, PhD
Paula Isabel Moreira	PhD
Sandra Isabel M. Cardoso	PhD
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Ana Raquel Esteves	PhD Student
Cristina Carvalho	PhD Student
Daniela M. Arduíno	PhD Student
Renato Xavier Santos	PhD Student
Rui Oliveira Costa	PhD Student
Sónia Correia	PhD Student
Sueli Cristina Marques	PhD Student
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Steve François Carvalho	MSc Student
Ana Isabel P.Fernandes	MSc Student
Diana F Gomes Pimentel	MSc Student
Diana FF Silva	MSc Student
Sílvia Catarina F. Gomes	MSc Student

Neuroendocrinology and Neurogenesis Group

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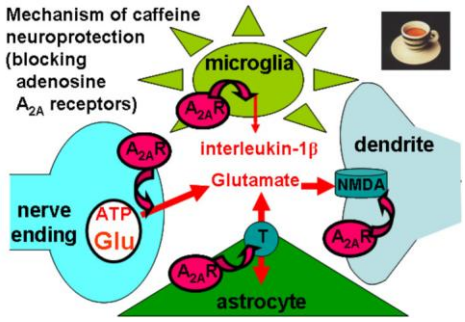
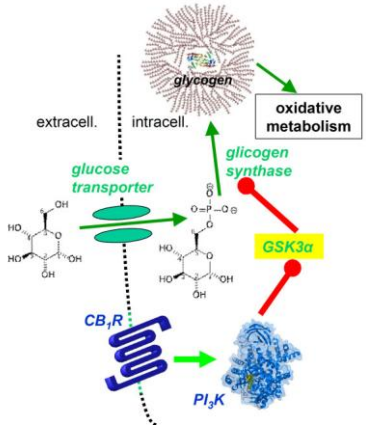
Caetana Carvalho	PhD
Inês Araújo	PhD
Joana Salgado	Post-Doctoral Fellow
Ana Rita Álvaro	Post-Doctoral Fellow
Bruno Carreira	PhD Student
Gabriel Costa	PhD Student
Maria Inês Morte	PhD Student
Ana S. Carvalho	PhD Student
Magda Santana	PhD Student
Mª João Catarino	PhD Student
Vera Raquel Cortez	MSc Student
Ana Sofia S. B. Baptista	Grant Technician

Neuromodulation Group | *Head: Rodrigo A. Cunha*

The mechanisms of brain dysfunction are at present unclear but accumulating evidence indicates that both metabolic dysfunction and synaptic dysfunction may be early events in neurodegenerative diseases. More that understanding the mechanisms of brain diseases, there is an urgent need to devise novel strategies to manage these diseases. Our group focuses on the study of modulators of synaptic activity that can also affect brain metabolism, namely purines (adenosine, ATP) and cannabinoids. We begun exploring the basic properties and function of the neuromodulation systems operated by adenosine and ATP in the nervous system: adenosine and ATP receptors (expression, binding characteristics, coupling to transducing systems, desensitisation), formation and inactivation of ATP and adenosine, physiological roles (control of neurotransmitter release, of ion channels and of synaptic transmission and plasticity) and we are now fostering understanding the role of these systems in physio-pathology using animal models of aging, hypoxia, epilepsy, diabetic neuropathies, stress, Alzheimer's and Parkinson's diseases and neuro-inflammation. Given the dual exploration of purines and on the other hand cannabinoids and brain metabolism, the area was split into two groups: 'Purines at CNC' (lead by RA Cunha) and, on the other hand, 'Neuromodulation and Metabolism' (lead by A Köfalvi).

The major achievement was the identification of adenosine A_{2A} receptors as the target operated by caffeine to afford neuroprotection and prevent memory impairment in models of Alzheimer's disease and diabetic encephalopathy. This supports epidemiologic studies showing an inverse correlation between caffeine consumption and dementia and paves the way to design clinical trials testing the therapeutic effects of A_{2A} receptor antagonists in dementia. The group Neuromodulation and Metabolism has pioneered and established new techniques at the laboratory, namely, parallel monitoring of glucose uptake and metabolism in brain slices, as well as regional mapping of the uptake of fluorescent glucose analogues. We have made the first explorations of the basic properties of cerebral glucose metabolism in the brain, and its control by the endocannabinoid system, and its alteration in diabetic encephalopathy. We are the first to claim for an early impairment of glucose uptake and metabolism in the cortex in animal model of Type-1 Diabetes. We also found the same alteration in the hippocampus of TgApp mice, a model of Alzheimer disease. As for the striatal projects, we pioneered a new highly sensitive technique, namely, the flow cytometric analysis of nerve terminals. We can for the first time rapidly evaluate quantitatively and qualitatively the bulk of nerve terminals for various proteins in the same time. With the help of this technique as well as binding and release experiments, we observed that the $A_{2A}R$ and the CB_1R receptor control each other's activity, possibly through a heterodimer formation, and like this, the beneficial mechanisms of the palliative medicine, istradefylline can be addressed with ease.





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Canas PM, Porciúncula LO, Cunha GMA, Silva CG, Machado NJ, Oliveira JMA, Oliveira CR, Cunha RA (2009) Adenosine A_{2A} receptor blockade prevents synaptotoxicity and memory dysfunction caused by β-amyloid peptides via p38 mitogen-activated protein kinase pathway. *J Neurosci* 29 14741-51 .

Duarte JMN, Carvalho RA, Cunha RA, Gruetter R (2009) Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. *J Neurochem* 111, 368-79.

Ferreira SG, Lomaglio T, Avelino A, Cruz F, Oliveira CR, Cunha RA, Köfalvi A (2009) N-acyldopamines control striatal input terminals via novel ligand-gated cation channels. *Neuropharmacol.* 56:676-683.

Glutamatergic Synapses Group | *Head: Ana L. Carvalho*

Neurons have a complex morphology, with branched dendrites exhibiting thousands of synapses, the contacts where communication between neurons occurs. We are interested in understanding these connections between nerve cells in the brain, and how they are modified with experience. The ability of synapses to change their strength is thought to be the cellular correlate of learning and memory, and synaptic dysfunction occurs in several neurodegenerative diseases. We focus on excitatory glutamatergic synapses, and study their regulation from a cellular and molecular biology viewpoint. We use a combination of primary neuronal cultures, molecular cell biology and biochemistry to address these questions.

Regulation of glutamatergic neurotransmission

Glutamate receptors of the AMPA type mediate the fast excitatory neurotransmission in the CNS, and play key roles in synaptic plasticity. The binding of these receptors to a variety of proteins is known to regulate their targeting to the synapse and consequently to modulate synaptic strength, as well as to modify receptor characteristics. We recently performed a proteomic screening (*Santos et al. J. Proteome Res. 2010*) and re-isolated known AMPAR partners, as well as identified novel interactors, such as motor proteins and proteins of the neuronal RNA granules (see Figure). We are currently addressing their function in regulating AMPAR synaptic expression and activity.

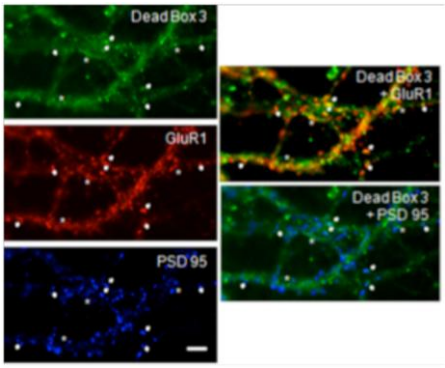
Recent work suggests that synapses employ different combinations of receptor subunits in response to changes in activity. One of the possible mechanisms for regulation of AMPAR composition is through local translation at dendrites of mRNA molecules for specific AMPAR subunits. We found evidence for post-transcriptional regulation of the mRNA levels for GluR1 AMPAR subunit, and are addressing the mechanisms and consequences of such regulation.

NMDA receptors are the coincidence detector in the induction of synaptic plasticity. We are studying the mechanism of synaptic accumulation of NMDARs by using neuronal cultures from knock-out mice for NMDAR subunits, and reintroducing mutated subunits of the NMDAR complex, to identify molecular determinants involved in NMDAR trafficking.

Ghrelin is an appetite-stimulating hormone which was shown to enhance memory processes and synaptic plasticity in the hippocampus. We are studying whether similarly to other appetite regulating hormones, such as leptin (*e.g. Moult et al. 2010*), ghrelin regulates glutamatergic transmission.

The cytoskeleton & synapse maturation

The number of dendritic spines, where excitatory synapses are located, is regulated by molecules that organize their actin cytoskeleton, e.g. cortactin. Cortactin is an F-actin binding protein which is enriched in dendritic spines, and has a role in spine morphogenesis. The simultaneous binding of cortactin to F-actin and the Arp2/3 polymerization machinery is thought to facilitate the nucleation of actin branches on the side of pre-existing filaments of actin. We are interested in how post-translational modifications of cortactin affect its role in promoting synapse maturation.



In a proteomic screening for interactors of long-form AMPA receptor subunits we identified RNA granule proteins (Santos et al. J. Proteome Res 2010), such as the RNA helicase DEAD box 3. It colocalizes with synaptic cell surface (arrows) and extrasynaptic (*) GluR1 in hippocampal neurons in culture. This suggests that the coupling of RNA granules to AMPARs may facilitate regulation of localized translation, in response to synaptic activity. Scale bar: 5 μm.

Synaptic dysfunction in Machado-Joseph disease

A collaborative project with Sandra de Macedo-Ribeiro (IBMC, Porto, Portugal) and Patrícia Maciel (Life and Health Sciences Research Institute, Braga, Portugal) has emerged, focusing on the cell biology of ataxin-3, a polyQ ubiquitin protease with an expansion in Machado-Joseph disease. We are studying the cellular transport of ataxin-3 (*Macedo-Ribeiro et al. PLoS ONE, 2009*) and how it is affected by the polyQ expansion in the disease protein. Moreover, we are interested in understanding how the expanded protein is toxic to selective populations of neurons, and in evaluating whether it causes synapse dysfunction.

Key References

Santos SD, Manadas B, Duarte CB, Carvalho AL. Proteomic analysis of an interactome for long-form AMPA receptor subunits. *J. Proteome Res.* (*in press*)

Santos SD, Carvalho AL, Caldeira MV, Duarte CB. (2009) Regulation of AMPA receptors and synaptic plasticity. *Neuroscience*. 158:105-125.

The research group "Neuroprotection and Neurogenesis in Brain Repair" seeks the identification of new cellular and drug targets to better understand mechanisms underlying neuroprotection and neuroregeneration towards brain repair.

The main specific objectives of the group for 2009 were the following:

1) To develop a new platform useful to functionally identify new oligodendrocytes differentiating from mice SVZ-derived neural stem cell cultures. We proposed to develop a new single-cell calcium imaging-based platform, extending our previously developed method that allows the functional discrimination of immature cells, astrocytes, progenitors and neurons.

2) To unravel the role of angiopoietin-1 in the functional crosstalk between blood vessels, endothelial cells and neural stem cells, in the SVZ neurogenic niche.

3) To reveal a role for inflammatory mediators and activated microglia in methamphetamine (METH)-induced neural cell toxicity.

4) To identify a role of histamine/ antihistamines in the subventricular zone neurogenesis, using phenotypic, functional and molecular approaches *in vivo* and *in vitro*.

5) To disclose the putative pro-neurogenic effects of the neuropeptides Galanin and Somatostatine in subventricular zone cell cultures.

4) To clarify the effects of Methamphetamine on neurogenesis in stem/progenitors cell cultures derived from both the subventricular zone and the dentate gyrus of the hippocampus.

5) To disclose whether the modulation of the endocannabinoid system, and particularly *via* the activation of CB1 receptors, is relevant for brain repair. This is particularly relevant in face of the new published data showing that hemoglobin-derived peptides may selectively activate CB1 receptors. Thus, we propose to investigate if hemoglobin-derived peptides can increase neurogenesis and oligodendrogenesis in the subventricular zone.

During 2009 and the beginning of 2010, several objectives were achieved.

Objective 1) We succeeded in developing a single cell calcium imaging method allowing, in real time, the functional identification of oligodendrocytes (*Grade et al., Rejuvenation Research,* (*in press*)). This method was developed on the basis of the selective response to thrombin application by O4+ and PLP+ cells. By using different agonists and antagonists for PAR receptors we have shown that the increase in intracellular calcium concentration elicited by thrombin involves PAR-1 receptor activation and downstream Gq/11 and PLC activity. This cascade of events triggers calcium release, presumably from the endoplasmic reticulum.

Objective 2) We disclosed the proneurogenic role of angiopoietin-1/Tie-2 receptor system on SVZ neurogenesis. (*Rosa et al., J. Neurosci.,* (*in press*)). The activation of Tie-2 receptor triggers a cascade of events inducing proliferation and neuronal differentiation. These processes involved MAPK including ERK1/2, SAPK/JNK and mTOR mobilization. SAPK/JNK and mTOR activation were shown, *in vitro*, to occur in parallel with neuronal differentiation and axonogenesis. Moreover, we were able to demonstrate that angiopoietin-1/Tie-2 receptor system is present in the neurogenic niche in a diversity of cell phenotypes, including recently divided cells (EGF-receptor positive cells and also doublecortin positive cells). In addition, Tie-2 positive neuroblasts were shown to be present in the rostral migratory stream (RMS) and also in periglomerular thyrosine hydroxylase neurons of the olfactory bulb (see Fig. 8 of the article presented in the present report).

Objective 3) We observed that METH caused an inflammatory response characterized by astrocytic and microglia reactivity, and TNF system alterations (*Gonçalves et al, Eur. J. Neurosci., (in press*)). Indeed, glial fibrillary acidic protein (GFAP) and CD11b immunoreactivity were upregulated, likewise TNF-α and TNF receptor 1 protein levels. Furthermore, the effect of METH on hippocampal neurons was also investigated, and we observed a downregulation in beta III tubulin expression. To clarify the possible neuronal dysfunction induced by METH, several neuronal proteins were analysed. Syntaxin-1, calbindin D28k and tau protein levels were downregulated, whereas synaptophysin was upregulated. We also evaluated whether an anti-inflammatory drug could prevent or diminish METH-induced neuro-inflammation, and we concluded that indomethacin (10 mg/ kg; i.p.) prevented METH-induced glia activation and both TNF system and beta III tubulin alterations.

The other objectives are currently in development.

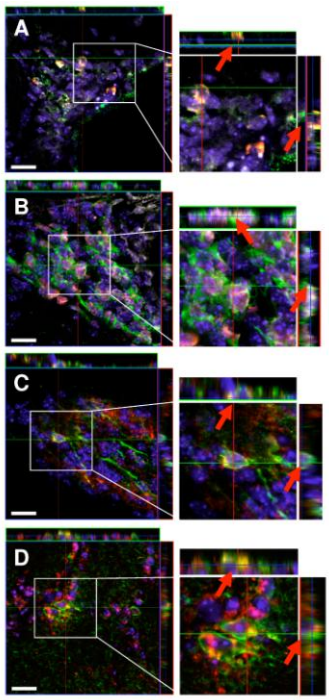


Fig. 8. Tie-2 is expressed in neurons along the SVZ, the RMS and the olfactory bulb. For further information see Rosa et al., J. Neurosci. (*in press*).

Key References

Gonçalves J et al.;; *Eur J Neurosci.*(*in press*). ; Grade S et al.; *Rejuvenation Res.* (*in press*).
Rosa AI et al.; *J. Neurosci.* (*in press*). ; Salgado AJ et al.; (2009) *J. Bioact. Biocomp. Polymers* 24:235-248.

Numerous disorders of the CNS are characterized by neuronal cell death, which may arise from the deregulation of the activity of neurotransmitter systems or insufficient neurotrophic support. In brain ischemia there is an excessive accumulation of glutamate, and the resulting overactivation of glutamate receptors causes neuronal death (excitotoxicity). The activity of glutamatergic synapses in the hippocampus is regulated by the neurotrophin BDNF (brain-derived neurotrophic factor), which is also an the toxic effects of glutamate. This group studies molecular mechanisms contributing to excitotoxic cell damage, particularly in the hippocampus, a brain region highly vulnerable to glutamate toxicity, and neuroprotection by BDNF.

Overactivation of glutamate receptors contributes to cell death in global brain ischemia due to an increase in Ca^{2+} entry through Ca^{2+}-permeable AMPA receptors (Ca-AMPARs). We found that excitotoxicity mediated by Ca-AMPARs involves the activation of the AP-1 transcription factor, which can be regulated by JNK (1). Recently, we observed that in HEK293 cells expressing Ca-AMPARs the excitotoxic stimulation of these receptors activates a cytotoxic JNK pathway in a calcium-dependent manner. In vivo studies showed that kainate injection (i.p.) promoted an interaction between the AMPARs GluR4 subunit and the JNK scaffold protein JIP-1 in the rat brain hippocampus. We identified the N-terminal of JIP-1 (aa 1-282) as the region responsible for the interaction with GluR4. We are currently investigating the molecular links coupling Ca-AMPARs to JNK activation in hippocampal neurons subjected to oxygen-glucose deprivation, an in vitro model of global ischemia.

Under excitotoxic conditions there is a deregulation of proteolytic systems and abnormal cleavage of key proteins. We found that excitotoxic stimulation of hippocampal neurons leads to cleavage of GAD65 and 67 (glutamic acid decarboxylase) by a mechanism sensitive to inhibitors of the ubiquitin-proteasome system (UPS), changing the subcellular distribution of the enzyme along neurites and its activity. This is the first time that the UPS has been implicated in events triggered during excitotoxicity. The vesicular glutamate and GABA transporters are also cleaved under excitotoxic conditions and current studies are addressing how the cleavage of neurotransmitter transporters affects their activity and intracellular trafficking, which may change the balance between excitatory and inhibitory neurotransmission.

Given the neuroprotective effects of BDNF, and its role in the regulation of synaptic activity, we conducted a proteome profiling of the effects of the neurotrophin in cultured hippocampal neurons. BDNF changed the abundance of proteins belonging to different functional categories (*Fig. 1*). The large majority of the identified proteins involved in translation activity were upregulated, but not all changes in the protein content were correlated with alterations in the corresponding mRNA. The increase in mRNA for proteins of the translation machinery in the soma was differentially coupled to the upregulation of neurite transcripts. (2). Studies will be performed based on this proteomics screening to further understand the mechanisms whereby BDNF provides neuroprotection and regulates synaptic activity.

In cell cultures and in a rat model of Parkinsons's disease we are investigating the neurotrophic support by glial cell line-derived neurotrophic factor (GDNF). We found that selective injury to dopaminergic neurons in culture can trigger GDNF upregulation by astrocytes upon release of soluble mediators by injured neurons. The cytokine profiling of intact and injured striatum and substantia nigra will be studied in vivo, and an assay in vitro will be used to test the effects of altered cytokines on GDNF expression. We found that the lesion of the nigrostriatal pathway increases the expression of adenosine A2a in striatal astrocytes, but only in areas of surviving dopaminergic terminals, which does not support the idea that A2a receptors contribute to the demise of dopaminergic neurons by stimulating neuroinflammatory pathways. We will test how the in vivo administration of A2a agonists or antagonists affects the survival and regeneration of dopaminergic terminals in striatum subregions and whether they affect GDNF expression in vivo.

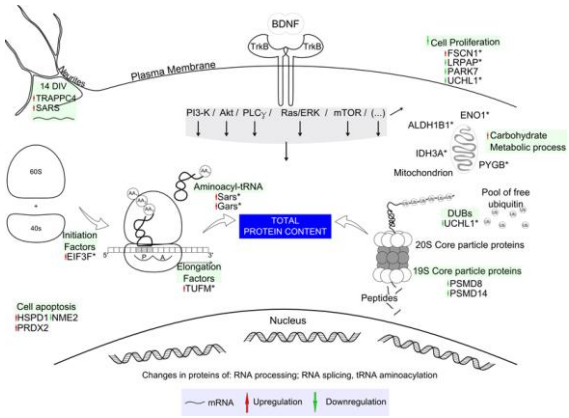


Fig. 1. Effect of BDNF on the proteome of hippocampal neurons. (J. Proteome Res. 8:4536-4552 [2009])

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In 2009 our research group explored the mechanism of 3-nitropropionic acid (3-NP, an inhibitor of succinate dehydrogenase) neurodegeneration in cortical neurons and cybrid lines and clarified the protective effects exerted by brain-derived neurotrophic factor (BDNF).

3-NP has been used to explore the primary mechanisms of cell death linked to mitochondrial dysfunction, metabolic impairment and neurodegeneration in Huntington's disease (HD). We defined the involvement of mitochondrial-dependent apoptosis in human HD and control cybrids (produced from the fusion of human platelets with mitochondrial DNA-depleted NT2 cells) exposed to 3-NP or staurosporine (STS). Apoptotic nuclei morphology, a moderate increase in caspase-3 activation and reactive oxygen species (ROS) formation were observed in HD cybrids upon 3-NP or STS treatment. 3-NP-evoked apoptosis in HD cybrids involved cyt c and AIF release, and mitochondrial Bax translocation (*Ferreira et al., Exp. Neurol., in press*).

Importantly, BDNF transcription and axonal transport are decreased in HD. In our studies, BDNF prevented 3-NP-induced mitochondrial-dependent neuronal death (*Fig. 1B*). By activating MEK1/2 signaling pathway, BDNF decreased the levels of the pro-apoptotic Bim, by increasing its degradation (*Fig. 1C*) (Almeida et al., *Neurobiol. Dis.,* 2009). We further investigated the roles of BDNF and nerve growth factor (NGF) in the dysregulation of transcription factors and histone modifying enzymes in 3-NP-treated cortical neurons. BDNF prevented 3-NP-induced decrease in CREB phosphorylation and in CBP. NGF and BDNF counteracted the increase in histone acetylation and reduced histone deacetylase (HDAC) activity induced by 3-NP (*Almeida et al, Neurotox. Res., 2009*). Our results support an important role for neurotrophins, particularly BDNF, in preventing detrimental changes in transcription and apoptotic cell death in cortical neurons subjected to selective mitochondrial inhibition.

By using mice postnatal neurosphere-derived cells, we further demonstrated that BDNF increase the number of differentiated neurons and decrease the number of neural precursors. Moreover, cells treated with BDNF and in combination with fibronectin acquired a GABAergic phenotype (*Fig. 1A*) (*Silva et al., J. Neurosci. Res., 2009*).

Concerning the research in the neurotoxicity of drugs of abuse, and knowing that chemical interactions between the heroin (Her) metabolite morphine (Mor) and cocaine (Coc) may result in Mor:Coc adducts, we analysed the effect of Coc and Her combinations in rat cortical neurons. Data showed that drug combinations potentiate cortical neurotoxicity and that chemical interactions occurring in Her:Coc (e.g. adduct formation) shift cell death mechanisms towards necrosis (*Cunha-Oliveira et al., submitted*).

Within the scope of Parkinson's disease, we determined the influence of ROS, induced by iron and rotenone, on wild-type and A53T alpha-synuclein phosphorylation on Ser 129, using human neuroblastoma cells (*Perfeito et al., in preparation*).

With the objective of clarifying ataxin-3 function and neurodegeneration caused by mutant ataxin-3 in Machado-Joseph's disease (MJD), we are investigating ataxin-3 deubiquitinating activity and its crosstalk with known interactors, in collaboration with H. Paulson (Univ. Michigan Med Sch, USA). We have been also assessing the changes in mitochondrial activity in different MJD cell models and transgenic mice (*Laço et al., in preparation*).

Concerning HD studies, we have been thoroughly examining oxidative stress in striatal cells expressing full-length mutant huntingtin (*Ribeiro et al., in preparation*).

We have previously demonstrated that insulin and insulin-like growth factor-1 (IGF-1) can be neuroprotective. Thus, in collaboration with Prof. P. Brundin (Lund University, Sweden), we analysed the effect of IGF-1 treatment on diabetic parameters, body weight and behaviour in an *in vivo* model of HD, the R6/2 mice (*Duarte et al., in preparation*).

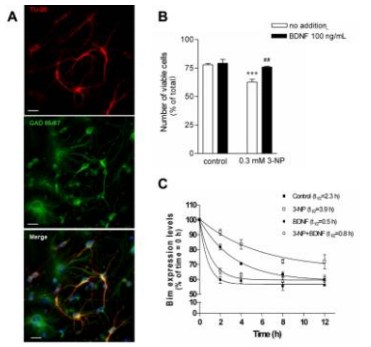

Figure 1. GABAergic phenotype of BDNF-treated mouse neural stem cells (Silva *et al.*, 2009) (**A**) and BDNF-mediated protection through decreased Bim levels in primary cortical neurons subjected to 3-nitropropionic acid, 3-NP (Almeida *et al.*, 2009) (**B,C**).

Exposure to oligomers of amyloid-beta peptide 1-42 was recently shown to decrease the levels of beta-III tubulin and of polymerized tubulin in mature hippocampal neurons. We are currently examining the role of *N*-methyl-D-aspartate receptor (NMDAR) activation on microtubule disassembly and neurodegeneration induced by the peptide. Preliminary data support the hypothesis that microtubule disassembly underlie excitotoxic neurodegeneration in Alzheimer's disease.

The influence of IGF-1, insulin and HDAC inhibitors will be examined in HD cell and mouse models expressing full-length mutant huntingtin. We will further examine the effect of BDNF on neuroprotection in HD striatal cells and on the differentiation potential of neural precursor cells. Additionally, we will determine the contribution of NR2A or NR2B subunits of NMDARs and the role of subunit phosphorylation and/or oxidation on amyloid-beta-induced disturbed calcium homeostasis and mitochondrial dysfunction in mature neurons and in the 3xTg-AD mice.

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Molecular Mechanisms of Disease Group | *Head: Cláudia Pereira*

Our research aim is to unravel the molecular mechanisms underlying neurodegeneration in pathologies characterized by aberrant peptide accumulation, namely Alzheimer´s, Parkinson´s and Prion´s diseases. Our goal is to identify novel strategies for therapeutic intervention that may delay or even stop the neurodegenerative process in these disorders.

Our *in vitro* studies showed that Aβ and PrP peptides, implicated in the pathogenesis of Alzheimer's (AD) and Prion's diseases activate the ER stress-mediated apoptotic pathway by a mitochondrial-dependent process. We described for the first time that a functional mitochondria is required for Aβ and PrP-induced apoptosis in studies conducted in mitochondrial DNA-depleted rho0 cells.

The role of mitochondrial dysfunction in AD was supported by data obtained in cultured neurons from the triple transgenic mice (3 x Tg-AD) and was further explored using cybrid cells (that recapitulate mitochondrial deficits of AD patients), which have a compromised ability to cope with Aβ-induced ER stress.

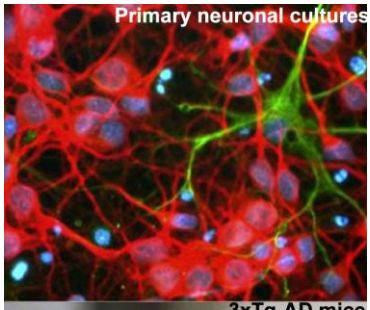
Data obtained by our group showed that mitochondrial impairment causes the loss of microtubule function, culminating in microtubule depolymerization that enhances α-synuclein aggregation, a pathological hallmark of Parkinson's disease (PD), via autophagic-lysosomal pathway alteration.

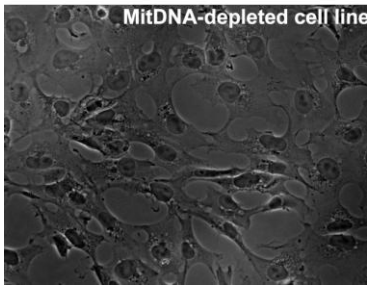
We provided evidence that mitochondria are a fundamental link between diabetes and AD, two age-related pathologies. Brain mitochondria isolated from diabetic rats have an increased susceptibility to Aβ injury and mitochondrial dysfunction is exacerbated in older animals. Data obtained with cultured fibroblasts and human brain tissue corroborates the existence of an age-related mitochondrial impairment that is more pronounced in AD. Furthermore, we found that mitochondrial dysfunction in AD is associated with increased degradation of these organelles by autophagy.

Mitochondrial-dependent impairment of intracellular trafficking in PD. Biochemical alterations in AD and MCI cybrids: development of new biomarkers. Mitochondrial signalling pathway in AD: disclosure of new therapeutic strategies.

Exploring the crosstalk between mitochondrial reactive oxygen species and the transcriptional factor hypoxia factor-1 in neuronal and brain endothelial cells. The pathological interaction between diabetes and Alzheimer's disease: exploring the role of brain endothelial mitochondria and uncoupling proteins.

Explore the role of different subunits of N-methyl-D-aspartate receptors (NMDARs) in Aβ-induced ER stress and analyze post-translational modifications of NMDAR triggered by oligomeric Aβ. Investigate the interplay between Aβ-induced endothelial cell dysfunction and neurogenesis in the subgranular zone of dentate gyrus (SGZ), and survival of mature hippocampal neurons, focusing on ER stress. Evaluate the neuroprotective effect of several drugs, including novel estrogen derivatives, statins and anorexigenic/orexigenic peptides (such as leptin and ghrelin) as potential disease-modifying therapeutics for AD. Search for epigenetic modulation of AD-related genes and correlation with signaling pathways involved in AD pathogenesis.





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The contribution of adrenal-hypothalamic axis and adipose tissue regulation as target systems to regulate neuronal protection and healthy lifespan is now a promising and emerging line of research of our group.

Our studies on proliferation of endogenous neural progenitor cells, as a strategy to promote neuronal repair, show that the antiproliferative effect of inflammation in rat neural stem cells is mediated by nitric oxide (NO) produced by microglia cells. Moreover, the exposure to antiepileptic drugs derived from 5*H*-dibenzo[*b,f*]azepine-5-carboxamide (carbamazepine) affects cell proliferation and the cell cycle of neural stem cells. Also in the context of developing new tools to neuronal repair, we isolated the progenitor cells from rat retina that grow in spheres (*Fig. 1*). And, we isolated progenitor cells from human adult adrenal gland that also grow in spheres. In differentiation medium, a small population of adrenal progenitor cells gained neuronal morphology with long neurites expressing the neuronal marker beta-3-tubulin. The isolation and characterization of human adrenal progenitor cells contributes to a better understanding of the adrenocortical and sympathoadrenal systems development, and their differentiation into adrenocortical cells, chromaffin cells or neurons could be relevant in the context of cellular regenerative therapy of neuroendocrine and neurodegenerative diseases.

Our group is also investigating the conditions that may negatively regulate neuronal protection and healthy lifespan, namely high food intake/obesity and stress, and we focused on adrenal-hypothalamic axis and adipose tissue as target systems. We showed that the cytokine IL1-beta increases the release of the stress hormone (adrenaline) from adrenal medullary cromaffin cells through neuropeptide Y (NPY) receptor and NO signaling pathways. On other hand, also adenosine A2A receptors modulate adrenalin release from mouse adrenal gland - *in vitro* (adrenal chromaffin cell culture) and also *in vivo* (mice subject to chronic stress) studies. These results unravel potential of $A_{2A}R$ antagonists to manage modifications induced by chronic stress.

In rat hypothalamus, the overexpressing of hypothalamic endogenous NPY, by using AAV vector approach, induces a high increase of food intake and obesity model.

The role of NPY on adipose tissue was also investigated. Our results show that NPY and NPY cleaved by DPPIV (dipeptidylpeptidase IV) induce adipogenesis, and this effect is inhibited by a DPPIV inhibitor. This study suggest that DPPIV inhibitors, the gliptins used as antidiabetic drugs, could be a new putative strategy to inhibit the increase of adipose tissue observed in overweighed diabetic type 1 patients.

We have found that rat retinal cell incubated in hyperglycemic conditions, a model of diabetic retinopathy, induces an increase on exocytotic release of ATP (1), that contributes to intracellular calcium concentrations increase observed in neurons and in microglial cells. Therefore, this purinergic activation of microglial cells as a result of the increased ATP release can be one of the mechanisms that contribute to the release of pro-inflammatory cytokines and the prevention of the purinergic activation in the retina may reduce the effects of diabetes on vision loss could be reduced, with a positive impact on the quality of life of diabetic patients. Targeting neuropeptide Y (NPY) system as a neuroprotective strategy in the retina, we observed in rat retinal neurons that NPY inhibits the toxicity induced by glutamate, the [Ca(2+)]i changes, through NPY Y1, Y4 and Y5 receptors, and the KCl-induced aspartate release (3). Therefore, these NPY receptors may be viewed as potential neuroprotective target in retinal degenerative diseases, such as glaucoma.

During the next year the specific objectives of our group are: (a) to investigate the role of adenosine receptors of adrenal gland in chronic stress conditions; (b) to investigate the impact of NPY modulation in rat hypothalamic neurons, by using AAV vectors, on food intake regulation and caloric restriction response. (c) To investigate the role of NPY and DPPIV on the expression of angiogenic factors in adipocytes, contributing for adipose tissue regulation. (d) to investigate the effect of prenatal exposure to antiepileptic drugs on cognitive and neuroendocrine functions; (e) to investigate the effect of diabetes or hyperglycemia on retina neuronal dysfunction and also to investigate the neuropeptide Y (NPY) and adenosinergic systems as neuroprotective strategies in the retina. (f) to study the potential effect of NPY on hypothalamic and progenitor cells.

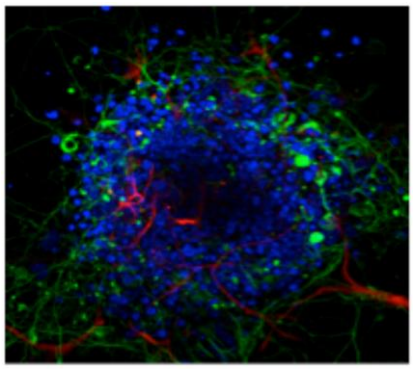

Fig. 1. Neurospheres of rat retinal progenitor cells, stained with antibodies against beta-3-tubulin (green), GFAP (red) and Hoescht 33342 (blue)

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Molecular Biotechnology and Health Area

Coordinator: Euclides Pires

The general objectives of this Area are: 1) unveal and understand normal interactions that occur in living organisms from a molecular up to a system level; 2) design vectors to deliver drugs and nucleic acids aiming to modulate or correct abnormal interactions; 3) develop new biomaterials for stem cell differentiation, tracking and transplantation as well biomaterials with anti-microbial properties.

This programme encompasses basic and translational research approaches which are conducted by five research groups. The research performed by the Molecular Systems Biology, Structural and Computational and Molecular Biotechnology groups is concerned with the first objective (unveal and understand interactions); the research performed by the Vectors and Gene Therapy Group is concerned with the second objective (design of vectors for drug and nucleic acid delivery);whereas the work performed by the Biomaterials and Stem Cell –Based Therapeutics group is concerned with the third objective (development of biomaterials).

Experimental validation of theoretically predicted design principles for moiety transfer cycles was extended to ADP/ATP – mediated phosphatransfer cycles in E. coli and S. cerevisial. The results obtained suggest that although some of the design principles are similar to those previously validated to NADP (M) redox cycles, others are quite different.

Functional consequences of sequestration of cycled intermediates in moiety-transfer cycles were shown to have opposite effects for sequestration of charged or uncharged moieties.

A large virtual screening effort to find new compounds with potential to be developed into drugs against amyloid diseases was launched on the Ibercivis platform.

A series of simulations detailing the different unfolding behavior of wild-type Transthyretin (TTR) and its highly amyloidogenic mutant L55R-TR was developed and published.

Through 2009, 51 Anabidopsis genes encoding aspartic proteinase of the pepsin-type were cloned in E-coli expression vectors. The recombinant enzymes revealed a strong dependence of redox conditions and an unexpected insensitivity to pepstation A.

Several pollens investigated were shown to contain serine and/or aminopeptidase activity capable of degrading airways active biopeptides, increasing transepithelial permeability and promoting cell detachment in vitro, by degrading intracellular adhesion proteins.

Significant reduction of miR-21 expression levels was achieved through the combination of cationic liposomes with anti-mi RNA LNA.

A novel triple targeting strategy involving cellular and molecular targeting at the BCR-ABL and Ber-Abl proteins level was developed and evaluated in terms of anti-leukemia activity.

Therapeutics involving non-allele –specific silencing were shown to be a promising strategy for safe and effective treatment of Machado-Joseph Disease patients.

Synthetic matrix metalloproteinase –responsive gels were shown to be very effective as bioactive co-encapsulating system of endothelial cells and thymosin beta 4.

Co-encapsulation of thymosin beta 4 was shown to significantly up-regulate endothelial genes involved in remodeling and survival of endothelial cells, facilitate cell attachment and induce endothelial like network formation.

The Molecular Biotechnology and Health Research Line, like the most of the Research Lines of LAs, was originally defined in a board sense to include and to report the activity of groups developing projects, that incorporated a substantial know-how in Molecular Biotechnology, aiming at the development of technologies or products of interest to health. The present main objectives of this Line which was stated at the beginning of this section, represent a continuous effort to incorporate new approaches to tackle the

central issue i.e. – development of products of interest to health on the basis of a deep knowledge of the interactions that occur in the living organisms from a molecular up to a system level. This trend led, more recently, to the incorporation in the Line of groups with an expertise in Systems Biology, Bioinformatics, Computational Biology and Biomaterials design.

Detailed descriptions of the future plans of each groups are presented in the section dedicated to groups. So far there are no constrains on the theme or direction of individual research projects, however, there is a feeling that soon, in addition to group projects, a large but focused bridging project, involving the expertise of all groups, must be devised and implemented.

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Structural and Computational Biology

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Nélio Gonçalves	PhD Student
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Biomaterials and Stem Cell-Based Therapeutics

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The Molecular Biotechnology group has a long-time interest in studying biotechnologically and/or biomedically relevant plant proteases. Understanding the structure-function relationship of plant aspartic proteases has been the main research objective. Initial studies used cardosins, the milk-clotting enzymes from the flowers of cardoon, as working models. Since the sequencing of Arabidopsis genome our interest shifted towards the study of aspartic proteases from this model organism, involved in disease resistance and stress responses. The goal is to understand the possible biological functions of this family of enzymes.

Another line of research is devoted to study serine proteases from allergenic pollens. The enzymes have been purified and caracterized in our laboratory and seem to play an important role in allergy. The overall goal is to understand the molecular mechanism underlying the possible involvement of these proteases in eliciting the allergenic response as well as to assess whether or not they can be good therapeutic targets.

In order to study the structure-function relationship of Arabidopsis aspartic proteinases there is a need to set out a reproducible and efficient method to produce the recombinant forms of these enzymes. Throughout 2009 the 51 Arabidopsis genes encoding protease aspartic proteinase of the pepsin-type were amplified and cloned into several E.coli expression vectors. Expression trails for six of the genes were successful and the recombinant enzymes were purified and characterized both at the structural and enzymatic level. The preliminary study revealed common enzymatic properties among them such a strong dependence of the redox conditions and a very surprising insensitivity to pepstatin A. In contrast, the Arabidopsis aspartic proteinases differ significantly on their specificity suggesting a very define and specific role in plant cells.

Allergic disorders, such as seasonal rhinitis and asthma, are increasing causes of morbidity worldwide and result often from exposure to airborne pollen. Over the past year we evaluated the presence of protease activity in several allergenic pollens and we assess the action of these proteases on the immunologic and inflammatory response to airborne allergens.

All pollen diffusates were shown to have high molecular proteases with low pI and predominant serine and/or aminopeptidase activity. These proteases were involved in the degradation of airways bioactive peptides. Moreover all pollen extracts, with distinct allergic potential, were able of increasing transepithelial permeability and cell detachment *in vitro* by degrading intercellular adhesion proteins. These results suggested that the proteases normally presented in the pollen grains might be involved in the sensitization to a range of airborne allergens by facilitating their contact to subepithelial immune cells. The identification and characterization of identical proteases within the majority of pollen types, accountable for the disruption of intercellular complexes, constitutes an important step to come across a therapeutic target in the treatment of allergic disorders.

Future research will focus more deeply on the study of Arabidopsis aspartic proteases and the proteases from pollens associated with allergic disorders. Using the recently identified atypical Arabidopsis aspartic proteases such as CDR1 (constitutive disease resistance 1) and PCS1 (promotion of cell survival 1) as working models, the main goals of our future research are the identification of their natural substrates and the understanding of their structure-function relationship. The research will be of crucial importance to better understand the diversity among plant aspartic proteases and the biotechnology potential of these enzymes involved in response to biotic and abiotic stress injuries.

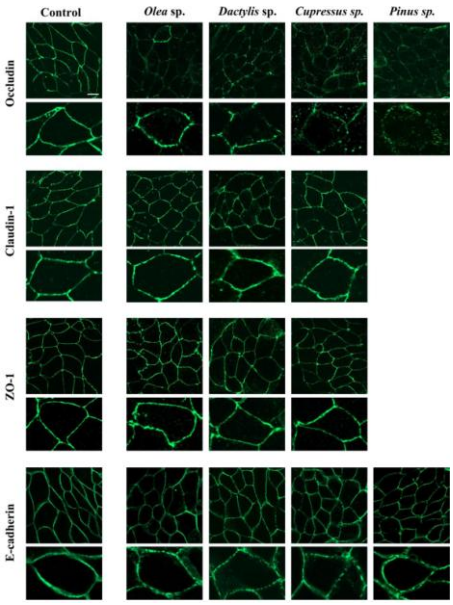

Fig. 1. Effect of pollen diffusates on intercellular junctions integrity. Distribution of transmembrane proteins, occludin and claudin-1, cytosolic complex ZO-1 (tight junctions), and transmembrane E-cadherin (adherens junctions) was studied by immunofluorescence. Representative images are shown for each stimulus. Bar = 10 µm

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Research at the Molecular Systems Biology Group is focused on the relationship between design and function in metabolic networks. Natural selection for organism's effective operation can strongly constrain the design of biochemical networks. For this reason, and because many of the functional requirements — *e. g.*, robustness, fast response times, buffering of critical concentrations — and physical-chemical constraints are similar across organisms and broad classes of biological circuits, one may expect some design principles to hold pervasively in naturally evolved networks. Through systems-theoretic analyses informed by knowledge of pertinent constraints and performance requirements we are revealing some of such biologically widespread rules relating aspects of the quantitative design of metabolic networks to their function.

About 70% of all documented enzyme-catalyzed processes in *Escherichia coli* and *Saccharomyces cerevisiae* participate in moiety-transfer cycles (MTC, *Fig. 1a*). Most MTC play a role analogous to that of power-supply units in electronic circuits. Namely, they couple supply of molecular parts (moieties) to demand, ensuring that moieties are transferred to metabolic acceptors at a rate that is proportional to demand and insensitive to fluctuations in the outside supply. We have derived the following set of design principles that must apply at the basal steady state so that redox cycles — a very representative class of MTC — perform as effective moiety-supply units. The demand-side enzyme must satisfy $K_M^{app}(C) < [C]$ and either $K_M^{app}(A) > [A]$ if demand is directly signaled by A (as in redox cycles involved in antioxidant protections) or $K_M^{app}(A) < [A]$ if moiety-transfer occurs at the committed step of a feedback-regulated pathway. The supply-side enzyme must satisfy $K_M^{app}(U) > [U]$. Finally, [U]/[C] must be low (*Fig. 1b*, red polygon). Detailed studies of well-characterized MTC in human erythrocytes (*Fig. 1b*) and a broad survey of K_Ms and metabolite concentrations in *Escherichia coli* (*Fig. 1c*) and *Saccharomyces cerevisiae* show that these design principles hold pervasively. A similar data survey for ADP/ATP phosphorylation-dephosphorylation cycles indicates that similar principles apply for the demand-side enzymes.

We are now seeking design principles for other simple and prevalent metabolic circuits and for modes of coupling, allosteric regulation, intermediate sequestration and enzyme's catalytic mechanisms in MTC. We are also developing numerical optimization methods that will allow us to analyze more-complex circuits.

Kinetic models help understanding the design and function of biochemical networks. However, many such models integrate data from very different biochemical domains of expertise and rely on some assumptions that are not fully informed by experimental data. Good tools for comprehensively documenting such assumptions and promo-ting effective collaboration between modelers and experimentalists are lacking. We are addressing this gap by developing WikiModels – a web-based platform for distributed collaboration in kinetic modeling.

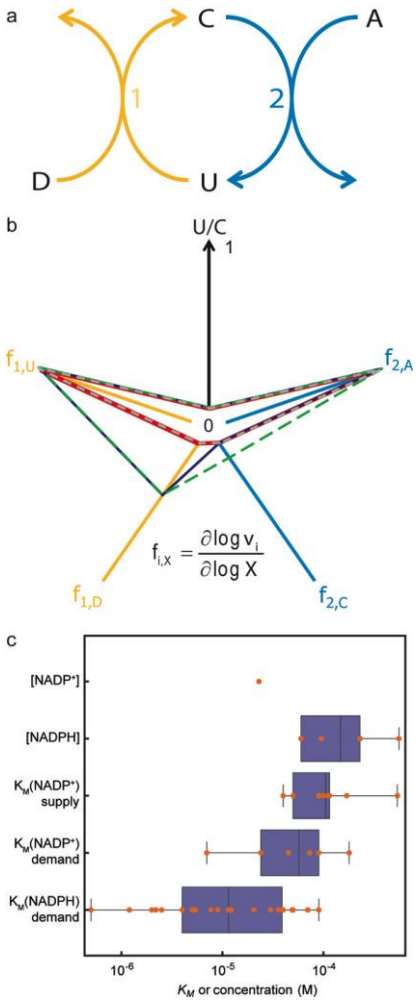

Fig.1: Design principles for moiety-transfer cycles. (a) Idealized moiety-transfer cycle. A, moiety acceptor; C, moiety-charged carrier; D, moiety donor; U, moiety-uncharged carrier. (b) Optimal design (red) and design of several redox cycles in human erythrocytes: NADPH cycling by glucose 6-phosphate dehydrogenase (G6PD) and glutathione reductase (violet), NADPH cycling by G6PD and NADPH-flavin reductase (dashed green), GSH/GSSG cycling by glutathione reductase and "classic" glutathione peroxidase (dashed gray). Each kinetic order (f_{iX}) or concentrations ratio (U/C) is represented in its own axis, extending from 0 (inner tip) to 1 (outer tip); values corresponding to the same cycle are joined. (c) Data for E. coli indicating that the enzymes involved in NADP cycling adhere extensively to the principles above. The transferred moiety is an electron pair (reducing equivalent). The concentration of $NADP^+$ (U) is higher than that those of NADPH (C) found in a range of conditions and lower than $K_M(NADP^+)$ for enzymes catalyzing NADPH regeneration, situation favors a high $f_{1,U}$. NADPH concentrations significantly exceed the K_M(NADPH) for the demand-side enzymes ($p<0.002$ by Mann-Whitney test), favoring low $f_{2,C}$. $K_M(NADP^+)$ for supply-side enzymes are significantly higher than both the K_M(NADPH) ($p<10^{-4}$) and the $K_M(NADP^+)$ for the demand-side enzymes ($p<0.07$). This further suggests evolutionary adaptation of the former enzyme's $K_M(NADP^+)$ towards high $f_{1,U}$ and is consistent with evolutionary adaptation of the latter enzyme's K_M(NADPH) towards low $f_{2,C}$.

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Structural and Computational Biology Group | *Head: Rui M. M. Brito*

The group is strategically focused on the use of experimental and computational methodologies to study the molecular basis of human and animal pathologies, in particular amyloid diseases. Combining the reach of experimental and computational approaches, the group has been working in four main inter-related topics:

1. The characterization of the kinetics and molecular species involved in the initial stages of amyloid formation by the protein Transthyretin (TTR), the causative agent of Familial Amyloid Polyneuropathy (FAP), a mostly fatal human disease with some incidence and social relevance in Portugal;

2. Virtual screening and rational design of inhibitors of TTR amyloidosis. The experience gained with TTR will also be used to model inhibitors of amyloid formation by the A-beta-peptide of Alzheimer´s, a project in collaboration with Doctor Claudia Pereira of CNBC;

3. Extension to Portugal and support of the volunteer computing network Ibercivis (*www.ibercivis.pt*) and development of the project AMILOIDE to run in this platform;

4. Development of computational tools for the storage and management (project P-found: *www.p-found.org*) and data mining of large data sets produced in protein folding and unfolding simulations.

The realization of these objectives has been strengthned by the recent addition to the group of 2 PhD researchers: one experimentalist in the area of protein stability, and one expert in protein modelling and molecular dynamics.

The group has been invited by UMIC – Agência para a Sociedade do Conhecimento IP – to coordinate the launching of a volunteer computing platform in Portugal, in close connection with the work being developped in Spain by the Institute of Biocomputing and Physics of Complex Systems, University of Zaragoza. After installation of dedicated infrastructures at FCCN – Fundação para a Computação Científica Nacional, the iniciative was launched and public anounced on July 30th, 2009, at the "Encontro Nacional da Ciência 2009", Fundação Gulbenkian, Lisboa, Portugal.

On October 12th, 2009, the project AMILOIDE, developped by the group, was launched on the Ibercivis platform (*www.ibercivis.pt*). The AMILOIDE project is based on a large virtual screening effort to find new compounds with potential to be develpped into drugs against amyloid diseases such as Familial Amyloid Polyneuropathy (FAP) or Alzheimer´s.

This effort on volunteer computing has allowed the group to participate in a series of science communication actions.

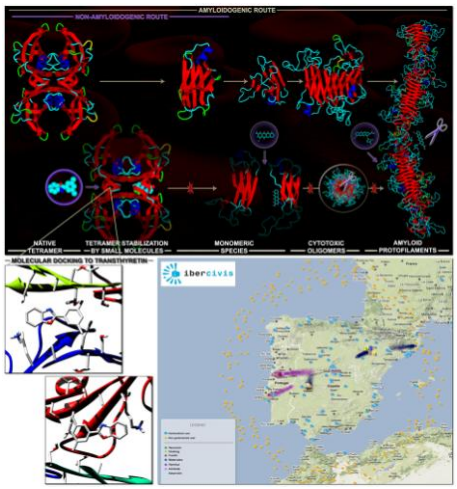
Based on the recent installation of a circular dichroism (CD) spectrometer, the group is now providing a service (or scientific collaboration) for researchers interested in the conformational characterization (and quality control) of their proteins. This service has been requesed by several researchers of institutions of the North and Center of Portugal, where there are no other CD spectrometer.

Additionally, the group published the results of a series of simulations detailing the different behavior on unfolding of wild-type Transthyretin (TTR) and its highly amyloidogenic mutant L55P-TTR.

The group will maintain its multidisciplinary approach to the study of the molecular mechanisms of disease, in particular of amyloid diseses, combining experimental and computational methodologies. This approach will be strengthned by the recent addition to the group of 2 PhD researchers: one experimentalist in the area of protein stability, and one expert in protein modelling and molecular dynamics.

The recent launch of the project AMILOIDE in the volunteer computing network Ibercivis (*www.ibercivis.pt*) will also continue to deserve an important impetus by the group and will develop in two main directions: i) scientific production and ii) science communcation. During 2010 we expect to finish the first screening of 2.5 million compounds for potential inhibitors of transthyretin amyloidogenesis. Concerning science communication, a series of events in secondary schools and other public forums are being planned in 2010 in order to publicize the AMILOIDE-Ibercivis initiative and its scientific projects among the general public, and in particular among the students of secondary schools.

Additionally, in the experimental front, special efforts will be made in the characterization of the aggregation kinetics of several Transthyretin (TTR) variants varying in amyloidogenic potential. It is known today that amyloid aggregates with different sizes may have different toxicity. Thus, it is critical to have a better grasp of the kinetics and structural identity of the molecular species populating the aggregation process during amyloidogenesis, in order to develop the most rational approaches to fight these diseases.



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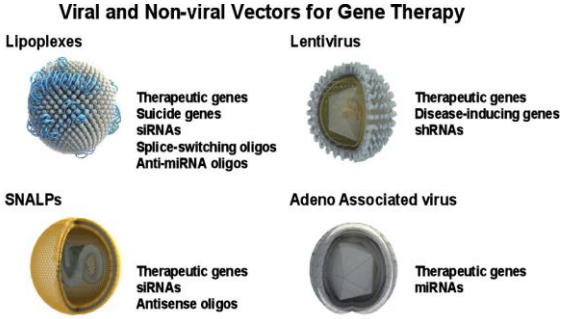
The CNC laboratory of vectors and gene therapy is devoted to the design of carriers, including viral and non-viral vectors, for nucleic acid and drug delivery aiming at their application as technological platforms for the establishment of disease models, study of disease mechanisms and development of new molecular therapeutic strategies. Our studies have been focused on the evaluation of the potential of these novel carriers for the treatment of both cancer and neurodegenerative disorders, and for the development of vaccines for infectious diseases.

Cancer has been the main target disease in which both gene silencing and gene delivery approaches have been evaluated. Non-viral vectors have been explored to deliver antisense oligonucleotides, siRNAs and anti-miRNA LNAs, aiming at promoting silencing of known oncogene proteins and cancer-related miRNAs (oncomirs). A splicing correction strategy has been developed using the $S4_{13}$PV CPP to mediate the intracellular delivery of splice-switching oligonucleotides and promote the modulation of the splice pattern of a target gene. This peptide has been used in combination with cationic liposomes to promote siRNA intracellular delivery and gene silencing. Moreover, silencing of the oncomir miR-21 has been achieved through combination of cationic liposomes with anti-miRNA LNA oligonucleotides, leading to a significant reduction in miR-21 expression levels and consequent decrease in tumor cell viability. In a different approach, the *in vitro* silencing efficacy of anti-*BCL2* siRNA sequences was tested in small lung cancer cells. In addition, the effects of these siRNAs on cytotoxicity and chemosensitization were adressed. Regarding a novel lipid-based nanosystem we have been working on for the treatment of breast tumors, we have demonstrated that, upon systemic administration, its accumulation in orthotopic breast tumor is 18-fold higher than its non-targeted counterpart.

Both non-viral and viral gene therapy approaches have been applied aiming at targeting neurodegenerative disorders,. Transferrin-lipoplex-mediated knockdown of the transcription factor c-Jun was found to improve neuronal survival and decrease inflammation in an animal model of excitotoxic injury, leading to a reduction of seizure activity and neuronal loss. RNA interference has also potential as a therapeutic approach for Machado-Joseph's Disease (MJD), but raises the issue of the role of wild-type ataxin-3 in MJD and of whether the expression of the wild-type protein must be maintained. To address this issue, we both overexpressed and silenced wild-type ataxin-3 in a rat model of MJD using lentiviral vectors. We showed that (i) overexpression of wild-type ataxin-3 did not protect against MJD pathology, (ii) knockdown of wild-type ataxin-3 did not aggravate MJD pathology and that (iii) non-allele-specific silencing of ataxin-3 strongly reduced neuropathology in a rat model of MJD. Our findings indicate that therapeutic strategies involving non-allele-specific silencing to treat MJD patients may be safe and effective.

Our research has also been focused on the identification of new disease-related molecular targets and design of innovative therapeutic strategies for cancer and neurological injury, using both viral and non-viral vectors. Ongoing and future work includes the modulation of the splicing pattern and expression levels of cancer-related proteins, such as survivin, using cationic liposomes and CPPs, as well as the association of tumor-specific targeting moieties to these formulations, aiming at improving their biocompatibility and efficacy following intravenous delivery. We are also currently working on the design of novel lipid-based nanosystems for efficient intracellular delivery of nucleic acids and drug combinations into tumor cells. We aim to combine therapy and molecular imaging within the same targeted nanoparticle. In addition, we plan to assess the therapeutic potential of a novel lipid-based nanosystem that targets breast tumor at two different levels of the tumor microenvironment. In parallel, mechanistic studies on the role of endogenous miRNAs in cancer and brain inflammation are being conducted, in order to advance our understanding on the physiological relevance of these molecules in the context of disease.

Mucosal vaccination (oral, nasal and pulmonary) with the antigen encapsulated in polymeric nanovectors, to target the lymphoid structures of the mucosal immune system is also addressed by the group. Related with this theme two projects are emerging: "Development of a mucosal anthrax vaccine: designing a prototypic multi-antigen polymeric delivery system" and "Development of chitosan-based nanoparticles for nasal immunization against hepatitis B".



Studies addressing disease modifying strategies for MJD involving the modulation of autophagy, proteolysis and adenosine antagonism are currently in progress. Other projects will be initiated soon regarding the study of the interaction of ataxin-3 with other polyQ expanded proteins, the modification of the delivery systems for intravascular administration to the brain, and the development of an induced pluripotent stem cell model of MJD.

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Biomaterials and Stem Cell-Based Therapeutics Group | *Head: Lino Ferreira*

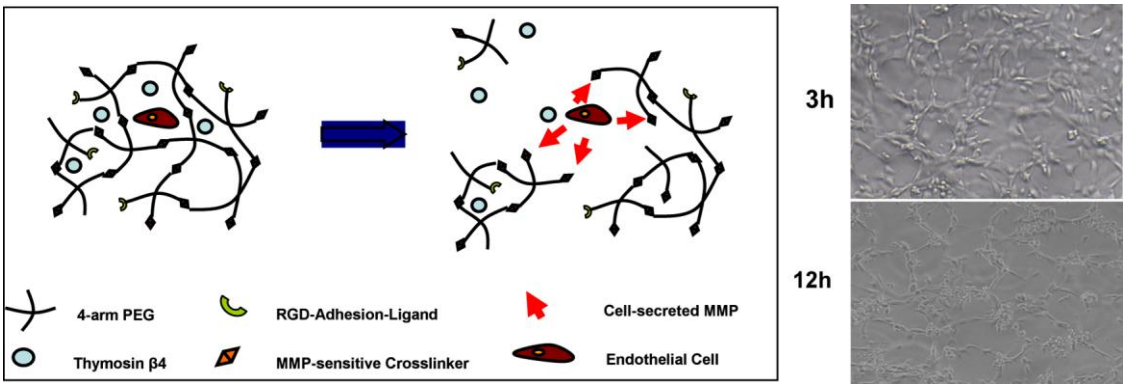
The group of biomaterials and stem cell-based therapeutics is an emerging group at CNC. The group has two major avenues of research: i) to develop new biomaterials for stem cell differentiation, tracking and transplantation, and ii) to develop biomaterials with antimicrobial properties. We are designing biomaterials which provide different types of information to stem cells, with the purpose of controlling their differentiation and enhancing their grafting after *in vivo* transplantation. In this context we are developing or modifying natural or synthetic polymers and to characterize their physico-chemical and biological properties. Another focus of our group is the design of biomaterials with antimicrobial properties. A major problem associated with the implantation of biomedical devices in the human body is the inherent risk of microbial infections. We are developing effective strategies to control antimicrobial infections by developing coating technologies to immobilize antimicrobial agents.

Recently we examined the *in vitro* potential of synthetic matrix metalloproteinase-responsive gels as a bioactive co-encapsulation system of endothelial cells and thymosin beta 4 (*Kraehenbuehl et al., Biomaterials 2009*). We showed that these bioactive gels supported endothelial adhesion, survival, migration and organization while serving as a controlled release system of thymosin beta 4. We further demonstrated that thymosin beta 4 significantly increased survival of the co-encapsulated endothelial cells and significantly up-regulated endothelial genes involved in vascular maintenance, remodeling and survival. Finally, we showed that thymosin beta 4 entrapped in three-dimensional poly(ethylene glycol) hydrogels facilitated cell attachment and induced endothelial-like network formation (see Figure).

Recently we reported the isolation of vascular progenitor cells (VPCs) that have the ability to differentiate into smooth muscle and endothelial cells (*Ferreira et al., Circulation Research 2007*). However, it is poorly understood the mechanism and bioactive molecules involved in this differentiation process and whether smooth muscle cells can be obtained from other sources than VPCs. We are performing studies to evaluate the ability of different cell populations isolated from human embryoid bodies to differentiate into smooth muscle cells. The isolated cells are cultured in media supplemented with several inductive signals. At the moment we are encapsulating these cells in three-dimensional scaffolds for future application in regenerative medicine.

Another area that we are actively involved is in the regeneration of chronic wounds (in collaboration with Eugénia Carvalho, CNC, and the Portuguese stem cell-banking company Crioestaminal) and myocardium after infarct (in collaboration with Robert Langer). An important obstacle for successful cell-based therapeutic angiogenesis is the low engraftment and viability of transplanted cells. Cell death is a multi-factorial phenomenon and might require the use of a "cocktail" of factors to improve cell survival. We are developing a platform to deliver efficiently the cells at the wounds or the myocardium while enhancing their therapeutic effect. The results of both studies will be reported during 2010.

Another focus in the group is the development of antifungal biomaterials (in collaboration with Matera, a company with headquarters at Biocant). These biomaterials can be used to coat surfaces or incorporated in the bulk of devices. We expect to file a patent during 2010 related to this technology, and publish the results in a peer review journal.



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Cell and Molecular Toxicology Area

Coordinator: Leonor Almeida

This Area maintains a focus on the study of cellular and molecular basis of drug- and disease-related cell dysfunction, in which mitochondria, lipid membranes or free radicals could be involved, for the purpose of translating this knowledge into disease treatment and prevention. Three groups accomplish such goals: *Mitochondrial Toxicology and Disease*, centered on the role of mitochondria as a primary cellular mediator of cell dysfunction and on its potential usefulness as a target in anti-cancer therapy; *Redox Biology in Health and Disease*, focused on mechanisms inherent to neuromodulation and aging involving nitric oxide and on action mechanisms of dietary polyphenols in terms of endothelial dysfunction protection, anti-inflammatory properties and nitrite-driven regulatory processes; *Membrane Toxicity*, a smaller group, aims to study the role of lipid membranes in drug-mediated cell dysfunction. A more recent group, *Pharmacometrics,* brings a great insight into the optimization of drug efficacy and safety, in order to prevent costly and life-threatening drug-induced toxicity.

The groups in this Area, by using *in vitro* and *in vivo* approaches, obtained a vast range of results, as indicated in their individual reports. In brief:

Mitochondria were identified as a mediator of several xenobiotics toxicity, including the herbicide metolachlor, and of clinically used anti-cancer agents, as Doxorubicin and cis-platin, but by different underlying molecular mechanisms.

Mitochondria were also pointed as a plausible and attractive target for novel chemotherapeutics, as phytochemicals, in cancer cells.

Mitochondrial dysfunction is involved in the pathogenesis of some diseases, including diabetes, cholestasis and metabolic syndrome; the rescue of this dysfunction by some natural compounds brought new insights into the pathogenesis and therapeutics of such diseases.

The structural order of membrane lipids was identified as a common target for the toxic effects of a variety of environmental pollutants on biological systems.

The evaluation of concentration dynamics of nitric oxide in rat hippocampal slices and in anesthetized rat brains provided a quantitative and temporal basis for understanding nitric oxide activity and its modulation by pharmacological tools along the glutamate-nNOS pathway. The demonstration in the CA1 region that this radical regulates oxygen consumption supports the current paradigm for oxygen and nitric oxide interplay in the regulation of cellular respiration.

Regarding the antioxidants research line, the mechanistic studies of dietary polyphenols as nitrite reductants in the stomach, and as modulators of vascular signalling pathways, beyond their antioxidant activity, supported new potential beneficial effects on nitric oxide metabolism and endothelial function, in the context of atherosclerosis prevention.

A bioanalytical framework was developed to quantify structurally related antiepileptic drugs and its main metabolites in human plasma and mice tissues, giving support to further clinical pharmacokinetics and therapeutic drug monitoring, to optimize drug efficacy and safety.

This Area will pursue the study of cellular and molecular basis of drug- and disease-related cell dysfunction, in which mitochondria, lipid membranes or free radicals could be involved, for the purpose of translating this knowledge into disease treatment and prevention. The work performed by the groups, “Mitochondria Toxicology and Disease”, “Redox Biology in Health and Disease” and “Membrane Toxicology”, will be concerned with this general objective. As specified by each group research plan, future research plans encompass the continuation of the competitive ongoing research, from the molecular and cellular level to *in vivo* animal models, and the implementation and fortification of new research lines. Of note, the Mitochondrial Toxicology and Disease group will be reorganized and fostered by the inclusion of two new research teams with new research lines concerned with i) mitochondria,

carcinogenesis and chromium toxicity and ii) metabolic profiling and toxicology. Moreover, the work of the Pharmacometrics team will proceed related with a more specific goal of the Area, focused on development and application of pharmacostatistical models of drug efficacy and safety from non-clinical and clinical data. This research line will bring into CNC new insights into optimizing drug efficacy and minimizing its toxicity. The potential synergism with other groups within CNC is maintained as a great challenge in future.

On the other hand, an effort is being done i) to strengthen the cohesion and synergism of the Area, by conjugating the expertise of groups; at present, several collaborative works are in course; ii) to maintain the organization of the annual International Courses on Toxicology at CNC, with the participation of highly recognized scientists, and to dynamize the organization of conferences and advanced courses, mainly in the scope of the CNC Doctoral Programme, by joining the efforts of the research groups.

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João Miguel das Neves Duarte	Post-Doctoral Fellow
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Ana Adelaide Caldeira Burgeiro	PhD Student
Ana Carolina dos Santos Moreira	PhD Student
Ana Filipe Roque Branco	PhD Student
Ana Francisca Soares	PhD Student
Ana Isabel V. Rafael	PhD Student
Ana Patrícia da Silva Gomes	PhD Student
Ana Teresa Inácio Ferreira Varela	PhD Student
Gonçalo de Castro Pereira	PhD Student
Sandro Lino Cardoso Pereira	PhD Student
Camile Woitiski	PhD Student
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Mitochondrial Toxicology and Disease Group | *Head: Paulo J. Oliveira*

Mitochondria are the energy powerplants of cells, producing the majority of the chemical energy cells use. Besides this role, mitochondrial are active and critical players in cell death signaling, calcium homeostasis, intermediate metabolism and reactive oxygen and nitrogen species production. It is pertinent to question if different molecules, which can interact with living systems, or even disease conditions, promote their biological effects through mitochondrial-mediated effects. In fact, numerous examples of mitochondria-mediated cell injury can be found in the literature; not only chemicals can negatively affect mitochondrial function but also the origin and progression of several pathologies, including cancer, is closely related with disruption of mitochondrial homeostasis. The main and general objective of the "Mitochondrial Toxicology and Disease Group" is to provide an insight into the role of mitochondria as a primary intracellular target in the initiation of drug- and disease-induced cell dysfunction.

Our group has several distinct lines of research including the following topics:

1) Mitochondrial toxicity of anticancer agents: We deciphered the apoptotic signalling pathways induced by the antineoplastic agent Doxorubicin on cardiomyoblasts which involve p53 and Bax translocation to mitochondria. Also, we have identified mitochondria as a mediator of the hepatotoxicity induced by the anti-cancer agent cis-platin, namely through induction of the thiol-sensitive permeability transition pore. We have been also investigating the mitochondrial effects of several phytochemicals and their possible use as selective anti-cancer agents.

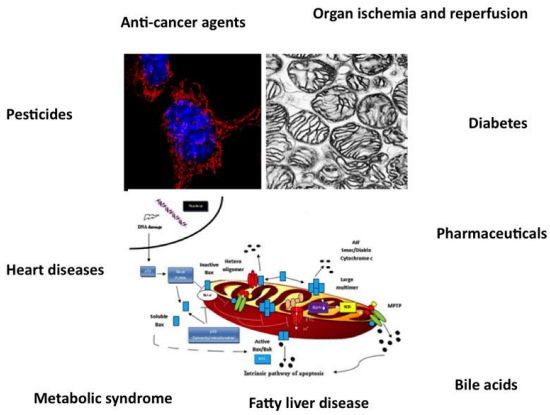
2) Mitochondrial biogenesis, modulation of metabolism and protection of hepatic function: We have established that Indirubin-3'-oxime, an indirubin analogue that shows favorable inhibitory activity targeting glycogen synthase kinase 3β, protects fatty liver from ischemia/reperfusion injury, by maintaining mitochondrial calcium homeostasis. We observed that berberine, a natural product with anti-diabetic properties, could rescue mitochondrial dysfunction in the liver and skeletal muscle of animals fed a high-fat diet for 12 weeks.

3) Modulation of mitochondrial function mediated by xenobiotics: We concluded that inhibitors of mono-amine oxidase A synergize with the toxicity of Ecstasy in an animal model. In order to develop methods to allow for a high-throughput analysis of mitochondrial toxicity of test compounds, we described a high-throughput method for measurement of mitochondrial oxygen consumption in living cells, based on the Becton-Dickinson Biosensor plates.

4) Mitochondria, carcinogenesis and chromium toxicity: We succeed at establishing an in vitro model of Cr(VI)-induced bronchial epithelial cells malignant transformation. The new cell line is aneuploid, induces tumours in nude mice and has increased expression of biomarkers of malignant transformation [(EGFR, c-MYC, LDHA, HIF-1α, MAPKinases (MAPK1, MAPK14, MAPK2K4, as well as RAD51, XRCC3 and OGG1 [homologous recombination (HR)], XRCC1 [base excision repair (BER)], XRCC5 (NHEJ) and MLH1 (MMR)] and do not show microsatellite instability.

5) Metabolic profiling and toxicology: We evaluated the effects of cardiac ischemia and ischemia followed by reperfusion in intermediary metabolic fluxes, namely in terms of alterations in substrate preferences and evaluation of metabolic remodeling taking place during those insults. Using the liver, we developed a protocol for determining *de novo* lipogenesis using deuterated water and ^{2}H NMR analysis of tissue triglycerides and glutamate/glutamine. In the brain a dual approach was undertaken, *in vitro* using hippocampal slices and *in vivo* using ^{1}H-MRS methodologies.

Among several lines of research, we are currently evaluating how key regulators of mitochondrial biogenesis and function, such as PGC-1alpha, sirtuin 1 (SIRT1) and nitric oxide (NO), are affected by exposure to high concentrations of fatty acids and glucose, under conditions of FXR activation. We are also conducting an exhaustive work regarding the mitochondrial targets of DOX in vivo treatment in the kidney, heart, liver and lung, as well as using 3D cancer cell cultures to investigate the anti-cancer potential of natural and synthetic molecules aimed at mitochondria. We are investigating how mitochondrial function and cell metabolism is altered during chromium-induced carcinogenesis.



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The production of reactive oxygen/nitrogen species and the occurrence of antioxidants are critically involved in the redox regulation of cell functions but their steady-state levels and dynamics may be connected to selective responses, including the extensive oxidative damage to biomolecules (oxidative and nitrosative stresses), leading to cell death, either by turning off vital processes or by upregulating toxic cascades.

We are interested in: (a) the study of the molecular mechanisms inherent in neuromodulation, and aging that critically involve nitric oxide, connecting the dynamic profiles of nitric oxide in the brain with its role as a neuromodulator and as the mediator of neurovascular coupling; (b) the analysis of the mechanisms of action of plant-derived dietary phenolic compounds, particularly those present in wine, in terms of protection against vascular endothelial dysfunction, anti-inflammatory properties, as well as their impact on nitrite-driven regulatory processes, encompassing the non-enzymatic production of nitric oxide from dietary nitrite in the gastric compartment.

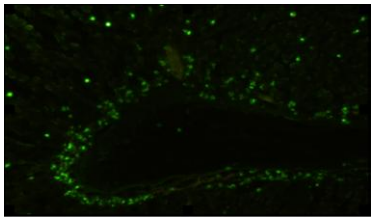
We have shown that the production of nitric oxide elicited by glutamate in rat hippocampus *in vivo* is the result of an integrated activation of ionotropic glutamate receptors NMDA and AMPA and that each pathway elicits distinct concentration dynamics. Further, the concentration dynamics of nitric oxide in the rat hippocampus *in vivo* measured in a real-time showed to be distinct in CA1 and dentate gyrus. These results provide a quantitative and temporal basis for the understanding of nitric oxide activity in the rat hippocampus and for its modulation by pharmacological tools along the glutamate-nNOS pathway.

We established the proof of concept that, in the presence of nitrite, polyphenol-containing dietary products induce a strong increase of nitric oxide in the stomach of humans. Nitric oxide produced in such a dietary-dependent way in the stomach may diffuse the gastric wall reaching mucosal blood vessels and elicits local relaxation.

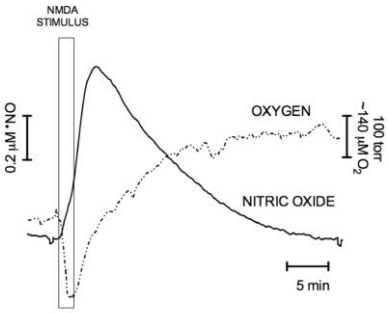
We demonstrated that, beyond their antioxidant properties, malvidin-3-glucoside, a typical anthocyanin, inhibits peroxynitrite-triggered endothelial cell apoptosis in a way similar to that of resveratrol, by disrupting the mitochondrial pathway through modulation of Bcl-2 intracellular levels. Furthermore, it inhibits peroxynitrite-triggered endothelial cells toxicity by up-regulating cellular nitric oxide and down-regulating NF-kB.

We have shown that endogenously produced nitric oxide regulates oxygen consumption in the CA1 region of rat hippocampal slices. The quantitative and dynamic assessment of nitric oxide in a complex biological preparation that retains cytoarchitectural and neuronal circuit integrity strongly supports the current paradigm for oxygen and nitric oxide interplay in the regulation of cellular respiration.

Recently, we have developed a tri-component microsensor array with a versatile geometry and comprising a nitric oxide-selective microelectrode and a laser Doppler sensor that inserted stereotaxically in the brain of rats and mice enabled to couple glutamate-dependent nitric oxide concentration dynamics with the profile of change of local microvascular blood flow. The significance of this research is that it establishes *in vivo* nitric oxide as a mechanistic and regulatory device coupling glutamatergic-dependent neuronal activity and local cerebral blood flow on a quantitative basis. Aging and transgenic models of Alzheimer´s disease will be used to assess whether an aberrant neurovascular coupling underlie such conditions.



Nitro-tyrosine in the mucosa layer of the stomach exposed to nitrite



Interacting profiles of nitric oxide and oxygen in hippocampus upon glutamate receptor stimulation

Furthermore, we will elaborate on the analysis of the biological mechanisms of polyphenols in terms of modulation of signaling pathways connected to gastric and intestinal inflammatory processes, as well as the redox modulation of gastric epithelial functions upon nitrite-driven nitric oxide production.

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Membrane Toxocity Group | *Head: Mª Amália Jurado*

The main purpose of our research is to find out more about the roles played by lipids and the lipid-bilayer component of cell membranes in cell physiological processes and in cellular dysfunction leading to disease. The emphasis is on deciphering the principles governing the dynamic organisation of lipid molecules in non-covalent supramolecular structures and the influence of physical and chemical properties of the lipid bilayer in membrane protein functioning, that is a quest for functional lipidomics.

Two basic questions, focusing on lipids and lipid biological relevance, have been tentatively clarified by Membrane Toxicity group: i. the functional implications of lipid diversity in biological membranes; ii. the mechanisms of regulation of membrane mediated biological functions by lipid membrane composition, structure and dynamics.

To investigate these central problems in lipid biology, different experimental strategies have been developed: a) to qualitatively and quantitatively analyse membrane lipid composition changes induced by physical and chemical agents, using bacterial cells as models; b) to study how membrane lipid composition alterations, induced by diet-manipulation, affect physiological functions and susceptibility to pharmaceutical drugs or toxicants, in animal models; c) to identify alterations of the physical properties of the lipid bilayer related with cellular dysfunction and disease; d) to clarify how the cellular processing of nanostructures, such as fullerenes and lipid-based drug-vectors, is influenced by their interaction with cell surface, depending on the characteristics of the nanoparticles (size, surface chemistry and charge) and the cell membrane physical and chemical properties.

A large experience has been accumulated in our lab concerning lipid analysis, membrane modelling and the study of membrane physical properties under the influence of lipid composition and the action of different physical or chemical agents. The area of research has included the study of a wide range of biological and chemical compounds, such as DNA, sterols, surfactants, drugs, environmental pollutants and nanomaterials.

On the basis of collected data and knowledge we emphasise the following conclusive aspects: a) a toxic action targeted on the structural order and organisation of membrane lipids has been identified as a common strategy for a variety of environmental pollutants (detergents, insecticides, herbicides, organometals) to induce adverse effects on biological systems. Subtle changes of membrane physical properties, including disturbance of the bilayer lateral pressure profile and induction of remodelling of the membrane microphase pattern may also constitute the molecular mechanisms for a variety of drugs (e.g. antiarrythmic, anticarcinogen and anti-inflammatory drugs) to alter the homeostatic equilibrium of biological systems, promoting adverse side-effects; b) bacterial and mitochondrial models can be used as a suitable experimental approach to correlate pesticide or drug/induced membrane physical disturbance and cytotoxic effects, reflected by inhibition of bacterial cell growth and viability or impairment of bacterial/ mitochondrial respiratory activity, allowing to establishing structure-activity relationships.

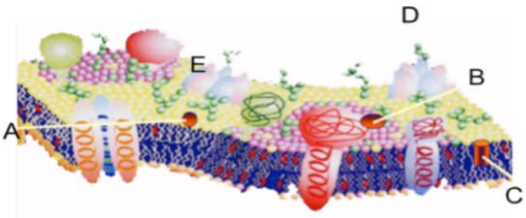

Fig.1. Interaction of chemical agents with membranes. Small molecules interact with the membrane surface, in fluid (A) and lipid raft (B) domains, or penetrate in the membrane core (C). Nanostructures such as fullerenes (D) or lipid-based DNA vectors (E) establish different interactions with the membrane, depending on their size, surface chemistry and charge.

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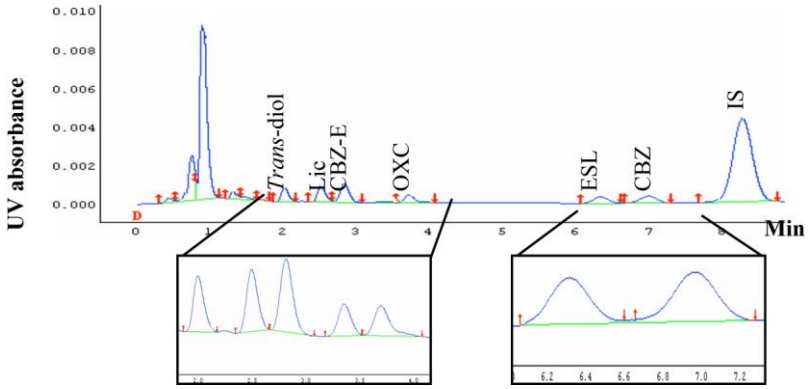
Pharmaceutical industries are profoundly changing the strategies of drug discovery and development (DDD) in order to reduce the late-stage failures that occur during that process. The integration of Pharmacometrics as an applied science in DDD and also in pharmacotherapy is increasing. Pharmacometrics interprets and describes the pharmacology in a quantitative fashion, targeting the characterization of pharmacokinetics and pharmacodynamics in preclinical and clinical studies. Obviously, the availability of reliable bioanalytical methods is required to support pharmacokinetics studies. Therefore, the development and validation of bioanalytical methods to quantify drugs and metabolites in biological matrices is a crucial line of research within the Pharmacometrics group.

Aiming the development of pharmacostatistical models to compare the potential three structurally related antiepileptic drugs (AEDs), carbamazepine (CBZ), oxcarbazepine (OXC) and eslicarbazepine acetate (ESL), we developed and validated a aquiral HPLC-UV method to quantify CBZ, OXC and ESL, as well as their main metabolites (*trans*-diol, licarbazepine and carbamazepine-epoxide) in human plasma. This method will be useful to support further clinical pharmacokinetics and it may be also applied in routine for therapeutic drug monitoring of CBZ, OXC and ESL. In addition, a similar chiral method, able to differentiate S-licarbazepine and R-licarbazepine was also developed and validated in several biological samples of mouse, which will support the execution of multiple *in vitro* and *in vivo* studies.

The bioanalytical chromatographic methods developed are simple and fast, and they were fully validated following the international guidelines for validation of bioanalytical methods intended for pharmacokinetics studies. The methods demonstrated to be sensitive and linear ($r^2 > 0.99$) over a wide drug/metabolite concentration range, including the therapeutic window usually established for epileptic patients. The methods also showed good selectivity, precision (coefficient variation < 15%) and accuracy (bias < 15%). The sample extraction procedure employed (solid-phase extraction) affords high recovery, and the stability of the analytes was also demonstrated.

Recently, we start *in vitro* and *in vivo* pharmacokinetic studies in mouse directed to get information about the drug disposition of CBZ, OXC, ESL and their metabolites, which will be essential to develop pharmacostatistical models able to compare and better describe the potential of such AEDs.



Chromatogram (aquiral HPLC-UV method)

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Microbiology Area

Coordinator: Milton Costa

The Extreme Environment Group will gain a better understanding of the microbial diversity in geothermal areas, hypersaline environments and extremely alkaline springs. One of the primary objectives involves the study of mechanisms that confer radiation and desiccation resistance in species of the genus Deinococcus and Rubrobacter. We are also studying the evolution of genes involved in the pathogenesis of Legionella species in natural environments to define clones that human disease. Another line of research focuses on the characterization of the pathways for the synthesis of compatible solutes in hyperthermophiles. This led us to examine the synthesis of the essential lipopolysaccharides in Mycobacterium species.

The Extreme Environments Group has described a large number of novel species has now isolated several extremely halophilic organisms from deep anaerobic Mediterranean brines which constitute new lineages of bacteria and archaea.

Among other organisms described were two new species of the thermophilic genus Meiothermus with anomalous glycolipid patterns, which due to the absence of synthesis of 2OH fatty acids did not produce one of the diagnostic glycolipids of the members of the genus.

Our research has lead to the discovery a novel pathway for the synthesis of the compatible solute mannosylglucosyl-glycerate was described in Persephonella spp. and Rhodopirellula spp. We have also discovered the function of the gene product of an essential gene in Mycobacterium spp. namely maltose-1-phosphate synthase that could lead to the development of a very specific antibiotic. The genetic evolution of the dotA gene in Legionella clones, from natural environments, indicates larger diversity and plasticity to infect several protozoan hosts.

The Yeast Research Group is unravelling the resistance of Candida albicans to macrophages as well as the epidemiology of yeast infections in a local hospital.

The Microbiology of Extreme Environments Laboratory will participate in the first Portuguese exploration of the Atlantic sea-floor at depths of 6000. The samples retrieved and others from the Red-sea deeps and hot springs from the Azores will be used for isolation of organisms and metagenomic studies. We will evaluate the functional diversity of an alkaline groundwater environment by screening genomic libraries of conserved genes involved in central metabolic processes. We aim to study the homeostasis of compatible solute (CS) pools in extremophiles through regulation of biosynthesis and catabolism since the regulation of catabolism/export is scarce. We will continue to study the pathways for recently identified CSs. Glucosyl-glucosylglycerate for example, found in a thermophilic bacterium, was detected in mycobacteria and proposed to be a precursor of methylglucose polysaccharides. We will elucidate the biosynthesis of the methylglucose polysaccharides from mycobacteria. After the identification of the genes involved we will obtain the structure of the corresponding enzymes, essential for probing the catalytic mechanism and design/development of specific inhibitors to act as anti-mycobacterial drugs. We will probe the importance of recombination events on speciation mechanisms within Legionella and the distribution of virulence-related genes as a driving force on the evolution of the pathogen L. pneumophila. We will additionally design of a universal, portable and unambiguous epidemiological tool capable of correlating L. pneumophila population structure and virulence.

The Yeast Research group has achieved the following: Yeast metabolic response to the presence of bacterial endotoxin (one paper submitted); Combined effect of anti-fungal cell wall inhibitors in A. infectoria: identification and cloning of the AiFKS gene and its regulator AiRHO; caspofungin susceptibility.

The Medical Mycology – Yeast Research Group will characterize the sensing mechanism by which yeasts are able to detect and respond to the presence of LPS, to study in vivo models of mixed infections and to assess yeast gene modulation by LPS. The in vivo and in vitro effect of purines in the interaction of C. albicans- macrophages will be studied, together with the molecular and pharmacological characterization of purine receptor and transporters in C. albicans. The inefficiency of single therapeutic strategies to eradicate dematiaceous infections prompts us to study the sinergism between caspofungin and chitin synthetase inhibitors and how this affects the A. infectoria-host interaction.

Microbiology of Extreme Environments Group

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Branca Margarida Silva	Undergraduate Student
Nelson A. Carvalho Cunha	Undergraduate Student

Microbiology of Extreme Environments Group | *Head: Milton Costa*

The general objectives of our group are:

Isolate and characterize novel organisms from extreme environments for basic studies and for their biotechnological potential;

Continue our studies on the mechanisms involved in stress adaptation of thermophilic, halophilic and desiccation-resistant bacteria and in members of the *Planctomycetes*, an unusual deep-rooted lineage of bacteria;

Identify new compatible solutes and elucidate their biosynthetic pathways and their role in stress tolerance;

Elucidate the pathway for the synthesis of methylglucose polysaccharides (MGLPs) exclusively found in mycobacteria and to probe the function of an enigmatic maltokinase essential for *Mycobacterium tuberculosis* growth;

Determine the contribution of natural environmental *Legionella pneumophila* strains into the molecular evolution of genes belonging to several secretion systems known to be related with virulence under distinct environmental conditions;

Determine the microbial diversity related to stalactite/stalagmite system in a subterranean karstic environment by culture-independent community analysis.

We have participated in the first Portuguese exploration of the Atlantic sea-floor and in the international expedition Middle and Mamba 09 to the deep brine basins of the Mediterranean Sea and retrieved a large number of samples from those unexplored environments. We have also isolated strains from thermal springs, saline ecosystems and other environments leading to the description of new Genera and new Species of bacteria.

The extremely gamma-radiation resistant *Rubrobacter xylanophilus* is the only actinobacterium known to accumulate the compatible solute mannosylglycerate (MG). Due to its uniqueness and ancestry we characterized the key-enzyme for MG synthesis. We have elucidated two alternative biosynthetic pathways for the rare compatible solute mannosylglucosylglycerate in *Petrotoga mobilis*, a bacterium isolated from petroleum environments. We have examined the compatible solutes pools in members of *Planctomycetes*, and found several unique compatible solutes that accumulate in response to different stresses.

An essential maltokinase from *M. tuberculosis* has been characterized. The enzyme was able to use several NDPs to phosphorylate maltose and may participate in the inactivation of aminoglycoside antibiotics.

We demonstrated that UV disinfection provides effective control of *Legionella* spp., with the advantage of being a method that does not affect the physicochemical composition of the water. These findings suggest that UV irradiation, applied at key points in therapeutic spas, can be used to control colonization of water distribution systems by *Legionella* spp.

We have determined that the virulence-related DotA alleles from *L. pneumophila* natural-environmental strains were the only under strong diversifying selection indicating that recombination and frequent nonsynonymous mutations are important evolutionary mechanisms to increase fitness of *L. pneumophila* strains in some environmental niches and towards distinct hosts, contrarily to what has been suggested for man-made and clinical-related strains.

Culture-independent community analysis performed on stalactite/stalagmite system revealed that the majority of the populations detected were very closely related to the populations previously isolated.

The samples retrieved from the Mediterranean deep brine basins and hot springs from the Azores will be used for isolation of organisms and metagenomic studies. We will also evaluate the functional diversity of an alkaline groundwater environment by screening genomic libraries of conserved genes involved in central metabolic processes.

We will continue studying the pathway leading to the synthesis of MGLPs from mycobacteria, by functional and structural characterization of the intervening enzymes. We will create *M. bovis* maltokinase conditional mutants and perform transcriptional studies to elucidate the role of this essential enzyme in mycobacterial metabolism and stress adaptation.

We will probe the importance of recombination events on speciation mechanisms within *Legionella* and the distribution of virulence-related genes as a driving force on the evolution of the pathogen *L. pneumophila*.

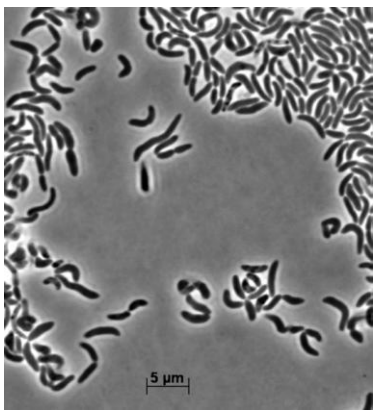

Fig. 1: Phase-contrast microscopy of Bacillus isabeliae (strain CVS-8^T). Bar, 5µm.

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The outbreak of individuals with immune diseases or physiological conditions, which weaken the immune system, has led to an increased incidence of opportunistic fungal infections, difficult to diagnose, to treat and with poor outcome. Our main goal is to understand fungal infections, its epidemiology and pathogenesis together with unraveling novel therapeutic approaches.

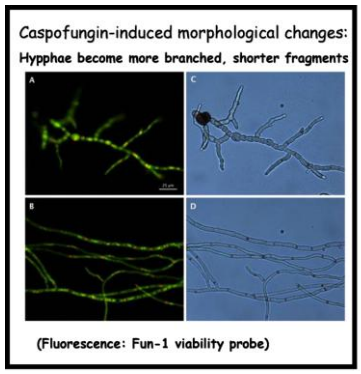
Alternaria infectoria, an agent of cerebral phaeohyphomycosis. The identification of this dematiaceous fungi as a cause of a cerebral abscess, prompt us to a project financed in 2006 by Merck, Sharp & Dohme (Medical School Grant). AiFKS and AiRHO genes were identifyied, isolated and cloned [Anjos et al., *Alternaria infectoria* FKS and RHO genes. MS under preparation]. A collaboration with Professor Neil Gow, Univ. of Aberdeen, resulted in a study showing a strong synergy between different types of cell wall inhibitors, β-1,3-D-glucan synthase and chitin synthase (CHS) inhibitors (MS under preparation) based on the approach recently published by this group. During 2009 a FCT grant was approved for financing, together with a Pos-doc Fellowship (to be initiated during 2010).

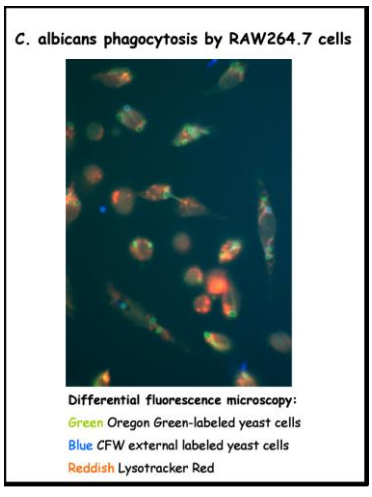
The ongoing work aims to identify the AiCHS genes and to study the influence of the melanin pathway in the pathogenesis of this melanin-containing mold, especially towards the central nervous system.

*Yeast infections epidemiology***.** A four year surveillance of yeasts isolated as etiological agents of infection was conducted in a hospital laboratory of the Centre of Portugal, aiming to evaluate the epidemiology of yeast infections. Clinical isolates and clinical data were gathered from 755 patients. The isolates were first identified using classical methods, routinely used at the hospital laboratory, and then re-identified using RFLP of the ITS 5.8S rRNA gene and sequence of the D1/D2 domain of the 26S rRNA gene. A statistical study was performed in order to assess the probability of a patient developing a blood stream yeast infection. The results showed that the variables with statistic impact were AIDS or haemodialysis, the parameter with a higher odds ratio when associated with patients aged over 65 years. Currently, an ongoing collaboration with FMUC and the School of Dentistry, aims to characterise the oral health of diabetic children in what regards yeast load.

Role of adenosine and adenosine receptors in the resistance of Candida albicans to macrophage attack. Macrophages have a primordial role in the host immune response to *Candida albicans* infection, but this yeast has developed strategies to overcome this initial line of defence by mechanisms still unsolved. This work was devised to test the novel hypothesis that purines, particularly adenosine, and their sensing devices may constitute a key system exploited by *C.albicans* to evade macrophage attack, thus explaining its success as a pathogen. This project is a multi-disciplinary collaboration between two groups at CNC, the "Medical Mycology Yeast Research Group" and the group "Purines at CNC".

During 2009, this 2-year exploratory project lead to the a major conclusion that the ability of *C. albicans* in disabling the activation of the adenosinergic system, via A_{2A} receptor, is crucial for the property of *C. albicans* to be successfully internalised into the macrophage but not destroyed by the enzymatic and oxidative machinery of this cell of the innate immune system. This opens an outstanding opportunity of further investigation, never explored, that will certainly give valuable hints on the resistance of fungal cells to phagocytosis, thus remaining silently inside the phagocytic cells, enabling its transport to other sites of body host, or its switching to an invasive phenotype.





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Biophysics and Biomedical NMR Area

Coordinator: Carlos Geraldes

Inorganic Biochemistry and Molecular Imaging: Study new diagnostic imaging tools - metal based nanoparticles and chelates as multimodal (MRI, nuclear imaging) targeted agents – *in vitro* and in animal models. Inorganic drugs for therapy – Li^+ in bipolar disorder and V(IV) complexes as oral insulin-mimetic agents–mechanisms of action in cell and animal models. NMR and DFT studies of ion-polymer complexes.

Intermediary Metabolism: Insulin resistance and Type 2 Diabetes are characterized by a global loss of metabolic flux control that disrupts carbohydrate, lipid and protein metabolism. To integrate the analysis of these effects into simple and practical metabolic flux assays, we are developing stable-isotope tracer measurements of glucose, fatty acid, and amino acid metabolism in humans and in animal models of diabetes. These measurements are providing new insights about the interactions between carbohydrate, lipid and protein metabolism in the setting of insulin resistance and diabetes.

Molecular Imaging: New Gd^{3+} complexes as potential MRI contrast agents with improved performance were studied: a) the complex of a new substituted DTTA ligand with accelerated water exchange and higher relaxivity; b) the complexes of five phosphinate and phosphonate monoester ligands with significant contribution of second-sphere water to relaxivity; c) a new versatile synthon with optimized water exchange for the synthesis of high relaxivity, targeted MRI contrast agents was obtained (Gd(DO3A-N-a-aminopropionate); d) PAMAM dendrimers conjugated with a neutral Gd^{3+} chelate with a fast water exchange were obtained and studied.

Inorganic Biochemistry: a) Li^+ effects on the metabolism of glucose and acetate in rat brain and primary cultures of neurons and astrocytes are mediated by reduction of neuronal glucose uptake resulting in decrease of glutamatergic and GABAergic neurotransmission (^{13}C NMR study); b) Cytotoxicity study of three vanadium (V) complexes in cell lines showed that V^V-MHCPE has potential antitumor activity.

Ion-polymer interactions: a) Structural NMR and DFT studies of polymers and complexes of transition metal ions; b) The effect of Al^{3+} on the flocculation and micellization of SDS and conjugated polyelectrolytes was studied.

Clinical Research Studies: Glutamine is a potentially important source of carbons for gluconeogenesis and may therefore play a role in hepatic glucose production. Hepatic glutamine may be derived from peripheral tissues such as muscle either by cataplerotic efflux from the Krebs cycle or by proteolysis. Proteolytic and cataplerotic sources of hepatic glutamine were determined by ^2H NMR analysis of urinary phenylacetylglutamine (PAGN) ^2H-enrichments in eight healthy subjects after ^2H_2O and phenylbutyric acid ingestion. Hepatic glutamine was noninvasively sampled as the urinary phenylacetylglutamine (PAGN) conjugate following ingestion of phenylbutyric acid.

1. Develop new multimodal targeted diagnostic tools, eg. MRI contrast agents, optimizing the efficacy and safety for small and middle molecular weight MRI contrast agents (CAs) and the the sensitivity of reporter groups, based on chelates and nanoparticles (NPs) (eg. Ln^{3+}-containing NPs, core-silica shell NPs, gold NPs bound to Gd^{3+} macrocyclic chelates).

2. Develop innovative targeted paramagnetic liposomes and lipoplexes for *in vivo* visualization of drug delivery/release by MRI.

3. Study the mechanism of the effects of new vanadium complexes as insulin-mimetic agents in adipocytes, in particular on the insulin signaling cascade – target proteins (western blot analysis and MS).

4. In vivo MRS and MRI studies of the effects of new vanadium complexes in animal models of type II diabetes. Metabolic studies using ^{13}C-labeled substrates.

5. Study fluorescent sensors of ions (Ga^{3+}, Al^{3+}) for environmental applications.

6. Study cationic conjugated diblock polyelectrolytes as potential biosensors, biological imaging agents and materials for optoelectronic devices

7. Blood glutamine enrichment from ^2H_2O as an early marker of cachexia. The development of an early maker for cachexia will improve survival in diseases such as heart failure, cancer and HIV. Our primary hypothesis is that the enrichment of blood glutamine from ^2H_2O is significantly decreased in cachexia due to an elevated release of unlabeled proteolytic glutamine. Our secondary hypothesis is that this occurs ahead of significant changes in lean body mass thereby providing an early marker for cachexia. These hypotheses will be tested as follows: Blood glutamine ^2H-enrichment from ^2H_2O and whole body glutamine kinetics will be measured in a rat model of cachexia. If our primary hypothesis is correct, plasma glutamine ^2H-enrichment will be significantly reduced following induction of cachexia. If the secondary hypothesis is correct, changes in glutamine ^2H-enrichment will occur before detectable loss of lean body mass.

Inorganic Biochemistry and Molecular Imaging Group

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Intermediary Metabolism Group

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Ivan Viegas	PhD Student
Cristina Barosa	PhD Student
Joao Andre Duarte	PhD Student
Daniela Pinheiro	MSc Student
Filipa Simoes	MSc Student
Ana Rita Gonçalves	MSc Student
Joana Barra	MSc Student
Patrícia Nunes	Research Technician

The group works in four main research lines: a) *Inorganic Complexes for Medical Diagnostics: MRI and Molecular Imaging*; b) *Inorganic species for therapy*; c) *Environmental effects of metal ions*; d) *Polymers and their complexes* – characterization and applications.

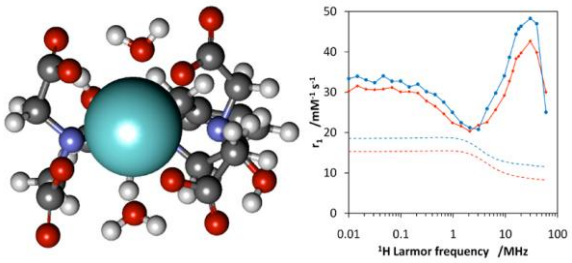
Lanthanide Chelates of (bis)-Hydroxymethyl-substituted DTTA with Potential Application as Contrast Agents in Magnetic Resonance Imaging

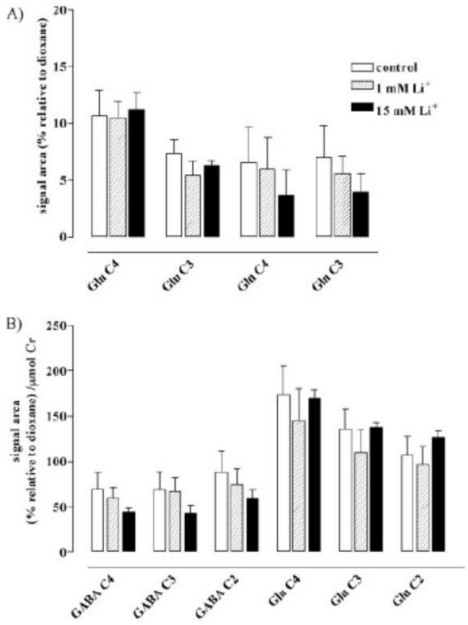
The Gd^{3+} complex of the new ligand N'-benzyl-*bis*-hydroxymethyl-substituted DTTA has improved properties as an MRI contrast agent relative to the parent DTTA: two hydration waters with accelerated exchange provide higher relaxivity, weak interaction with HSA but not with bidentate ligands. This endows it with improved relaxivity and optimized properties as a sensitive reported for targeted MRI contrast agents

Mechanisms underlying Li^+ effects in glutamatergic and GABAergic neurotransmissions in the adult rat brain and in primary cultures of neural cells as revealed by ^{13}C NMR

We investigated by ^{13}C NMR the mechanisms underlying Li^+ effects on glutamatergic and GABAergic neurotransmission systems in the adult rat brain and in primary cultures of cortical neurons and astrocytes during the metabolism of (1-^{13}C) glucose or (2-^{13}C) acetate. Adult male rats receiving a single dose of Li^+ intraperitoneally were infused with (1-^{13}C) glucose or (2-^{13}C) acetate. ^{13}C NMR spectra of brain extracts prepared after the infusion revealed that Li^+ significantly decreased the incorporation of 13C in glutamate and GABA carbons from (1-13C) glucose, but not from (2-^{13}C) acetate. Our results indicate that the effects of Li^+ are mediated through a reduction of neuronal glucose uptake resulting in a decrease of glutamatergic and GABAergic neurotransmission without apparent effects on astrocytic metabolism.

In the future we will: a) develop new multimodal targeted diagnostic tools, eg. MRI contrast agents, optimizing the efficacy (relaxivity) and safety (stability) for small and middle molecular weight MRI contrast agents (CAs), the sensitivity of reporter groups, based on chelates and nanoparticles; b) develop innovative targeted paramagnetic liposomes for *in vivo* visualization of drug delivery/release by MRI; c) study the mechanism of the effects of new vanadium complexes as insulin-mimetic agents in adipocytes; d) study conjugated polymers as biosensors.





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Silvério S, Torres S, Martins AF, Martins JA, André JP, Helm L, Prata MIM, Santos AC, Geraldes CFGC (2009) (*bis*)-Hydroxymethyl-substituted DTTA skeleton: a new lead for the synthesis of high relaxivity MRI contrast agents? *Dalton Trans*. 4656-4670.

Fonseca CP, Sierra A, Geraldes CFGC, Cerdán S, Castro MMCA (2009) Mechanisms underlying Li^+ effects in glutamatergic and gabaergic neurotransmissions in the adult rat brain and in primary cultures of neural cells as revealed by ^{13}C NMR. *J. Neuroscience Res.* 87: 1046–1055.

Insulin resistance and Type 2 Diabetes are characterized by a global loss of metabolic flux control that disrupts carbohydrate, lipid and protein metabolism. To integrate the analysis of these effects into simple and practical metabolic flux assays, we are developing stable-isotope tracer measurements of glucose, fatty acid, and amino acid metabolism in humans and in animal models of diabetes. These measurements are providing new insights about the interactions between carbohydrate, lipid and protein metabolism in the setting of insulin resistance and diabetes.

A) Clinical Research Studies: Glutamine is a potentially important source of carbons for gluconeogenesis and may therefore play a role in hepatic glucose production. Hepatic glutamine may be derived from peripheral tissues such as muscle either by cataplerotic efflux from the Krebs cycle or by proteolysis. Proteolytic and cataplerotic sources of hepatic glutamine were determined by ^2H NMR analysis of urinary phenylacetylglutamine (PAGN) ^2H-enrichments in eight healthy subjects after ^2H_2O and phenylbutyric acid ingestion. Hepatic glutamine was noninvasively sampled as the urinary phenylacetylglutamine (PAGN) conjugate following ingestion of phenylbutyric acid. Enrichment of hepatic glutamine from ^2H_2O is preserved in the glutamine moiety of PAGN. The glutamine hydrogens of PAGN have well resolved NMR signals allowing positional enrichment in the backbone hydrogens to be analyzed by ^2H NMR. We demonstrated that analysis of the PAGN ^2H-enrichment pattern following ^2H_2O ingestion provides a practical and novel insight on the sources of hepatic glutamine in humans. Our results indicate that in healthy postabsorptive subjects, at least 50% of hepatic glutamine molecules had originated from proteolytic C5 amino acids. In conclusion, we present a simple and noninvasive method for resolving the contributions of whole-body metabolic and proteolytic activities to the supply of hepatic glutamine carbon skeletons. This analysis may allow the role of peripheral intermediary metabolism and protein synthesis/degradation on the sources of hepatic glutamine carbons to be better defined. PAGN recovery and analysis can be integrated with ^2H_2O measurements of hepatic gluconeogenesis therefore in principle, the relationship between hepatic glutamine sources and gluconeogenic activity can be explored in a variety of physiological and pathophysiological settings.

B) Basic Research Studies: Triglycerides (TG) are secreted by the liver in the postabsorptive state, and are synthesized by esterification of free-fatty acids (FAs) – a significant portion of which are produced by hepatic de novo lipogenesis (DNL). Therefore, the study of DNL becomes crucial to understand dysfunctions of lipid metabolism in disease. To quantify DNL by stable isotope tracer methods, it is necessary to measure the acetyl-CoA precursor and TG product enrichments. The objective of this work was to develop novel approaches for quantifying hepatic acetyl-CoA precursor enrichments from deuterated water (^2H_2O) tracer and applying these to quantify DNL in isolated perfused livers. Hepatic acetyl-CoA enrichment from ^2H_2O was inferred from the hydrogen 4 enrichments of hepatic glutamate/glutamine, which are assumed to be derived from the methyl hydrogens of acetyl-CoA. Our studies revealed that the ^2H enrichment of acetyl-CoA pool was only about half that of bulk water (52.7 ± 2.7%) and the assumption that acetyl-CoA and bulk water hydrogens are equally enriched with ^2H is incorrect for our experimental conditions. Thus, the DNL contribution during the 2 hours of perfusion, based on the ratio of product TG-methyl to acetyl-CoA precursor enrichment, represented 1.40 ± 0.09% of total triglyceride, corresponding to a synthesis rate of 5.42 ± 0.42mg/2 hr.

Our studies demonstrated that it is possible to quantify DNL using the deuterated water (^2H_2O) method in an isolated perfused liver. This represents a relatively simple and therefore useful model for improving our understanding of factors that control or alter hepatic DNL.

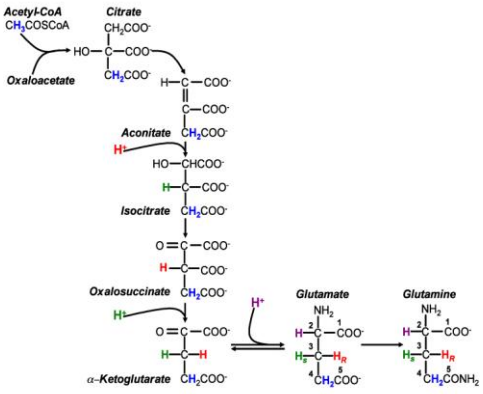

Fig. 1. Positional enrichment of glutamine hydrogens from ^2H_2O via the metabolic steps of the Krebs cycle.

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Barosa C, Almeida M, Caldeira MM, Gomes F, Jones JG. Contribution of proteolytic and metabolic sources to hepatic glutamine by ^{2}H MR analysis of urinary phenylacetylglutamine ^{2}H enrichment from $^{2}H_2O$. M*etabolic Engineering* 12: 53-61. (*in press*)

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Cell and Development Biology Area

Coordinator: João Ramalho Santos & Maria Celeste Lopes

The key identifying feature of the "Cell and Development Biology" area is CNC Researchers whose programs involve close partnerships with clinicians at FMUC/HUC, both in terms of basic research with human samples, setting up novel clinically-relevant services and trials, and hopefully furthering translational research. Partnerships already in place include: Immunology, Oncobiology, Genetics, Neurology, Dermatology, Reproduction, Endocrionology (Obesity, Diabetes), and likely others.

One of the major strengths of the groups, included in the "Cell and Development Biology" area, is the strong collaboration with clinical departments, allowing the collection of human tissues and samples for the development of translational investigation in several distinct, yet interconnected research lines. In line with this, the major goal in 2009 was the consolidation of the research projects being carried out, which was achieved as the publication record for the various groups in this area demonstrates.

As mentioned in the previous report, the main purpose for this area was to continue the consolidation of the research carried out, as well as the recruitment of new researchers to address specific needs. In this regard, in 2009, the Reproduction group has now established solid grounds in the fields of stem cell biology and tissue engineering.

The Cellular Immunology and Oncobiology group was able to strengthen national and international collaborations established in previous years, which will become more apparent in the near future when collaboration manuscripts already submitted become published.

The Chronic Inflammation group was established and initiated its expansion in line with the process of new recruitments and solidified its national and international cooperation networks.

The Phagocytosis and Pathogens group reached a significant dimension in line with the process of new recruitments initiated in the previous year.

The Metabolism, Insulin Resistance and Complications group is now more firmly established within CNBC, especially due to collaborations with HUC services and CNBC's groups.

There is an enormous wealth of expertise in terms of healthcare, medical know-how; sample collections and patient groups at HUC/FMUC, which could be explored further, provided there are common interests and the partnerships are mutually potentiating. However, the CNC should conduct organized prospecting in terms of novel possibilities for clinical research.

The "pitfalls" of the approach include encroachment of both clinical and research perspectives (i.e. "territorial" issues), which must be made to dialogue with vocabularies that are not exactly the same, although they may sound similar. An important point is that value-frames and time-frames also are different, from day-to-day clinical care, to long-term research approaches. It is thus crucial to identify willing partners on both sides, and nurture the dialogue continuously. It can be done.

Some examples of possible joint approaches are:

- Using Induced Pluripotency to create Stem-cell-like cells from patients with different pathologies, thus enabling the creation of human cell line models on which the disease can be modeled, for drug and gene expression screens, etc.

- Tissue engineering for tissue repair in cardiology or other specialties.

- The development of new animal models for specific diseases of interest (e.g. transgenic rats or mice).

- Development of trans-services core facilities (microscopy, flow cytometry, sequencing, gene expression, etc) that are not involved in day-to-day operations and are therefore available for research purposes.

The groups in the "Cell and Development Biology" area will continue to develop the research lines in which they are engaged, further strengthening existing collaborations and seeking new ones, both

national and international. In terms of funding, all groups will continue to apply for grants from FCT and other national and international institutions

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Ana Teresa Rufino	PhD Student
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Michelle Stump	PhD Student
Inês Santarino	MSc Student
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Molecular and Translacional Medicine: Metabolism, Insulin Resistance and Complications Group

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Ermelindo Leal	Post-Doc Fellow
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Maria Joao Pereira	PhD student
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Emerging Group

Chronic Inflammation Group

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Paulo Jorge R. dos Santos	PhD Student

Cellular Immunology and Oncobiology Group | *Head: Mª Celeste Lopes*

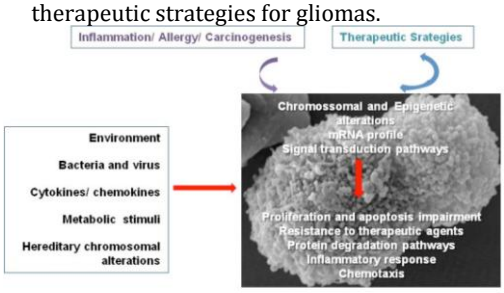
The researchers of this group share common interests in identifying the cellular mechanisms that regulate the function of normal human cells and in understanding how disruption of these processes leads to disease, namely to allergic contact dermatitis, osteoarthritis, autoimmunity and cancer. A major strength of this group is the variety of approaches, ranging from *in vitro* studies in human primary cell cultures and established cell lines, to *in vivo* experiments with animal models and analysis of clinical samples made in close collaboration with hospital departments.

The research projects of the cellular immunology sub-group focused in evaluating: i) how *Leishmania infantum* modulates the functions of antigen presenting cells and the anti-inflammatory properties of polyphenols from *Cymbopogon citratus*: *L. infantum* successfully infect mouse bone marrow-derived dendritic cells (DC) without inducing cell maturation. The sustained AKT activation and the induction of an atypical NF-κB signaling pathway are strategies by which *Leishmania* parasites subvert DC immunogenicity. Polyphenols prevent NF-kB activation by inhibiting the proteasome activity. ii) age-related metabolic changes in human chondrocytes that contribute to osteoarthritis development and progression: regulation of the facilitative glucose transporter-1 in human chondrocytes involves ATP-sensitive K^+ channels and is impaired in OA chondrocytes leading to intracellular glucose accumulation and induction of catabolic responses. Insulin, through its specific receptor, promotes cartilage matrix-specific collagen II expression in OA chondrocytes, overcoming age-related IGF-I resistance. iii) the role of the CD38 on the regulation of immune responses, namely infection and autoimmunity: CD38 plays a role in the development of productive immune responses against *Mycobacterium tuberculosis* and its absence compromises leukocyte recruitment and macrophage activation during responses to mycobacteria.

The research projects of the oncobiology sub-group evaluated: i) the molecular changes relevant to the carcinogenesis of the thyroid and breast cancers and the genetic risk factors in HPV-mediated cervical cancer: common and rare human Papillomaviruses in Portuguese women were identified and correlated with the incidence of cervical cancer. Novel phylogenetic and viral pathogenesis concepts of Human Papillomavirus were described and their contribution to cervical cancer unraveled. ii) the cell signalling pathways involved in cancer and chemoresistance and their contribution to the identification of new molecular therapeutic targets: Oxidative stress and mitochondrial dysfunction are involved in neoplastic development and determine the levels of apoptotic modulators, probably contributing to cell death resistance in haematological malignancies. The farnesyltransferase inhibitor, α-HFPA, promotes cancer cell death independently of Ras mutations. iii) Genomic and phenotypic abnormalities of human gliomas: the results show genetic heterogeneity among human gliomas and support the existence of different cytogenetic pathways of intratumoral evolution in high versus low grade tumours.

In the future *Cellular Immunology will try:* i) to identify a) modifications on the proteomic, lipidomic and intracellular signalling profiles of skin dendritic cells differentially induced by chemical sensitizers and irritants to establish *in vitro* tests to predict the sensitizing potential of chemicals, b) mechanisms of *Leishmania infantum* immune evasion to explore the potential of dendritic cell-based vaccination, and c) the pharmacological activity of polyphenols from *Cymbopogon citratus* and their potential as anti-inflammatory drugs. ii) to identify a) the mechanisms that allow normal human chondrocytes to resist the deleterious effects of hyperglycemia and how those processes are affected by aging and osteoarthritis, b) molecules in essential oils with potential anti-osteoarthritic activity, and c) conditions of mechanical stimulation that promote the maintenance of the differentiated chondrocyte phenotype to optimize the production of articular cartilage *in vitro*. iii) *In collaboration with the Portuguese Oncology Institute of Coimbra*, we are studying the role of multifunctional ectoenzymes CD38 and CD157 in mycobacterial infection and systemic lupus erythematosus.

In the future, *Oncobiology will:* i) *In collaboration with the Portuguese Oncology Institute of Coimbra*, to study: a) the immunological risk factors of HPV-mediated cervical cancer; and b) the molecular changes relevant for the carcinogenesis of the thyroid (LRP1B) and breast (Claspin). ii) To identify potential molecular targets that can be used in prognostic definition and therapeutic development for haematological neoplasias, by studying the signal transduction pathways, namely those involving the Ras gene. iii) To continue the genetic characterization of gliomas in order to identify prognostic markers and to develop effective therapeutic strategies for gliomas.



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The main goals involve determining the metabolic cues that govern gonad homeostasis, proper mammalian gamete function, and pluripotent stem cell status, with the goal of increasing the success rates of Assisted Reproduction in humans and endangered species, as well as to develop efficient methods to improve stem cell propagation and differentiation into specific fates.

Recently concluded research includes:

1- Characterization of testicular mitochondrial bioenergetics and the finding that they are very distinct from that of mitochondria from other tissues, both in terms of basic function and how it is modulated by different substances. The effects of both diabetes and aging on testicular mitochondria were also evaluated. While the former seems to have little effect, aged testes showed a severe decrease in mitochondrial function coupled with an increase in reactive oxygen species (ROS) production, which seemed to be partially counteracted by an up-regulation of proton leak, probably via uncoupling proteins. This suggests that both ROS and proton leak may have a role in modulating the deleterious effects of testicular aging.

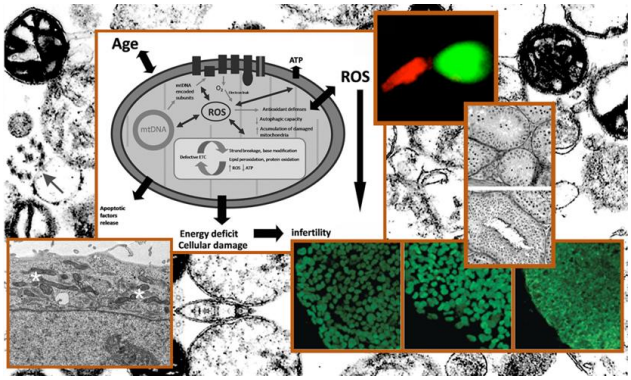
2- Development of novel assays to improve the analysis of human sperm function and the diagnosis of male infertility, given that the methodology currently employed is unreliable. Namely, a simple assay to monitor human sperm DNA status, an important parameter that is not usually quantified in routine semen analysis, was introduced. This assay was derived from previous work carried out in the cat, and its usefulness in predicting treatment outcomes has been validated in a multi-center collaboration. Additionally the analysis of mitochondrial functionality in mature human sperm, and how it can be efficiently monitored routinely, was also perfected (see Biomedical Inter-Institutional Research Collaborations).

3- Discovery of a role for mitochondria in maintaining human embryonic stem cell pluripotency and in inhibiting stem cell differentiation into specific fates via a ROS-dependent mechanism. Mitochondrial inhibition results in a specific up-regulation of pluripotency markers (e.g. Nanog) in stem cells, alleviates their need for exogenous growth factors (such as bFGF) while maintaining the pluripotent state, and prevents differentiation towards cardiomyocytes and neurons, suggesting that metabolic modulation may play an important role in stem cell biology.

Current projects include continuing research to characterize the most viable human gametes, both in terms of basic science and for application in Assisted Reproduction. In this regard more functional sub-populations of sperm from a heterogeneous ejaculate are being isolated and characterized (by classical methods or flow cytometry), and long-term sperm *in vitro* culture systems perfected in order to prolong the time window in which male gametes can be used following collection. Evaluation of oocyte quality using novel simple non-invasive assays is also underway.

Other projects involve both the preservation of the male germline by testicular xenotransplantation using the cat as a model, and further characterization of testis bioenergetics, with an emphasis on mitochondrial function and how it can be affected by xenobiotics, such as dioxins or pesticides. Given that testicular mitochondria seem to be completely different from other mitochondrial normally used for *in vitro* assays (namely liver mitochondria), we postulate that they may more accurately serve as models for toxicology studies involving substances thought to impair male reproductive function. Parallel studies are being carried out with mature sperm, and also include other candidate substances that may modulate sperm function.

Finally the group is pursuing the modulation of stem cell pluripotency and differentiation using metabolic cues, and this work is being expanded to also included the generation, propagation and differentiation of induced pluripotent cells (iPS cells).



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Infection and Pathogens Group | *Head: Mª Otília Vieira*

In 2009 we continued to work on the following projects:

Project 1: Surfactants in the Prophylaxis of Sexually Transmitted Infections and in Oral Hygiene

The ultimate goal of this project is to find new surfactants that have virucidal and bactericidal activities at sub-toxic concentrations towards mammalian cells.

Project 2: A systematic functional analysis for Rab proteins in phagocytosis and phagosomal maturation of Mycobacterium tuberculosis.

The main objective of this project is to explore the function of some Rabs (small G proteins), previously identified in proteomic studies, on phagocytosis and phagosomal maturation of *Mycobacterium tuberculosis*-containing phagosomes.

Project 3: LDL charge and lipid droplets formation: implications for atherosclerosis

The objective is to clarify if different negatively charged LDL models originate similar lipid droplets organelles in macrophages and their contribution to apoptosis and atherosclerosis.

In project 1 we systematically examined the toxicity of different surfactant groups toward mammalian columnar epithelial cells (MDCK and Caco-2 cells) and non-epithelial cells (HeLa and dendritic cell lines). Polarized epithelial cells seem to be more resistant to toxicity induced by these molecules than non-polarized cells (manuscript in preparation). Among the surfactants tested there are a new family of surfactants designed and synthesized by us which, according to our preliminary results, are capable of inhibiting virus infection of epithelial cells at concentrations that are 5-20 times lower than the toxic levels for the epithelial cells.

In project 2 we started to identify by which mechanism(s) Rab10 contributes to the phagosomal maturation. Rab10 was found to be involved in recycling of phagosomal components back to the plasma membrane an event required for phagolysosome biogenesis (2). This project is currently the object of a collaboration with researchers at Harvard Medical School.

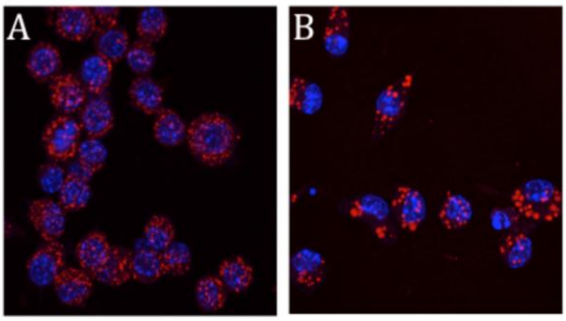
In project 3 our results show that the lipid droplets obtained with acetylated-LDL and cholesteryl hemi-ester enriched-LDL are not equivalent and do not resemble each other (Figure). More important the latter model of negatively charged LDL particles is pro-atherogenic in contrast with the former model.

We expect to:

-Synthesize and test newly amphiphile compounds for bactericidal and virucidal activities and for toxicity towards mammalian cells.

-Continue the systematic functional analysis of Rab proteins in the regulation of phagocytosis and phagosomal maturation. We are at moment investigating Rab8, that like Rab10 is a member of the group V Rabs.

-Continue the characterization of the lipid droplets induced by the different LDL models in macrophages.



Lipid droplets in macrophages incubated with acetylated- (**A**) and cholesteryl hemi-ester enriched- LDL (**B**). Blue, nuclei staining. Red, lipid droplets staining.

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Molecular and Translational Medicine: Metabolism, Insulin Resistance and Complications Group | *Head: Eugenia Carvalho*

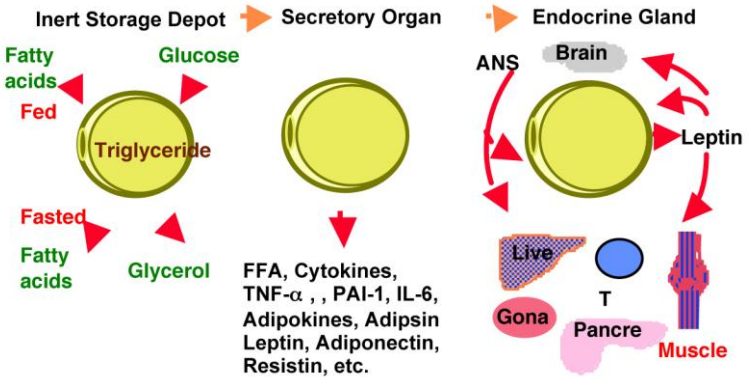
- Mechanisms of insulin resistance, pathogenesis of type 2 diabetes and obesity
- Signal transduction and cross talk pathways in the cardiovascular system
- The effects of glucocorticoids and immunosuppressive agents on insulin action and metabolism
- Complications of diabetes – diabetic foot ulcers

The adipocyte is emerging as participant in regulating physiologic and pathologic processes, including immunity and inflammation, as a secretory and endocrine organ, modulating appetite, energy expenditure, insulin sensitivity, endocrine, reproductive systems and bone metabolism. It stores excess energy in the form of lipids and is able to dramatically change its size in agreement with changing metabolic needs, with obesity as the result, increasing in both adipocyte number and size. The obese state has been characterized by a deregulation of the adipose tissue that can cause a state of low-grade, chronic, systemic inflammation that can link both the metabolic and vascular pathologies. Better understanding of the mechanisms of adipose tissue regulation and identification of the molecular basis of the deregulated adipose tissue may provide new insights into the causes of insulin resistance, diabetes and the associated complications.

• *The role of glucocorticoids (GCs) and immunosuppressive agents (IA) in the impairment of glucose and lipid metabolism in the metabolic syndrome.* The induction of insulin resistance by GCs and IA is a process that is still poorly understood. The main hypothesis is that GCs and IA are associated with insulin resistance, causing major metabolic chages in adipocytes leading to impaired insulin sensitivity. Our preliminary results indicate that the treatment of isolated rat fat cells with IA (cyclosporin A, tacrolimus, Prednisolone and Dexamethasone) causes a significant decrease in the insulin stimulated glucose uptake. These results demonstrate that both CsA, FK, P and D can inhibit insulin stimulated glucose uptake ex-vivo, promoting insulin resistance and causing major metabolic chages in adipocytes. *Increased knowledge on the mechanisms responsible for the development of insulin resistance caused by GCs and IA is of great importance to find new and more efficient treatments for post-transplant diabetes.*

• *The role of neuropeptides in wound healing in diabetes.* Impaired wound healing is a major clinical problem in diabetes. Peripheral neuropathy is a major contributing factor to tissue ischemia. We study wound healing in models that mimic the human condition by using Streptozotocin induced diabetes and NK-1R deficient mice. We are looking at the importance of mast cells and the role of Circulating endothelial progenitor cells (EPCs) in the diabetic state, both in humans and in animal models.

We plan to further investigate the molecular mechanisms of action of different neuropeptides in wound healing at the skin level both in animal models and in humans with diabetes and to develop biomaterials for in vivo peptide delivery to the wound site. In a new project this year we will study the Molecular mechanisms involved in Diabetic Cardiomyopathies in collaboration with the Division of Cardiology at the Hospital.



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A- The role of CD8+ T lymphocytes in rheumatoid arthritis and experimental chronic polyarthritis

CD8+ T lymphocytes play a major role in destroying tumor cell or cells infected by virus or cytosolic bacteria. However, they comprise up to 40% of the T lymphocytes infiltrating the synovial membrane in rheumatoid arthritis, and make 50% of the T lymphocytes present in the rheumatoid synovial fluid. These results suggest that CD8+ T lymphocytes might play a relevant role in the pathogenesis of rheumatoid arthritis.

Using the K/BxN mouse model of chronic spontaneous polyarthritis we have found that the articular tissue is infiltrated by activated effector CD8+ T lymphocytes, which are producing high levels of pro-inflammatory cytokines (IL6, IL17, TNFa, INFg) and cytolytic mediators (Granzyme B and perforin). Moreover, systemic depletion of CD8+ T lymphocytes in a mouse model of chronic spontaneous polyarthritis lead to disease amelioration according to several clinical and serological parameters. These results, suggest that CD8+ T lymphocytes play a central role in maintaining disease chronicity, and that manipulation CD8+ T lymphocytes has a strong therapeutic potential in arthritis. These results have been published in Arthritis and Rheumatism.

Therefore, our aim is to characterize the mechanisms by which CD8+ T lymphocytes contribute to the chronic inflammatory process of rheumatoid arthritis, using animal models of the disease, as well as samples from rheumatoid arthritis patients, in order to identify the potential role of CD8+ T cell manipulation in rheumatoid arthritis and other chronic inflammatory diseases.

B- Memory B lymphocyte and NK differentiation in chronic inflammatory diseases

Memory B lymphocytes are responsible for protecting the organism from recurrent infections by the same pathogenic agent. Some recent studies show that in chronic inflammatory diseases (e.g. AIDS; systemic lupus erythematosus; multiple sclerosis, chronic granulomatous disease) present a significant drop in the levels of circulating memory B lymphocytes, in particular the IgD+CD27+ subset. Unfortunately, the origin and immunological function of this IgD+CD27+ subset are still ill characterized. Therefore, our project aims at characterizing this particular memory B lymphocyte subset both in healthy donors and in the context of rheumatoid arthritis and multi-drug resistant tuberculosis, and understand its role in the chronic inflammatory process.

Our team has shown that in patients with very early (symptoms for less than 6 weeks) and established rheumatoid arthritis, similarly to other systemic autoimmune diseases, the memory B lymphocyte pool is reduced when compared to age and gender matched healthy individuals. In particular, the IgD+CD27+ memory B lymphocyte subset is the one in which we observe the most dramatic reduction. We have also shown that the memory B lymphocytes expressing pro-inflammatory chemokine receptors seem to accumulate in the synovial membrane. Additionally, rheumatoid arthritis patients undergoing anti-TNFa therapy have a significant recovery of the peripheral blood memory B lymphocyte pool, thus suggesting that TNFa plays a role in the homing of memory B lymphocytes in the arthritic joints. These results have been published in two different journals: Arthritis Research and Therapy (where it is tagged as "Highly Accessed" by the Biomed-Central) and in The Journal of Rheumatology.

Our future aims are:

1- Explore the potential of $IgD^{+}CD27^{+}$ memory B cells to control chronic inflammation

2- Identify the functional diversity of NK cell subsets in health and disease to be used in the design of new therapeutic / diagnostic strategies. (to be coordinated by Paulo Rodrigues-Santos).

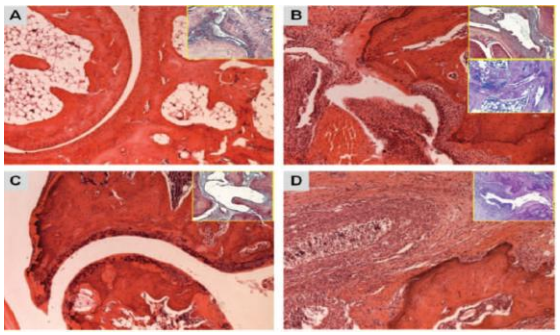

Fig. 1. Histologic assessment of articular tissue shows clearance of the inflammatory infiltrate in anti-CD8 monoclonal antibody–treated K/BxN mice.

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Biomedical Inter-Institutional Research programme

1. Psychiatry Research

Carlos Pato, Michele Pato (University of Southern California.), Maria Helena Azevedo, António Ferreira de Macedo, (HUC, FMUC, CNC)

1.1. Molecular Genetics Studies of Complex Disorders

Our team has over 20 years experience in population studies of schizophrenia (Sz) and Bipolar Disorder (BP) focusing on the identification of susceptibility genes for these disorders through the use of linkage and the more recent state-of-the art association analysis with genome wide association studies (GWAS). For this purpose several populations have been analyzed: a relatively homogenous population from Azores, augmented by a similarly homogenous subsample from Madeira, and a mainland Portuguese population. To date we have collected over 3000 DNA samples, including 700 schizophrenic patients, 500 bipolar patients, and 1400 unaffected family members. Additionally, 350 unaffected (i.e. no history of psychiatric disorder) subjects of Azorean descent have been collected as a control group. The schizophrenic sample includes 100 multiplex (2 or more affected members) families, and the bipolar sample includes 120 multiplex families. This sample is being expanded by Dr Pato at The University of Southern California (USC-Center for Genomic Psychiatry), with a project integrating a US- wide network of academic medical centers that have created the Genomic Psychiatry Cohort (GPC). The aims of this project are to assemble a cohort of 10,000 patients with schizophrenia and 10,000 controls without schizophrenia or a family history of schizophrenia, from 8 sites and in the future, assemble a similar sample of bipolar patients. The collection of the initial 5000 Caucasian patients and 5000 Caucasian controls is now under way to perform a GWA study.

In the GPC as well as in the International Schizophrenia Consortium (ISC) that we have also formed we intend to use whole genome approaches to define the genomics of schizophrenia and later develop similar collaborative efforts for bipolar disorder.

Our studies have utilized the more recent DNA and RNA microarray technology to identify chromosomal regions of linkage to each disorder, genetic association information, as well as areas of differential gene expression in the presence of illness. This convergent genetic-genomic approach has led to the identification of several areas in the human genome that may harbour susceptibility genes for Sz or BP. In Sz, our group identified a region on 5q31–5q35 with a NPL score of 3.28 which was replicated in the BP sample with psychosis. Further study of this region showed positive SNP associations with several GABA receptor subunit genes in patients with SZ. In BP, the identification of a region on 6q22 (NPL-Z=4.2), was also an important finding. In our case-control studies a number of significant associations were reported for several genes: syntaxin 1A; NRG1, GABA receptor subunit genes; Neurogranin; CHRNA7, and DRD2. More recently, as published in *Nature*, our studies with copy number variants (CNVs) led to the identification of 22q11.2, 15q13.2 and 1q21.1 as regions with excess CNVs in Sz.

An exploratory WGA study in the Portuguese Sz probands was carried out on the Affymetrix GeneChip® Mapping 500K Assay. We identified a total of 55 SNPs that showed nominally significant associations with schizophrenia at a threshold of $P < 1 \times 10^{-4}$. Two of these SNPs survived FDR correction (rs6638512 on chromosome X, and rs4907606 on chromosome 13). However, in this study, when considering the region of maximal linkage on Chromosome 5q31-35, only one of the 22 candidate genes, glutamate receptor, ionotropic, AMPA 1 (GRIA1) was found to have multiple SNPS showing significant association at $p<10^{-4}$.

However, the problem of the phenotypic heterogeneity in the area of psychosis still remains to be solved and we have to face the possibility that it could even be increased in samples of the magnitude used in GWAS. It is necessary, in parallel with these large GWAS, to implement nested studies, using clinical covariates that shows high familiality and are potentially under the control of a smaller set of genes, defining more homogeneous sub-samples. One of the areas of expertise of our team is precisely in phenotypic definition, and in this context, we intend to use phenotypic measures potentially more adequate to dissect the underlying pathologic mechanisms.

We are applying for a FCT grant to study a sample of 250 probands with Sz and BP (150 from multiplex families and 100 unrelated cases), in order to allow careful assessment of phenotype and alternative phenotypic measures. Some of the phenotypes that have received greatest attention to date are those relating to psychosis because both population-based studies and molecular genetic studies, either linkage or association studies, show evidence that SZ and BP partly share a common genetic cause. Thus, based on the assumption that we can expect substantial overlaps of genetic susceptibility across diagnostic categories and substantial heterogeneity within diagnostic categories our central objectives will be to investigate some key phenotypic measures/symptom dimensions selected for their heritabilities in order to better characterize the genetic architecture of psychosis and guide the search for susceptibility genes.

1.2. Phenotypic Studies of Complex Disorders

In parallel with the genetic studies of schizophrenia and bipolar disorder, we have developed a range of clinical investigations in areas in which a more clear understanding of the phenotypic definitions and boundaries were needed. These studies have focused in the area of personality, namely studying the perfectionism and the relationship between this trait and some disorders of the obsessive-compulsive spectrum (eating disorders and OCD) and sleep problems. Another important topic under investigation is in the area of affective disorders, specifically perinatal depression, and for this purpose a funded project from the *Fundação para a Ciência e Tecnologia* has been completed. The main objectives of this present project were to (1) to determine the effect of postpartum on mothers sleep, mood and symptoms of depression; (2) to establish the predictive significance of sleep loss in mothers depressive mood after childbirth; (3) to identify personality dimensions (such as Perfectionism) which could predict the severity of depressive symptoms associated with postpartum.

Another of the areas of expertise of our team is in the field of diagnostic methodologies and tools, and in this context several scales have been developed and validated to be used in the above mentioned studies, namely to assess depression in the postpartum and pregnancy periods, which have been neglected until now. This opens the possibility of screening for depression in these periods and consequently, to treat this disorder more precociously.

PUBLICATIONS

Carvalho Bos S, Gomes A, Clemente V, Marques M, Pereira AT, Maia B, Soares MJ, Cabral AS, Macedo A, Gozal, D, Azevedo MH (2009) Sleep and Behavioral and Emotional problems in children: A population-based study. *Sleep Medicine* 10: 66-74.

Carvalho Bos S, Pereira AT, Marques M, Maia B, Soares MJ, Valente J, Gomes A, Macedo A, Azevedo MH (2009) The BDI-II factor structure in pregnancy and postpartum: two or three factors? *European Psychiatry,* 24: 334-340.

International Schizophrenia Consortium[1]. (2009) Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature advance* | doi:10.1038/nature08185

Macedo A, Marques M, Maia B, Soares MJ, Pereira AT, Gomes A, Valente J, Azevedo MH, Bos S (2009) The Portuguese Multidimensional Perfectionism Scale factor structure in pregnant women: is Conditional Acceptance a distinct feature of Socially-Prescribed Perfectionism? *Archives of Women Mental Health* 12(1):43-52.

Maia BR, Soares MJ, Gomes A, Marques M, Pereira AT, Cabral AS, Valente J, Bos S, Pato M, Pocinho F, Azevedo MH & Macedo A (2009) Perfectionism in Obsessive-Compulsive and Eating Disorders. *Revista Brasileira de Psiquiatria*, 31(4): 322-327.

Soares MJ, Macedo A, Bos S, Marques M, Maia B, Pereira AT, Gomes A, Valente J, Pato M, Azevedo MH (2009) Perfectionism and eating attitudes in Portuguese students: A longitudinal study. *European Eating Disorders Review* DOI: 10.1002/erv.926.

2. Neurology Research

Luis Cunha (FMUC, HUC), Catarina Oliveira (CNC, FMUC), Manuela Grazina (CNC, FMUC) Maria do Rosário Almeida (CNC), Maria Helena Ribeiro (FMUC), Inês Baldeiras (FMUC), Isabel Santana (FMUC, HUC)

2.1. Biochemical studies in neurodegenerative disorders

Recent work from our group has shown that oxidative stress is an early event in Alzheimer´s disease (AD) pathology, as patients with mild cognitive impairment (MCI) already present with levels of oxidative damage similar to AD patients, but with a small decrement of antioxidant defenses. We hypothesized that the progression to AD may be related to an inadequate capability of the antioxidant system to counterbalance the oxidative attack.

To test this hypothesis, we conducted a longitudinal study on a well characterized group of MCI patients. Changes in peripheral levels of a broad spectrum of non-enzymatic and enzymatic antioxidants, nitrogen oxidative species and lipid and protein oxidation markers were followed as well as cognitive performance. At baseline, there were no differences in any of the indexes of oxidative damage between stable MCI patients (MCI-St) and patients that progressed to AD (MCI-AD). Intracellular levels of lipid peroxidation markers increased in both groups and this was accompained in MCI-AD, but not in MCI-St patients, by a significant decrease in intracellular antioxidant defenses

[1] autores contribuintes da Faculdade de Medicina de Coimbra: António Macedo, Maria Helena Pinto de Azevedo

(oxidyzed / reduced gluthatione ratio and vitamin E). Among MCI-AD patients, the longitudinal decrease in intracellular vitamin E was associated with the deterioration in cognitive performance. These results suggest that accumulation of oxidative damage may start in pre-symptomatic phases of AD pathology and that progression to AD might be related to deplection of antioxidant defenses.

Cerebrospinal fluid (CSF) biomarker identification in AD has been one of our areas of interest. Recently, research effort has aimed at evaluating the performance of CSF markers in MCI cases. We have studied a group of 98 patients with AD, 34 individuals with MCI and 72 controls (43 fronto-temporal dementia patients and 29 normal controls), where CSF levels of tau protein, amyloid $\beta_{(1-42)}$ protein (Aβ42) and tau protein phosphorylated at threonine-181 (p-tau181) were determined. Apolipoprotein E (ApoE) genotyping was performed in peripheral blood.

Preliminary data from our group shows increased levels of CSF t-tau and p-tau 181 in AD and MCI patients in relation to other types of demented and non-demented patients, while the levels of Aβ42 were only decreased in AD patients. In the group of MCI patients, the presence of at least one ApoE-ε4 allele resulted in lower Aβ42 levels, similar to the AD group. A on-going longitudinal study is aimed at evaluating the utility of these protein markers in identifying MCI cases that latter will progress to AD.

PUBLICATIONS

Baldeiras I, Santana I, Proença MT, Ribeiro MH, Pascoal R, Rodrigues A, Duro D, Oliveira CR (2008) Peripheral oxidative damage in mild cognitive impairment and mild Alzheimer's disease. *JAD* (1):117-28.

Baldeiras I, Santana I, Proença MT, Ribeiro MH, Pascoal R, Rodrigues A, Duro D, Oliveira CR. Oxidative damage and progression to Alzheimer´s disease in patients with mild cognitive impairment. *Submitted*.

2.2 DNA studies in neurodegenerative disorders

Neurodegenerative disorders are complex and the mechanisms underlying the phenotypic expression of this group of diseases are not clearly understood. Finding genetic risk factors, either from nuclear or mitochondrial genome origin, will contribute to identify new tools for early diagnosis, as well as to support the development of more rationale therapies, including the implementation of pharmacogenetic approach.

We have performed the evaluation of mtDNA *ND1* sequence variations in a larger sample of FTD patients, following the evidences of the involvement of MRC complex I in FTD, reported in 2004 (Grazina M, Silva F, Santana I, Santiago B, Oliveira M, Cunha L, Oliveira C. Frontotemporal dementia and mitochondrial DNA transitions. *Neurobiol. Dis.* 2004; 15-2: 306-311). So far, the sequencing of nucleotide regions corresponding to genes coding for remaining ND genes (2, 3, 4, 6, 7) has been initiated. The MRC complexes activity was also evaluated in more 14 FTD patients. We have found 20 sequence variations in 40% of patients, pointing to the involvement of mtDNA and MRC in FTD. The role of mtDNA needs further examination, but our results support mitochondrial cascade hypothesis in FTD etiopathogeny.

Additionally we have continued the genetic characterization of dementias related to 5HTR2A, aiming to perform a pharmacogenomic characterization of the patients. The analysis of the coding exons and the flanking intronic regions of 5HTR2A gene started to be performed ("Genetic Regulation of 5HT2A receptor in Frontotemporal Dementia", (SFRH/BD/45387/2008), The first results (3 PCR reactions and 6 sequencing reactions per sample) allowed the identification of 4 sequence variations previously described on genetic databases, being two intronic and the other two within exonic coding sequences. The analysis of 84 other samples from FTD patients were initiated.

A pharmacogenomic project in Alzheimer's disease was initiated. The CYP2D6 is involved in the oxidative metabolism of many different classes of commonly used drugs including donepezil. The present study is part of a MSc study with the final purpose of evaluating the impact of CYP2D6 genetic background on the plasma and cerebrospinal fluid (CSF) concentrations and the clinical outcome of donepezil, in patients with AD. Accordingly, optimization of PCR reactions for entire CYP2D6 gene amplification is under process and analysis of 100 samples from AD patients will be performed by sequencing of CYP2D6 coding exons and contiguous regions, aiming to identify sequence variations that may influence CYP2D6 enzymatic activity. Additionally, analysis of plasma and CSF donepezil concentrations will be performed in the previously mentioned 100 AD patients. Finally, correlation of genomic and biochemical data will be performed.

The pharmacogenomics approach was extended to other areas. The analysis of the genetic profile and correlation with response to anesthetics started to be performed. Genetic analysis of polymorphisms 118A>G, gene OPRM1, and val150met, gene COMT, has shown allele frequencies of 0,538 and 0,463 for val158 and 158met; and 0,837 and 0,162 for A118 and 118G variants, respectively. This is a preliminary, but original study that observed the frequency variation according to secondary effects. The results are being gathered for publication.

Mutations in the progranulin gene (*GRN*) are an important cause of frontotemporal lobar degeneration (FTLD), the second most common form of early-onset dementia after Alzheimer's disease. Up to 50% of patients with FTLD report a family history of dementia, and in some cases FTLD segregates as an autosomal dominant trait in families. At the

present, two genes have been identified, the *microtubule associated protein tau* (*MAPT*) and the *granulin gene* (*GRN*). Therefore, we have available the molecular diagnosis of FTLD in a routine basis, which involves the mutation search of these two known genes. To date, 63 different pathogenic *GRN,* scattered over the gene, have been reported in 169 unrelated FTLD families. However, sequencing the whole gene has been revealed a very laborous, time consuming and expensive procedure. Additionally, the clinical presentation associated with *GRN* mutations is highly heterogeneous including behavioral variant Frontotemporal Dementia, Progressive Aphasia, Corticobasal degeneration, Alzheimer's disease, Amnestic Mild Cognitive Impairment and Parkinson's disease. Therefore, it seemed important to find a non-invasive and realiable method to identify progranulin mutation carriers without sequencing the entire GRN gene. Based on the notion that all *GRN* mutations share the same pathogenic mechanism, i.e. the loss of 50% functional GRN, suggesting a haploinsufficiency disease mechanism, a study has been outlined to set up the dosage of serum progranulin protein in FTLD patients and their asymptomatic at-risk family members. We also aim to extend the study to other forms of early-onset dementia, such as probable Alzheimer's disease, in order to confirm/validate this procedure as a reliable biomarker.

PUBLICATIONS

Ferreira IL, Nascimento MV, Ribeiro M, Almeida S, Cardoso SM, Grazina M, Pratas J, Santos MJ, Januário C, Oliveira CR, Rego AC. Mitochondrial-dependent apoptosis in Huntington´s disease human cybrids. *Exp Neurol*. (*in press*)

3. Pediatric Research

Luísa Diogo (CHC), Catarina Oliveira (CNC, FMUC), Manuela Grazina (CNC, FMUC)

3.1. Metabolic disorders

Mitochondrial respiratory chain diseases (MRCD) are a diverse group of disorders with a broad spectrum of clinical manifestations, characterised by defects in mitochondrial energetic function. Inherited defects causing mitochondrial dysfunction can be due to mutations either in nuclear DNA (nDNA) or mitochondrial DNA (mtDNA). Each mitochondrion contains its own DNA that codes for 13 peptides of the mitochondrial respiratory chain (MRC) system, where the oxidative phosphorylation (OXPHOS) occurs, plus the two structural rRNAs and 22 tRNAs necessary for mtDNA genes expression. Novel concepts of mitochondrial inheritance, such as mtDNA heteroplasmy, tissue distribution and threshold effect, have explained many of the clinical characteristics. Different gene mutations of mtDNA origin that produce MRC defects have been identified and have been classified as point mutations, large-scale mtDNA deletions, duplications or insertions. Additionally, other mutations affecting nDNA genes (either coding for MRC subunits or assembly/mtDNA stability factors) have also been recently identified; in particular, autosomically inherited disorders have been identified in cases with multiple mtDNA deletions. The major laboratory criteria for the diagnosis of MRCD include: ragged red fibbers (RRF's) on muscle biopsy, lactic acidosis, a specific deficiency in a mitochondrial respiratory enzyme complex and nDNA/mtDNA abnormalities. However, not all MRCD cases display RRF's, biochemical analyses of muscle tissue may show no apparent defects and, in a large proportion of patients with MRC enzyme deficiencies, no mutations have been found. Taking into account these facts, our main objective is to provide tools for the diagnosis of MCRD and a better understanding of the pathogenic mechanisms leading to the clinical phenotypes. This will provide new insight into mitochondrial dysfunctions and will be the basis for more rational therapies for the patients. The precise pathogenic mechanisms by which these biochemical abnormalities induce tissue dysfunction are not clearly understood and diagnosis of these disorders is complex, requiring specialised techniques and correlation between clinical and biochemical/ genetic data.

The implementation of mtDNA copy number/mutation quantification by real time PCR was an important step for patients' diagnostic workup, but also for translational research projects, and represents a major advance for our centre in this area.

A collaborative project was established with Dr. Fernando Scaglia and Prof. Lee-Jun Wong (Baylor College of Medicine, Houston, Texas, USA) for the study of autism patients. We have screened mtDNA copy number, total mtDNA sequence and *POLG1,2* genes. Additionally, we have screened plasma ATP and aminoacid levels as possible biomarkers in 32 autistic patients. The results are being gathered for publication.

We have continued the set up of the evaluation of Pyruvate dehydrogenase and Krebs cycle enzyme activities for diagnostic and research purposes.

PUBLICATIONS

Diogo L, Grazina M, Garcia P, Rebelo O, Alte Veiga M, Cuevas J, Vilarinho L, Tavares Almeida I, Oliveira CR (2009) Pediatric Mitochondrial Respiratory Chain Disorders in the Centro Region of Portugal. *Pediatr Neurol* 40(5):351-6.

Cordeiro, M, Scaglia F, Lopes da Silva S, Garcia P, Grazina M, Moura C, Diogo L (2009) The Brain-Heart Connection in Mitochondrial respiratory Chain Diseases. *The Neuroradiology Journal* 22: 558-563.

4. Dermatology Research

Margarida Gonçalo (HUC), Américo Figueiredo (FMUC, HUC), Teresa Cruz (FFUC, CNC), Rosário Domingues (UA), Pedro Domingues (UA), Celeste Lopes (FFUC, CNC)

4.1. Contact dermatitis

In collaboration with the Dermatology Department of the University Hospital of Coimbra and the Chemistry Department of the University of Aveiro, we are investigating the effect of chemical sensitizers and irritants on the chemokine/cytokine release and on the proteomic profile of skin dendritic cells. We observed that chemical sensitizers selectively modulated the cytokine IL-17 and the chemokines CCL17 and CCL22. In addition, the antioxidant and detoxifying proteins thioredoxin and thioredoxin reductase were also up-regulated by skin sensitizers. Moreover, and by proteomic analysis, we observed that chemical sensitizers selectively modulate proteins involved in the carbohydrate metabolism.

PUBLICATIONS

Francisco V, Neves BM, Cruz MT, Gonçalo M, Figueiredo A, Duarte CB, Lopes MC (2009). Effect of lipopolysaccharide, skin sensitizers and irritants on thioredoxin-1 expression in dendritic cells: relevance of different signalling pathways. *Arch Dermatol Res*., Sep 16 [Epub ahead of print].

Neves BM, Cruz MT, Francisco V, Garcia-Rodriguez C, Silvestre R, Cordeiro-da-Silva A, Dinis AM, Batista MT, Duarte CB, Lopes MC (2009). Differential roles of PI3-Kinase, MAPKs and NF-kappaB on the manipulation of dendritic cell T(h)1/T(h)2 cytokine/chemokine polarizing profile. *Mol Immunol*., 46:2481-92.

5. Arthritis Research

José António. P. da Silva (HUC, FMUC), Fernando Judas (HUC, FMUC), Alexandrina Mendes (FFUC, CNC) Carlos Cavaleiro (FFUC, CEF), Ali Mobasheri (U. Nottingham,U.K.), Margarida Carneiro (CNC), Celeste Lopes (FFUC, CNC); Anabela Mota Pinto (FMUC), Lina Carvalho (HUC, FMUC), João Eurico da Fonseca (IMM, FMUL), Paulo Rodrigues dos Santos (CHC, CNC), Peter Lipsky (NIH)

5.1. Studies on osteoarthritis

In collaboration with the Orthopedic and Bone Bank Departments of HUC, we are using normal and osteoarthritic (OA) human articular cartilage and chondrocytes to 1) establish cryopreservation protocols that improve the clinical outcome of implanted osteochondral allografts; and 2) identify cellular and molecular mechanisms relevant in OA pathogenesis that can be translated into new therapeutic strategies. After identifying arbutin as a new more effective cryoprotective agent, we are investigating its ability to preserve the anabolic functions of cryopreserved human chondrocytes. In the second project, we found that human chondrocytes express functional insulin receptors which are decreased in OA chondrocytes contributing to their resistance to insulin, even in non-diabetic patients. Finally, we identified pinane-derived compounds as NF-kB and NO production inhibitors. Current work is underway to further elucidate their mechanism of action and their potential as disease-modifying osteoarthritis drugs.

5.2. Studies on rheumatoid arthritis

Our common projects explored the roles of CD8+ T cells, and B cells and oxygen radicals in the pathogenesis of rheumatoid arthritis (RA). Affecting about 1% of the world population, rheumatoid arthritis is a severely disabeling autoimmune disease, leading to joint destruction and marked reduction of life-span. With women being 4 times more prone than men to develop the disease, RA also acts as a factor of social and economical exclusion.

Current therapies fail to permanently cure and reverse the disease. Moreover, it is still unkown what triggers the disease and maintains the chronic inflammatory process.

Several studies have presented evidence for the presence of CD8+ T cells in the synovial membrane and synovial fluid of RA patients. However, they did not explore the possible roles of these cells in the pathogenesis of RA. Based on our recent findings in the K/BxN mouse model of chronic arthritis, we believe that CD8+ T cells play a crucial role in maintaining arthritis, both by releasing cytotoxic molecules like Granzime B and recruiting other inflammatory cells into the joint. Currently, using both human samples and mouse models, we are studying the cellular and molecular pathways of CD8+ T cell involvement in RA.

We have recently published that RA patients have a defective homeostasis of memory B cells, and that this homeostasis can be reversed by anti-TNF therapy. On the other hand, we had previously published that defective production of oxygen radicals was directly correlated with lower frequency of circulating memory B cells. Moreover, a recent mouse model of chronic collagen-induced arthritis stressed the importance of a normal oxygen radical production in preventing arthritis severity and chronicity. Therefore, our other project aims at identifying the relationship between oxygen radical production and memory B cell development, and how this can influence disease progression in RA.

PUBLICATIONS

Caetano-Lopes J, Nery AM, Henriques R, Canhão H, Duarte J, Amaral PM, Vale M, Moura RA, Pereira PA, Weinmann P, Abdulghani S, Souto-Carneiro M, Rego P, Monteiro J, Sakagushi S, Queiroz MV, Konttinen YT, Graça L, Vaz MF, Fonseca JE. (2009) Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. *Clin Exp Rheumatol*. 27(3):475-82. PubMed PMID: 19604441.

Neves A, Rosa SC, Gonçalves J, Rufino A, Judas F, Lopes MC, Salgueiro L, Cavaleiro C, Mendes AF. Screening of five essential oils for identification of potential inhibitors of IL-1-induced NF-kB activation and NO production in human chondrocytes: characterization of the inhibitory activity of alpha-pinene. *Planta Med*. doi: 10.1055/s-0029-1186085. (*in press*)

Rosa SC, Gonçalves J, Judas F, Lopes MC, Mendes AF (2009) Assessment of strategies to increase chondrocyte viability in cryopreserved human osteochondral allografts: evaluation of the glycosylated hydroquinone, arbutin. *Osteoarthritis Cartilage*.; 17:1657-61.

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Souto-Carneiro MM, Mahadevan V, Takada K, Fritsch-Stork R, Nanki T, Brown M, Fleisher TA, Wilson M, Goldbach-Mansky R, Lipsky PE. (2009) Alterations in peripheral blood memory B cells in patients with active rheumatoid arthritis are dependent on the action of tumour necrosis factor. *Arthritis Res Ther.* 11(3):R84. PubMed PMID: 19500335

6. Research in brain cancer

Alberto Orfão (CSIC, Univ. Salamanca), Fernando Gomes (HUC), Hermínio Tão (HUC), Olinda Rebelo (HUC), Celeste Lopes (FFUC, CNC) Maria do Rosário Almeida (CNC), Catarina Oliveira (CNC, FMUC)

6.1. Studies on genetic heterogeneity of gliomas

The project entitled "Whole human genome analysis of genetic imbalance and numerical abnormalities by single-nucleotide polymorphism (SNP)-arrays in gliomas: correlation with clinical and biological features of the disease" is being developed in collaboration with Neuropathology Laboratory and Neurosurgery Service of the University Hospital of Coimbra and with Center for Cancer Research of Salamanca. In this project, chromosome aberrations and allelic imbalances in chromosome regions of human gliomas have been evaluated using interphase fluorescence *in situ* hybridization (iFISH). The gene expression profiling is performed using cDNA oligonucleotide micro-arrays, and a full screening of the tumoral cell genome is being done by single-nucleotide polymorphism (SNP)-array analysis. The tissue samples are obtained from patients diagnosed with gliomas undergoing surgery at the University Hospital of Coimbra. Results obtained from iFISH evaluation revealed a complex cytogenetic heterogeneity in this type of tumours, and distinct clonal pathways of glioma evolution were also found. Moreover, distinct gene expression profiles were found between tumors of different histological origin and grade of malignancy. In addition, genome-wide allelotyping is being performed in gliomas and this analysis will facilitate the identification of new genetic/chromosomal changes, relevant for the understanding of the pathogenesis of the disease.

6.2. Predictive and prognostic markers evaluation in Gliomas

Gliomas are the most common primary brain tumors, in which the glioblastoma multiform is the most lethal adult brain tumor, representing 20% of all primary brain neoplasms. However, current diagnostic techniques based on clinical examination, neuro-imaging and neuro-pathology are far from straightforward concerning an accurate diagnosis and consequently they are insufficient to predict the prognosis of individual cases. Therefore, it seems crucial to develop different strategies to help these patients's outcome. Thus, over the past few years, molecular alterations in gliomas have been identified as conferring a predictive value on tumor aggressiveness, tumor response to therapy and patient survival. In this context, a study was outlined to evaluate specific tumor subtype and tumor-grade molecular alterations such as: the 1p/19q loss in oligodendroglial tumors, the somatic mutations in *IDH1* and *IDH2* genes in astrocytomas, oligodendrogliomas and oligoastrocytomas and the hypermethylation of the *MGMT* gene in glioblastomas. To date, we have isolated DNA from paraffin-embedded materials (94 cases) and from frozen tissue whenever available (23 cases). The evaluation of these markers in this sample set will allow us 1) to characterize tumor samples at the genomic level 2) to determine whether the genetic and epigenetic data correlates with the imaging and pathology data and subsequently tumor behaviour 3) to identify robust predictive markers with clinical applicability and 4) to develop, implement and validate a feasible molecular strategy to an accurate diagnosis.

PUBLICATIONS

Scientific journals

Vital AL, Tabernero MD, Crespo I, Rebelo O, Tão H, Gomes F, Lopes MC, Orfao A. Intratumoral patterns of clonal evolution in gliomas. *Neurogenetics*. (*in press*)

Carmo A, Patricio I, Cruz MT, Carvalheiro H, Oliveira CR, Lopes MC (2009) CXCL12/CXCR4 promotes motility and proliferation of glioma cells. *Cancer Biology and Therapy*; 9: 1-10.

Tabernero MD, Maillo A, Gil-Bellosta CJ, Castrillo A, Sousa P, Merino M, Orfao A (2009) Gene expression profiles of meningiomas are associated with tumor cytogenetics and patient outcome. *Brain Pathol.*;19(3): 409-420.

Conference proceedings

Carmo A, Carvalheiro H, Cruz MT, Lopes MC (2009) CXCL12/CXCR4 modulates glioma cell survival and motility. *IUBMB Life,* 61(3): 326.

Carvalheiro H, Carmo A, Crespo I, Lopes MC (2009) Study of glioma cell survival in response to temozolomide treatment. *IUBMB Life* 61(3): 349.

Vital AL, Tabernero MD, Castrillo A, Rebelo O, Tão H, Gomes F, Lopes MC, Órfão A (2009) Analysis of large-scale gene expression profiles in gliomas: association with tumor grade. *EORTC-EANO Conference*. *Trends in Central Nervous System Malignancies.*

7. Yeast nosocomial infections

Cidália Pina-Vaz (FMUP, Hospital S. João), Acácio Gonçalves Rodrigues (FMUP, Hospital S. João), Elizabete Ricardo (FMUP), Teresa Gonçalves (CNC)

Worldwide, in the last two decades, invasive fungal infections in hospitalized patients have increased significantly. According to data obtained from USA and Europe Candida species are, respectively, the 4th and 6th cause of systemic infections related to healthcare, representing 8 to 15% of the hospital-acquired sepsis. Associated to this type of infections are high morbidity and mortality rates. Although *C. albicans* is the most prominent agent of these infections, other species assume particular importance due to the inneficency of the available therapeutic tools. Outbreaks in hospital units are a serious health problem, especially in intensive care units. Facing a possible outbreak in a hospital unit, molecular methods represent a valuable tool to clarify transmission pathways, helping to design prevention and/or therapeutic strategies or, conversely, to exclude the hypothesis of the outbreak occurrence. Restriction endonuclease analysis (REA) has been described in the last decade as a valuable tool for *Candida* strains identification. REA of the mitochondrial DNA (mtDNA) was first applied in the biotechnology industry, being used to characterize yeast strains used in wine fermentation, and lately, it was used to discriminate Candida clinical strains. This ongoing study is currently being undertaken under the leadership of the Faculty of Medicine of the University of Porto and the Hospital de S. João, in Porto. The main objective of this research programme is to type yeast isolates using REA of mtDNA, in order to trace and prevent possible yeast infection outbreaks.

PUBLICATIONS

Ricardo E, Silva AT, Gonçalves T, de Oliveira SC, Granato C, Martins J, Rodrigues AG, Pina-Vaz C. *Candida krusei* reservoir in a neutropenic unit: molecular evidence of a foe? MS submitted

Ricardo E, Costa-de-Oliveira S, Silva AP, Gonçalves T, Pina-Vaz C, Rodrigues AG. (2009) Candidaemia: antifungal susceptibility and molecular typing profiles of concomitant isolates from blood and other biological products. *4th Trends in Medical Mycology (TIMM), Athens, Greece, October*.

Lisboa C, Ricardo E, Azevedo F, Costa AR, Goncalves T, Rodrigues AG, Pina-Vaz C. (2009) Typing of genital Candida isolates from couples using mitochondrial DNA typing. *4th Trends in Medical Mycology (TIMM), Athens, Greece, October*.

Pina-Vaz C, Ricardo E, Silva A, Granato C, Berganti R, Gonçalves T, Rodrigues AG. (2009) Typing analysis of consecutive *C. krusei* isolates among Hemato-oncology patients. *19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Helsinkia, Finlândia, Maio*.

7.1 Oral yeast carriage in type I diabetic children

M Santos-Rosa (FMUC), Ana Luísa Costa (FMUC, Dentistry Department), Alice Mirante (CHC), João Maló de Abreu (FMUC, Dentistry Department) Teresa Gonçalves (FMUC, CNC)

Diabetes is a condition that favors the occurrence of oral yeast infections, usually due to elements of the normal flora of patients. This collaboration, under the leadership of Faculty of Medicine (FMUC), aims to characterize the yeast species of normal and type I diabetic children, together with the yeast load in each individual. A number of factors have been associated with oral carriage of yeasts in diabetic children, such as the type and duration or metabolic control degree. Unfortunately, the exact mechanism by which type I diabetes predisposes to high oral carriage of Candida is multifactorial and not well established. In the ongoing study, the Medical Mycology Yeast Research Group is currently identifying, using molecular biology tools, the yeast isolates obtained from saliva and mucosal specimens from 200 patients. In these specimens it was quantified the yeast load, using a CFU based methodology. It is intended to execute this quantification directly in saliva, using a quantitative real-time PCR methodology.

7.2. HIV-1 Vpr variants in mother-child pairs. Using a yeast model to predict AIDS progression

Graça Rocha (CHC, FMUC), A. Meliço-Silvestre (HUC, FMUC), Teresa Gonçalves (FMUC, CNC)

People newly infected with HIV have widely variable courses. This also includes infants with perinatal acquired HIV-1 infection. The long-term non-progressors, should not need Highly Active Antiretroviral Therapy (HAART), and remain asymptomatic for over 5 years, while in the fast progressors the therapy should immediately be initiated, once the HIV infection is detected. ARV therapy, particularly HAART, results in noxious side effects, especially in children, since their bodies are still developing and they are likely to be exposed to HAART for prolonged periods of time, increasing the vulnerability to colateral complications.

The HIV Vpr protein is determinant to the disease progress, depending on variants of this protein. Its pathogenesis is, among other factors, related to the ability of certain variants to cause mitochondrial dysfunction. The mitochondrial dysfunction was described both in lower and higher eukaryotes. The mutations with higher potential of inducing mitochondrial dysfunction were described as being associated with fast progressors.

This ongoing study in the portuguese population of mother-child pair's showed the mutation R77Q in the protein Vpr is associated with a delayed progression of HIV1 disease. The Vpr variants, from long-term non-progressors and from fast progressors, have now been selected, and cloned. The yeast *Saccharomyces cerevisiae* will be used as a model to express the Vpr variants identified in the target population. The study of the influence of these variants in the mitochondrial dysfunction, independently of therapies, will be interpreted as a signal of cell damage. The correlation between disease progression and mitochondrial impairment will be used as a marker to predict the potential progression of the disease. Until now a total of 80 samples from neonatal infected children and 20 mother-child pairs have been characterized. Recently, this study was extended to the infected adult portuguese population, with the inclusion in the project the Infecciology Department of Hospitais da Universidade de Coimbra. The goal is to study, in the portuguese HIV-1-infected population, the naturally occurring genetic variants of HIV-1 vpr gene and to assess the resulting functional variability, using yeast as a cell model, and its potential impact on disease progression. For these purposes, samples will be obtained from adult patients attending the Serviço de Infecciosas of the Hospitais da Universidade de Coimbra.

PUBLICATIONS

Paulo C, Gonçalves T, Anjos J, Rocha G. (2008) The R77Q Vpr mutation and HIV Disease Progression in Children. *Accepted for presentation at the "25th European Society for Paediatric and Infectious Diseases", 2 - 4 May, Porto, Portugal.*

Paulo C, Rocha G, Anjos J, Meliço-Silvestre A, e Gonçalves T. (2007) Prevalência de mutações na posição 77 da Vpr num grupo de crianças infectadas verticalmente e relação com a progressão da doença. *Accepted for presentation at the Meeting of the European Society for Peadiatric Infectious Diseases, Porto , Maio 2-4.*

8. Novel Techniques for the Diagnosis and treatment of Human Infertility

Teresa Almeida Santos (HUC, FMUC), Ana Paula Sousa (HUC, CNC), Alexandra Amaral (CNC), Renata Tavares (CNC), Marta Baptista (CNC), Raquel Brito (HUC), J. F. Velez de la Calle (Clinique Pasteur, Brest, France), Helena Figueiredo (Gaia Hospital, Portugal), Vasco Almeida (University of Oporto, Portugal), João Ramalho-Santos (CNC, FCTUC)

Infertility is a growing problem, affection about 15% of couples worldwide. A partnership has been established between CNC and the Assisted Reproduction Laboratory of the University Hospitals of Coimbra (HUC) to develop novel assays to monitor human sperm and oocyte quality with the ultimate goal of improving Assisted Reproduction.

For sperm analysis the focus has been on complementing traditional analysis by including new parameters with a higher predictive value in terms of defining proper sperm function. These parameters include sperm viability, sperm mitochondrial activity, and sperm chromatin status, monitored using simple, easy and quick assays that can be implemented clinically with minimal effort. The collaboration has recently been extended to two other Portuguese labs (University of Oporto and Gaia Hospital) and one in France (Clinique Pasteur, Brest) for a multi-center evaluation and validation of procedures. Papers describing a novel methodology to assess sperm chromatin routinely, and how to correctly determine sperm mitochondrial function have been published (below).

In terms of oocyte evaluation novel non-invasive techniques are being pioneered to select the best oocytes (and, ultimately, the best embryos) to be used in Assisted Reproduction.

In addition, the collaboration also involves improving the cryo-banking and subsequent use of ovarian tissue for patients undergoing chemotherapy, as this type of treatment often leads to female infertility.

PUBLICATIONS

Sousa AP, Tavares RS, Velez de la Calle JF, Figueiredo H, Almeida V, Almeida-Santos T, Ramalho-Santos J. (2009) Dual use of Diff-Quik-like stains for the simultaneous evaluation of human sperm morphology and chromatin status. *Hum. Reprod.* 24:28-36.

Amaral A, Ramalho-Santos J. Assessment of mitochondrial potential: Implications for a correct monitoring of human sperm function*. Int. J. Andrology* (*in press*)

I**nternationalization**

Internationalization has been a permanent concern of the CNC strategy. To attain this goal the researchers have been encouraged to establish colaborations and joint projects with laboratories abroad, and to colaborate in the organizaton of international scientific meetings. A third action line of the Internationalization strategy is the Graduate Studies Programme which is described in the next section of this report.

Projects jointly with laboratories abroad

Neuroscience and Disease

Alteration of hippocampal synaptic function and plasticity in models of Alzheimer's disease. Christophe Mulle (CNRS, Univ.Bordeuax2, France); Rodrigo Cunha (CNC, Portugal).

Axonal transport of mitochondria in the triple transgenic mouse model of Alzheimer disease. J. Busciglio (Univ. California, USA); Cláudia MF Pereira (CNC, Portugal).

Characterization of adenosine neuromodulation in the development of hippocampal circuits. Christophe Bernard (CNRS; Univ.Méditerrannée, France); Rodrigo Cunha (CNC, Portugal).

Characterization of the BDNF-induced changes in the proteome of cultured hippocampal neurons. Michael Fountoulakis (Foundation for Biomedical Research of the Academy of Athens, Greece); Carlos Duarte (CNC, Portugal).

Control by ATP P2X receptors of NMDA receptor. Juan Lerma (Neuroscience Inst., Alicante, Spain); Rodrigo Cunha (CNC, Portugal).

Effects of BDNF on the mitochondrial proteome of a mouse model for Huntington's disease. Lisa Ellerby (Buck Institute for Age Research, Novato, CA, USA); Ana Cristina Rego (CNC, Portugal).

Effect of the Contactin/Caspr complex on AMPA receptor-mediated excitatory postsynaptic currents in hippocampal neurons in culture. Christophe Mulle (University of Bordeaux, Bordeaux, France); Ana Luisa Carvalho (CNC, Portugal).

Effect of the Neuropeptide Y (NPY) in rat subventricular zone cell cultures. Coronas V. (Poitiers); João Malva (CNC, Portugal).

Hypoxic preconditioning as a trigger of neurovascular protection in Alzheimer's disease and diabetes: role of HIF signalling pathway and mitochondria. Mark A. Smith (Institute of Pathology, Case Western Reserve University, USA); Paula I. Moreira (CNC, Portugal).

Interaction between A2A and CB1 receptors in striatal nerve terminals. Patrizia Popoli (Institut Sanitá, Rome, Italy); Rodrigo Cunha (CNC, Portugal).

Interaction between cannabinoid CB1 receptor and A2A receptors in the control of striatal glutamatergic transmission. Laurent Venance (Collège de France); Rodrigo Cunha (CNC, Portugal).

Interactions between the purinergic and the endocannabinoid system in pain sensation. László Köles (Semmelweis University, Budapest, Hungary); Rodrigo Cunha (CNC, Portugal).

Localization and function of dopamine D4 receptors – interaction with adenosine A2A receptors. Sergi Ferré (NIDA, NIH, Bethesda, USA); Rodrigo Cunha (CNC, Portugal).

Localization and role of adenosine receptors in amygdalar circuits. Ki Ann Goosens (MIT, Boston, USA); Rodrigo Cunha (CNC, Portugal).

Mapping the metabolic and neuromodulator role of insulin in the hippocampus. Tibor Harkany (Uni. Aberdeen, Scotland); Rodrigo Cunha (CNC, Portugal).

Mechanisms involved i the ability of caffeine to prevent memory impairment. Diogo Souza (UFRGS, Brazil); Rodrigo Cunha (CNC, Portugal).

Mitochondrial Respiration and Respiration Associated Proteins in Cell Lines Created through Parkinson's Subject Mitochondrial Transfer. Russell H Swerdlow (Kansas University, USA); Sandra M. Cardoso (CNC, Portugal).

Modulation of the glutamatergic synapses by BDNF. Clive Bramham (University of Bergen, Norway); Carlos Duarte (CNC, Portugal).

Neural stem cell cultures as a potential source of repairing cells in the pilocarpine mice model of temporal lobe epilepsy. Cavalheiro E. (São Paulo); João Malva (CNC, Portugal).

Neurophysiological role of the dopamine-adenosine systems in attention-deficit hyperactivity disorder: a new therapeutic target for caffeine. Francisco Ciruela (Univ.Barcelona, Spain); Rodrigo Cunha (CNC, Portugal).

Protein cleavage in the ischemic rat brain. Takaomi C. Saido (Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Wako, Saitama); Tadeusz Wieloch (Wallenberg Neuroscience Center, Lund Sweden); Carlos Duarte (CNC, Portugal).

Rab7 rescues the Parkinson's disease related autophagic pathology. Ana Maria Cuervo (Albert Einstein College of Medicine, USA); Sandra M. Cardoso (CNC, Portugal).

Redirection of neuroblast migration from the rostromigratory stream into the ischemic striatum. Saghatelyan A. (Québec City); João Malva (CNC, Portugal).

Regulation of glutamatergic transmission by ghrelin in the hippocampus. José Esteban (Centro de Biologia Molecular Severo Ochoa, Universidad Autonoma de Madrid/CSIC, Madrid, Spain); Ki Ann Goosens (McGovern Institute for Brain Research, MIT, Cambridge, MA, USA); Claudia Racca (Newcastle University, Newcastle); Ana Luisa Carvalho (CNC, Portugal).

Remodelling induced by pro-neurogenic factors. Bragança J. (Faro); João Malva (CNC, Portugal).

Retinal progenitors and Muller cells as source of potential repairing cells in retinal excitotoxicity; AMPAkines are modulators of neurogenesis in SVZ-derived cultures. Mello F. and de Melo RR. (Rio de Janeiro); João Malva (CNC, Portugal).

Role of astrocytic adenosine A2A receptors in the control of neurodegeneration in animal models of Parkinson's disease. Michael Schwarzschild (MGH, Harvard Univ., Boston, USA); Rodrigo Cunha (CNC, Portugal).

Role of calpains in excitotoxic neuronal damage. Ben A. Bahr (University of Connecticut, Storrs, USA); Carlos Duarte (CNC, Portugal).

Role of calpains in neural stem cell migration. Alan F. Horwitz (University of Virginia, Charlottesville, VA, USA); Claudia Cavadas (CNC, Portugal).

Role of calpains in neural stem cell migration: Role of nitric oxide in adult neurogenesis. Patrik Brundin (Lund University, Lund, Sweden); Claudia Cavadas (CNC, Portugal).

Role of cortactin in AMPA receptor traffic. Andras Kapus (The St. Michael's Hospital Research Institute, University of Toronto, Toronto, Ontario, Canada); Ana Luisa Carvalho (CNC, Portugal).

Role of galanin and somatostatin in neural differentiation of subventricular zone (SVZ)-derived cultures; impact of seizure activity in NPY and NPY receptor expression in the hippocampus and SVZ; methamphetamine causes alteration in NPY and NPY receptor expression levels. Woldbye D. (Copenhagen); João Malva (CNC, Portugal).

Role of the JNK/C-Jun pathway on excitotoxic cell death. Michael Courtney (Molecular Signalling Laboratory, Department of Neurobiology, A. I. Virtanen Institute, University of Kuopio, Finland); Armanda Santos (CNC, Portugal).

Role of microglial adenosine A2A receptors in the control of neurodegeneration. Jiang Fan Chen (Boston Univ., USA); Rodrigo Cunha (CNC, Portugal).

Role of NMDAR subunits in endoplasmic reticulum stress induced by ADDLs. William L Klein (Northwestern University, Chicago, USA); Cláudia MF Pereira (CNC, Portugal).

Structure-function analysis of the NMDA receptor domains involved in synaptic delivery under basal conditions and during synaptic plasticity. Ann Marie Craig (Brain Research Centre, University of British Columbia, Vancouver, BC, Canada); Ana Luisa Carvalho (CNC, Portugal).

The neuronal ischemic response through Ca^{2+}-permeable AMPA receptors: genetic expression profile and mechanisms of receptor trafficking. Luís Miguel Martins (Cell Death Regulation Laboratory, MRC Toxicology Unit, Leicester LE1 9HN, UK); Armanda Santos (CNC, Portugal).

The neurogenic niche and brain tumour stem cells; cross-talk between the neural stem cell niche and vascular endothelial cells. Hofman F (Los Angeles); João Malva (CNC, Portugal)

The Neuropeptide Y (NPY) and Dipeptidyl-peptidase IV (DPPIV) as new promising targets on the adipose tissue regulation in obesity. Eric Grouzmann (Division of Clinical Pharmacology and Toxicology, Lausanne University Medical School, Switzerland); Claudia Cavadas (CNC, Portugal).

The pathological interaction between diabetes and Alzheimer's disease: exploring the role of brain endothelial mitochondria and uncoupling proteins. George Perry (College of Sciences, University of Texas at San Antonio, USA); Paula I. Moreira (CNC, Portugal).

The role of growth hormone in proliferation and neural differentiation of hippocampal progenitors. Arce V. and Devesa J. (Santiago de Compostela); João Malva (CNC, Portugal).

The role of OPA1 proteolytic processing in mitochondrial fission/fusion and mitophagy in Alzheimer's disease. Xiongwei Zhu (Institute of Pathology, Case Western Reserve University, USA); Paula I. Moreira (CNC, Portugal).

Toxic pathways triggered by activation of Ca^{2+}-permeable AMPA receptors. Lloyd Greene (Dept. of Pathology, Columbia University Medical Center, New York, USA); Jonhatan Ham (Institute of Child Health, University College of London, London, UK); Armanda Santos (CNC, Portugal).

Molecular Biotechnology and Health

AAV vectors-mediated gene therapy. Sebastian Kugler (Department of Neurology, Faculty of Medicine, University of Göttingen, University of Gottingen, Gottingen, Germany); Luis P. Almeida (CNC, Portugal).

Advancing the field of drug delivery - combined targeted treatments against human breast cancer and human leukemia (The OncotargetNanoMed network). María Jesús Vicent (Centro de Investigación Príncipe Felipe, Medicinal Chemistry Unit, Polymer Therapeutics Laboratory, Valencia, Spain); João Nuno-Moreira (CNC, Portugal).

Alginate Coated Chitosan Nanoparticles as Adjuvant for Mucosal Vaccination With Hepatitis B Antigen. Professor Gerrit Borchard (University of Genève, Switzerlan and Centre Pharmapeptides, Archamps, France); Professor Hans Junginger (Former Professor at Leiden University, Netherlands and visiting Professor at Naresuan University, Phitsanulok, Thailand); Olga Borges (CNC, Portugal).

Analysis of the structure of metabolic networks. George Stephanopoulos (M.I.T., U.S.A.); Armindo Salvador (CNC, Portugal).

Antimicrobial coatings. Andreas Zumbuehl (Department of Organic Chemistry, University of Geneva, Switzerland); Lino Ferreira (CNC, Portugal).

Application of non-viral suicide gene therapy approaches in animal models for cancer and mechanisms associated with the antitumor response. Valérie Pierrefite-Carle (Unity INSERM, Faculty of Medicine, Nice, France); Conceição P. Lima (CNC, Portugal).

Bioinformatics. Professor Werner Dubitzky (University of Ulster, UK); Doctor Christian Borgelt (European Centre for Soft Computing, Mieres, Spain); Professor Alfonso Tarancon Lafita (University of Zaragoza, Spain); Cândida Silva, Rui Brito (CNC, Portugal).

Cell internalization mechanisms of anti-HIV peptides. Abraham Loyter (Department of Biological Chemistry, Institute of Life Sciences, Hebrew University of Jerusalem, Israel); Conceição P. Lima (CNC, Portugal).

Controlling the differentiation of stem cells by bioactive molecules released from biocompatible micro- and nanotechnologies. Tariq Enver (Weatherall Institute of Molecular Medicine – University of Oxford, UK); Lino Ferreira (CNC, Portugal).

Design principles of biochemical circuits, mathematical methods for systems analysis of biochemical networks. Michael Savageau (U.C. Davis, U.S.A.); Armindo Salvador (CNC, Portugal).

Development of Chitosan-Based Nanoparticles for Nasal Immunization Against Hepatitis B. Professor Gerrit Borchard (University of Genève, Switzerlan and Centre Pharmapeptides, Archamps, France); Olga Borges (CNC, Portugal)

Development of lipid-based nucleic acid delivery systems for application in gene therapy. Nejat Duzgunes (University of the Pacific, San Francisco, USA); Conceição P. Lima (CNC, Portugal).

Development of non-viral vectors for siRNA delivery to the central nervous system. Ernst Wagner (Department of Pharmacy, University of Munich, Germany); Conceição P. Lima (CNC, Portugal).

Development of three-dimensional matrices for differentiation and transplantation of human stem cells for regenerative medicine. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA); Lino Ferreira (CNC, Portugal).

Dissecting the pathogenesis of Machado-Joseph disease. Henry Paulson (Veteran's Hospital, Ann Harbor, USA); Luis P. Almeida (CNC, Portugal).

Encapsulation of viral vestors into targeted nanolipid-based carriers: evaluation of therapeutic activity in animal models of ischemia. Mauro Giacca (Laboratory of Molecular Medicine, ICGEB - International Centre for Genetic Engineering and Biotechnology, Trieste, Italy); Sergio Simões (CNC, Portugal).

Energetic constraints on gene expression in S. cerevisiae, methods and software for kinetic modeling, factors shaping proteins' aminoacid usage. Rui Alves, Albert Sorribas, Ester Villaprinyó (University of Lleida, Spain); Armindo Salvador (CNC, Portugal).

Gecko-inspired tissue adhesive. Robert Langer (Department of Chemical Engineering, Massachusetts Institute of Technology, MIT, EUA), Jeffrey Karp (Harvard-MIT Division of Health Science and Technology, USA); Lino Ferreira (CNC, Portugal).

Lentiviral vectors-mediated ataxin-3 gene silencing. Nicole Déglon & Philipe Hantraye (Service Hospitalier Frederic Joliot, MIRCen Program, Departement de Recherches Medicales, Direction des Sciences du Vivant, Commissariat a l'Energie Atomique (CEA), Orsay, France); Luis P. Almeida (CNC, Portugal).

Lipoplex- and cell penetrating peptide-based delivery of steric-block oligonucleotides and application in splice correction. Bernard Lebleu (University of Montpellier, Montpellier, France); Conceição P. Lima (CNC, Portugal).

Microcalorimetry. Doctor Adrian Velasquez Campoy (University of Zaragoza, Spain); Rui Brito (CNC, Portugal).

Models of Machado-Joseph disease. Veronica Colomer, John Hopkins (School of Medicine, Baltimore, USA); Luis P. Almeida (CNC, Portugal).

Nanomaterials for cell tracking. John Martin (Centre for Cardiovascular Biology and Medicine, University College of London, UK); Lino Ferreira (CNC, Portugal).

Nuclear Magnetic Resonance. Doctor Christina Redfield (Oxford University, UK) Daniela Vaz (CNC, Portugal).

Plant proteases for food industry. Dra. Nora Priolo, Sandra Vairo (LIPROVE, Universidade de La Plata, Argentina); Carlos Faro (CNC, Portugal).

Pollen Proteases and Allergy. Dr. Sónia Barbensis and Cristina Barcia (Universidade San Luís, Argentina); Carlos Faro (CNC, Portugal).

Profiling of the metabolism of proliferating cells. Craig Thompson (University of Pennsylvania, U.S.A.); Armindo Salvador (CNC, Portugal).

Silencing Machado-Joseph disease e Autophagy in Machado-Joseph disease. Arnulf Koeppen (University of Michigan, Albany, USA); Conceição P. Lima (CNC, Portugal).

Ultrastructural and biophysical studies of the interaction of cell penetrating peptides with cellular membranes. Margus Pooga, (Institute of Molecular and Cell Biology, University of Tartu, Tartu, Estonia); Conceição P. Lima (CNC, Portugal).

Virtual screening and docking. Doctor Richard Jackson (University of Leeds, UK); Carlos J. V. Simões (CNC, Portugal).

Cell and Molecular Toxicology

A biophysical approach to the role of lipids in hepatic mitochondrial toxicity. Teresa Pinheiro (Department of Biological Sciences, University of Warwick, UK); Catherine Brenner (University of Versailles/St Quentin, France); Mª Amália Jurado, PJ Oliveira (CNC, Portugal).

Anticancer Effects of of Phytochemicals. Jon Holy (University of Minnesota, Duluth, USA); PJ Oliveira (CNC, Portugal).

Apoptosis Signaling as a Therapeutic Target in Melanoma. Faustino Mollinedo (Universidad de Salamanca-CSIC, Spain); PJ Oliveira (CNC, Portugal).

Cancer Stem Cell Responses to DNA Damage. Edward Perkins (Mercer University School of Medicine, Savannah, USA); PJ Oliveira (CNC, Portugal).

Development of microsensors for nitric oxide measurement in tissues. Greg Gerhardt (Dept. Anatomy and Neurobiology, and Center for Microlelectrode Technology (CenMet) University of Kentucky, Lexington, Kentucky, USA); João Laranjinha (CNC, Portugal).

DNA in lipoplexes: bilayer properties and adsorption factors. Rita Dias and Tommy Nylander (Department of Physical Chemistry 1, Lund University, Sweden); Mª Amália Jurado (CNC, Portugal).

Doxorubicin-induced Mitochondrionopathy. Kendall B. Wallace (University of Minnesota, Duluth, USA); PJ Oliveira (CNC, Portugal).

Effects of Caffeine Consumption in the Neurochemical Profile of the Hippocampus of Streptozotocin-induced Diabetic Rats. Rolf Gruetter (EPFL, Lausanne); RA Carvalho (CNC, Portugal).

Influence of Cardioplegic Solution Composition on the Metabolic Profile of the Reperfused Working Heart. Gary Lopaschuk (Mazankowski Alberta Heart Institute, University of Alberta, Canada); RA Carvalho (CNC, Portugal).

Interplay Between Sirtuins and Nitric Oxide: PGC-1alpha as a Common Mediator for Mitochondrial Biogenesis and Hyperglycemic Memory. David A. Sinclair (Harvard Medical School, USA); Kendall B. Wallace (University of Minnesota, Duluth, USA); CM Palmeira, AP Rolo (CNC, Portugal).

Mesenchimal Stem Cells as Anti-Cancer Weapons. Teresa Rose-Hellekant (University of Minnesota, Duluth, USA); VA Sardao (CNC, Portugal).

Mitochondrial Dynamics and Metabolic Diseases. Luca Scorrano (University of Padua, Italy); AP Rolo, CM Palmeira (CNC, Portugal).

Mitochondrial Involvement in Neural Stem Cell Differentiation: Role of Morpho-functional Alterations and Relevance for Pos-Transplant Neuronal Death. Ernest Arenas (Karolinska Institute, Sweden); PJ Oliveira (CNC, Portugal).

Mitochondrial Tolerance and Liver Ischemic Preconditioning: Pathophysiological Mechanisms. Joan Rosseló (CSIC, Barcelona, Spain); AP Rolo, CM Palmeira (CNC, Portugal).

New biological functions for wine polyphenols: Cellular regulation and anti-inflammatory actions via nitric oxide production from nitrite. Rafael Radi, Homero Rubbo (Facultad de Medicina, Universidad de la República, Montevideo, Uruguay); Jon O. Lundberg (Department of Physiology and Pharmacology, Karolinska Institutet, Sweden); João Laranjinha (CNC, Portugal).

Nitric oxide in neurodegeneration and aging. Enrique Cadenas (Dept. Pharmaceutical Sciences, University of Southern California, USA); João Laranjinha (CNC, Portugal).

Polyphenols and vascular cells redox signaling. Anne Nègre-Salvayre (INSERM-U, Institut Louis Bugnard CHU Rangueil, Toulouse, France); João Laranjinha (CNC Portugal).

The Effect of Ubiquitous Silver and Gold Nanoparticles: Evaluation of Mitochondrial Toxicity. Saber Hussain (Wright State University School of Medicine, Dayton, USA); AP Rolo, CM Palmeira (CNC, Portugal).

Microbiology

Cloning, expression and regulation of genes for the synthesis of compatible solutes in Thermus thermophilus. José Berenguer (Universidad Autónoma de Madrid, Spain); Milton Costa (CNC, Portugal)

Combined effect of anti-fungal cell wall inhibitors in Alternaria infectoria. Neil A. R. Gow (Institute of Medical Sciences of the University of Aberdeen, UK); Teresa Gonçalves (CNC, Portugal).

Extremophilic enzymes. Garo Antranikian (Institute of Technical Microbiology, Hamburg University of Technology, Hamburg, Germany); Milton Costa (CNC, Portugal)

Gamma radiation-resistant bacteria: taxonomy, diversity and physiology. Fred Rainey (Louisiana State University, Baton Rouge LA, USA); Milton Costa (CNC, Portugal).

Legionella genetics and modulation of host cell biology. Yousek Abu Kawaik (Department of Microbiology and Immunology, University of Louisville Medical Center, Louisville, USA); Joana Costa (CNC, Portugal)

Mediterranean deep-sea brines biodiversity. Michail M. Yakimov (Consiglio Nazionale delle Ricerche - Istituto per l'Ambiente Marino Costiero (CNR-IAMC), Messina, Sicilia, Italy); Milton Costa (CNC, Portugal)

Biophysics and Biomedical NMR

Characterization of Ga-based chelates as tracers for gamma and PET imaging. Frank Roesch (Institute of Nuclear Chemistry, Johannes Gutenberg Universitaet, Mainz, Germany); Carlos Geraldes (CNC, Portugal).

Characterization of Ga-based chelates for imaging. Imre Tóth (University of Debrecen, Hungary); Carlos Geraldes (CNC, Portugal).

Characterization of Gd-based MRI Contrast Agents. A.D. Sherry (U.T. Southwestern Medical Center, Advanced Imaging Center, Dallas, TX); Carlos Geraldes (CNC, Portugal).

Chemical and in vivo animal characterization of MRI contrast agents for Alzheimer's disease. Eva Tóth (Centre de Buiophysique Moléculaire, CNRS, University of Orleans, France); Carlos Geraldes (CNC, Portugal).

Effect of Transaldolase Enzyme Pathway on Gluconeogenesis in People with Prediabetes. Rizza, Rita Basu (Mayo Clinic); John Jones (CNC, Portugal).

EU Network of Excellence (NoE) "European Molecular Imaging Laboratory" (EMIL) (LSHC –2004-503569). Bernard Tavitian, CEA, Orsay, Paris); Carlos Geraldes (CNC, Portugal).

Functionalized Iron oxide and silica nanoparticles as targeted MRI contrast Agents. Robert Muller (University of Mons-Hainaut, Belgium); Carlos Geraldes (CNC, Portugal).

Functionalized liposomes and nanoparticles as responsive multimodal molecular imaging agents for image guided therpy (Teranostics). Silvio Aime and Enzo Terreno (Center of Molecular Imaging, University of Torino, Italy); Carlos Geraldes (CNC, Portugal).

Interaction of lanthanide ions with polyelectrolytes. K. Kogej (Universidade de Ljubljana, Eslovénia); Carlos Geraldes (CNC, Portugal).

Lanthanide binding tags for NMR of proteins: exploiting paramagnetic shifts and residual dipolar couplings. Claudio Luchinat (CERM, Universidade de Florença, Itália); Carlos Geraldes (CNC, Portugal).

NMR and relaxometry of Gd-based complexes and nanoparticles as MRI contrast agents. Joop Peters (Technical University Delft, Netherlands); Carlos Geraldes (CNC, Portugal).

NMR and relaxometric characterization Gd-based MRI Contrast Agents. Ivan Lukes (Charles University of Prague, Chech Republic); Carlos Geraldes (CNC, Portugal).

Non-invasive NMR studies of organ function with stable isotope tracers and contrast agents. Sebastian Cerdán and Pilar lopez-Larrubia (Laboratorio de RMN, Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC, Universidade Autónoma de Madrid, Espanha); Carlos Geraldes (CNC, Portugal).

Hepatic leptin action and leptin resistance in obesity. Robert O´Doherty (University of Pittsburgh School of Medicine); John Jones (CNC, Portugal).

Relaxometric characterization of potential MRI contrast agents. Lothar Helm (EPFL, Lausanne, Switzerland); Carlos Geraldes (CNC, Portugal).

Cell and Development Biology

A systematic functional analysis for Rab proteins in phagocytosis and phagosomal maturation of *Mycobacterium tuberculosis*. Prof. Marino Zerial (Director of the Max-Planck Institute for Molecular and Cell Biology, Dresden, Germany); Victor Hsu (Associate Professor of Medicine, Harvard Medical School, Boston, MA); Heinz Remold (Senior Immunologist and Professor of Medicine, Harvard Medical School, Boston, MA). Mª Otilia Vieira (CNC, Portugal).

Assessment of genetic heterogeneity in gliomas: impact on the clinical and biological behaviour of the disease. Alberto Orfão (Center for Cancer Investigation, University of Salamanca, Spain); Mª Celeste Lopes (CNC, Portugal)

CD38 and immune regulation. Fran Lund (Rochester University); Mª Celeste Lopes (CNC, Portugal).

CD38 and immune responses against *Mycobacterium tuberculosis*. Andrea Cooper (Trudeau Institute, Saranac Lake, USA); Mª Celeste Lopes (CNC, Portugal).

Characterization of a new mucosatropic HPV type: HPV 108. Ethel de Villiers (DKFZ, Heidelberg, Germany); Mª Celeste Lopes (CNC, Portugal).

Cross talk between the adipocyte and the heart. Prof Gary Lopaschuk (University of Alberta, Canada); Eugenia Carvalho (CNC, Portugal).

Glucose Uptake into Cardiomyocytes. Dr. Dale Abel (University of Utah School of Medicine, USA); Eugenia Carvalho

Immunosuppressors and insulin resistance. Dr. Jan Eriksson (University of Gothenburg, Sweden);Eugenia Carvalho (CNC, Portugal).

Implications of Claspin mutations in DNA replication, cell cycle checkpoints and oncogenesis. Raimundo Freire (University Hospital of Canarias, Tenerife, Spain); Mª Celeste Lopes (CNC, Portugal).

IMMUNOX Network: research network to study on the role of reactive oxygen species in regulating the immune response in arthritis. Rikard Holmdahl (Karolinska Institute, Sweden); Riitta Lahesmaa (Turku Center for Biomedical Research, University of Turku, Finland); Stephen Kilfeather (Aeirtec Ltd, UK); Margarida Carneiro (CNC, Portugal).

LDL charge and lipid droplets formation: implications for atherosclerosis. Paul Verkade (University of Bristol, United Kingdom); Mª Otilia Vieira (CNC, Portugal).

Metabolic activity and viability of chondrocytes in cryopreserved human osteochondral allografts. Ali Mobasheri (School of Veterinary Science and Medicine, University of Nottingham, England) Mª Celeste Lopes (CNC, Portugal).

Mitochondria and metabolism in pluripotent embryonic and induced stem cells, Gerald Schatten (University of Pittsburgh, USA); Christopher Navara (University of Texas San Antonio, USA); Miguel Ramalho-Santos (University of California, San Francisco, USA); João Ramalho-Santos (CNC, Portugal).

Study of the cytokine release profile, by protein arrays, of dendritic cells. Carmen García-Rodriguez (Institute of Biology and Molecular Genetic. CSIC-University of Valladolid, Spain); Mª Celeste Lopes (CNC, Portugal).

Testicular organization and xenotransplanting of testicular tissue in cats. Stefan Schlatt (University of Muenster, Germany); João Ramalho-Santos (CNC, Portugal).

The role of neuropeptides in wound healing. Dr. Aris Veves (Harvard Medical School, USA)“ Eugenia Carvalho (CNC, Portugal).

The role of protein tyrosine phosphatase 1B in inflammation. Dr. Janice Zabolotny (Harvard Medical School, USA); Eugenia Carvalho (CNC, Portugal).

Participation in the organization of scientific meetings

January 2009

"Microbiology" Doctoral Programme in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Date: 5-9 January, Coimbra

CNC members involved in the organization: Milton Costa, Nuno Empadinhas

"Cell Death" Course run within the context of the Ph.D. Programme in Experimental Biology and Biomedicine, Center for Neurosciences and Cell Biology, University of Coimbra

Date: 26-30 January, Coimbra

CNC members involved in the organization: Carlos Duarte

"Protein interactions - possible therapeutic targets for neurodegenerative diseases", *Michael J. Courtney* - Seminar run within the context of the Seminars Programme of the Center for Neurosciences and Cell Biology, University of Coimbra

Date: 30 January, Coimbra

CNC members involved in the organization: Carlos Duarte

February 2009

Principles and practice in drug development, Advanced course for the MIT-PT and BEB Ph. D. programmes; CNC, University of Coimbra.

Date: 2-13 February, Coimbra

CNC members involved in the organization: João Nuno Moreira

"Neurodegenerative disorders" - Advanced Course for the PhD Programme in Experimental Biology and Biomedicine, Center for Neuroscience and Cell Biology, Coimbra, Portugal.

Date: 16-20 February, Coimbra

CNC members involved in the organization: Ana Cristina Rego, Paula Agostinho, Cláudia Pereira, Luís Pereira de Almeida

"Stem cell replacement therapy for Parkinson's disease and regeneration", *Prof. Ernest Arenas* Seminário no âmbito do Programa Doutoral em Biologia Experimental e Biomedicina (PDBEB) organizado pelo Centro de Neurociências e Biologia Celular (CNC) da Universidade de Coimbra.

Date: 20 February, CNC, Coimbra

CNC members involved in the organization: Doutores Ana Cristina Rego, Cláudia Pereira, Luís Pereira de Almeida e Paula Agostinho

March 2009

"IdeaSpring – bioinnovation teams", (MIT - Portugal programme)

Date: 2 March, Biocant, Cantanhede

CNC members involved in the organization: João Nuno Moreira

"Biomedical Imaging and Metabolism" - CNC BEB Program Optional Course

Dates: 3-6 March, Coimbra

CNC members involved in the organization: John G. Jones & Rui A. Carvalho

"Fuelling of Obesity and Type 2 Diabetes" - CNC BEB Program Optional Course

Dates: 9-13 March, Coimbra

CNC members involved in the organization: John G. Jones, Cristina Barosa, Madalena Caldeira, Ivana Jarak, Patrícia Nunes, Daniela Ribeiro, Pedro Coxito, Eugenia Carvalho

"Oncobiology Course" Advanced course on Oncobiology (PhD programme on Biomedicine and Experimental Biology, University of Coimbra, Portugal)

Date: 17–20 March, Coimbra

CNC members involved in the organization: Anália do Carmo, João Nuno-Moreira

"Purines and related substances in brain research." - Organization of the symposium entitled at the 29th European Winter Conference of Brain Research

Dates: March 2009, Les Arcs, France

CNC members involved in the organization: Rodrigo Cunha

May 2009

"V Encontro Luso-Brasileiro de RMN- III Encontro Ibero Americano de RMN" - AUREMN, Brasil

Dates: 4-8 May, Angra dos Reis, Brasil

CNC members involved in the organization: Carlos F.G.C. Geraldes

"Mitochondria: between life and death" . International courses of Toxicology at Center for Neurosciences and Cell Biology.

Dates: 5-8 May, Coimbra,

CNC members involved in the organization: Paulo Oliveira, Vilma Sardão, Leonor Almeida, Anabela Rolo e Carlos Palmeira, João Laranjinha

"Integrated Approaches for the Study of Mitochondrial Dynamics", Organization of the Practical Course at the Center for Neurosciences and Cell Biology

Dates: 11-15 May, Coimbra

CNC members involved in the organization: Paulo Oliveira, Vilma Sardão, Carlos Palmeira, Anabela Rolo, João Laranjinha, Leonor Almeida, Ana Ledo

"2nd Workshop on "Bioinformatics' Challenges to Computer Science", ICCS-2009: International Conference on Computational Science, Baton Rouge, Louisiana

Dates: 25-27 May, Louisiana

CNC members involved in the organization: Rui Brito

June 2009

"Challenges in Cell and Gene Therapies" MIT-Portugal Program, CNC, Biocant

Dates: 02-06-09, Biocant

CNC members involved in the organization: Helena Vazão, Dora Pedroso, Cristiana Paulo, Renata Gomes, Maria Pereira.

"2nd ESF/UB European Summer School in Nanomedicine". Quinta da Marinha, Cascais, Portugal,

Dates: 12–16 June, Cascais

CNC members involved in the organization:

"FEMS 2009, 3rd Congress of European Microbiologists", organized by the Federation of European Microbiological Societies.

Dates: 28 June - 2 July, Goteborg, Sweden

CNC members involved in the organization: Milton Costa

July 2009

"10th FIGIPAS Meeting in Inorganic Chemistry", Sociedade Química Italiana - Palermo, Italy,

Dates: 1-5 July, Palermo, Italy

CNC members involved in the organization: Carlos F.G.C. Geraldes

"Metabolic Aspects of Chronic Brain Diseases" - 2009 PENS Summer School, Reisensburg Castle, Günzburg, and Ulm University, Ulm, Germany

Dates: 9-15 July, Germany

CNC members involved in the organization: Ana Cristina Rego

October 2009

"Short Course of the Portuguese Society of Biophysics on Systems Biology" - Portuguese Society of Biophysics

Dates: 30 October – 1 November, Santarém

CNC members involved in the organization: Armindo Salvador

"International Workshop on Practical Applications of Computational Biology & Bioinformatics" IWPACBB 2009 – Salamanca, Spain

Dates: 9-12 October, Spain

CNC members involved in the organization: Rui Brito

"8.ª Conferência de Química Inorgânica da SPQ" - Sociedade Portuguesa de Química

Dates:16-17 October 2009, Curia, Portugal.

CNC members involved in the organization: Carlos F.G.C. Geraldes

November 2009

"Jornadas de Bioinformática" - IGC

Dates: 3-6 de November, Lisboa

CNC members involved in the organization: Armindo Salvador

"Talks in Free Radical Biology" - Center for Neurosciences and Cell Biology - *Rafael Radi and Silvina Bartesaghi* (Departamento de Bioquímica and Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay).

Dates: 6 November, Coimbra

CNC members involved in the organization: João Laranjinha and Bárbara Rocha.

"Reunião de Outono do GEIR (Grupo de Estudo da Insulino-resistência) 2009" - Grupo de Estudo da Insulino-resistência

Dates: 19 November, Coimbra

CNC members involved in the organization: John G. Jones, Cristina Barosa, Madalena Caldeira, Ivana Jarak, Patricia Nunes, Daniela Ribeiro, Pedro Coxito.

"MicroBiotech09", organized by The Portuguese Society of Microbiology and The Portuguese Society of Biotechnology.

Dates: 28-30 November, Vilamoura, Algarve

CNC members involved in the organization: Milton Costa

December 2009

"Microbiology" - Doctoral Programme in Experimental Biology and Biomedicine, organized by the Center for Neuroscience and Cell Biology, University of Coimbra, Portugal

Dates: 7-11 December, Coimbra

CNC members involved in the organization: Milton Costa, Nuno Empadinhas

"TT viruses – the still elusive human pathogens", *Ethel-Michele de Villiers* - Organization of the Conference at CNC; from the German Cancer Research Center, Heidelberg, Germany.

Date: 10 December, Coimbra

CNC members involved in the organization: Conceição P. Lima

Graduate Studies Programme

During 2009, CNC organized 24 Advanced Courses and hosted 35 seminars. The seminars were attended by local graduate students and researchers, whereas the advanced courses also met the interest of people from other Portuguese Universities. Besides the organization of courses and seminars, CNC also supported the ongoing research work for Ph.D. and M.Sc. thesis. Throughout this year, 14 Ph.D. and 42 M.Sc. thesis were concluded.

In October 2002 CNC, with the financial support of FCT, launched an International Doctoral Programme in Experimental Biology and Biomedicine to provide advanced, multidisciplinary, research-oriented training in emerging areas of modern Biology and Biomedicine. The programme included advanced courses in top research areas, taught by forein scientists in collaboration with local investigators, laboratory rotations and research work o be carried out within international networks organized by CNC. The programme provided fellowships to 12 students.

Advanced Courses 2009

January 2009

Microbiology
January 5 - 9
Nuno Empadinhas, Milton Costa

Immunobiology
January 12 - 16
Celeste Lopes

Neuroscience
January 19 - 22
Robrigo Cunha

Basic Neuroscience
January 19 - 23
BEB and MIT-Portugal PhD programmes

Cell Death
January 26 - 30
Armanda Santos

February 2009

Principles and Pratice in Drug Development
February 2 - 13
João Nuno Moreira, Conceição Pedroso de Lima, Sérgio Simões, Luís Almeida

Visual Neuroscience: From photons to perception
February 9 - 13
Miguel Castelo-Branco, Francisco Ambrósio

Neurodegenerative Disorders
February 16 - 20
Ana Cristina Rego, Paula Agostinho, Cláudia Pereira, Luís Pereira de Almeida

RNA Biology
February 23 - 24
Manuel Santos

Vascular Physiology
February 25 - 27
João Laranjinha, Giovanni Mann, Paul Frazer

March 2009

Biomedical Imaging and Metabolism
March 3 - 6
Carlos Geraldes, Margarida Castro, Rui Carvalho, John Jones

Obesity & Type II Diabetes
March 9 - 13
John Jones and Eugénia Carvalho

Oncobiology
March 17 - 20
Anália Carmo, João Nuno Moreira

Advanced course in Neurogenesis
March 23 – 27
João Malva, Fabienne Agasse

September 2009

Biostatistics
September 29 - October 2
Chris Palmer

October 2009

Cell Biology
October 6 - 9
Edgar Gomes

Molecular Biotechnology
October 12 - 23
Paula Veríssimo, Sandra Ribeiro Sukalyan Chaterjee

Vascular Biology
October 26 - 30
Joao Laranjinha, Ana Ledo

November 2009

CNC Cores
November 3 - 5
Isabel Nunes, Luisa Cortes, Bruno Manadas; John Jones, Carlos Geraldes

Lab Rotations
November 9 – 20

Mitochondrial Bioenergetics
November 23 - 27
Paulo J. Oliveira

Systems Biology
November 30 - December 4
Armindo Salvador

December 2009

Microbiology
December 7 - 11
Milton Costa, Nuno Empadinhas

Science Communication
December 15 - 18
Sofia Araújo, João Ramalho-Santos

Seminars

2009 Series | CNC Auditorium 16:00 h

January

20.1.2009

Disentangling molecular interaction networks for Chorea Huntington
M. E. Futschik | *Centre for Molecular and Structural Biomedicine, University of Algarve, Campus of Gambelas Faro, Portugal*

30.1.2009

Protein interactions - possible therapeutic targets for neurodegenerative diseases
Michael Courtney | *Molecular Signalling Laboratory, Department of Neurobiology, A.I. Virtanen Institute, University of Kuopio, FINLAND*

February

13.2.2009

Multisensory perception and the interaction between auditory and visual processes

Beatrice de Gelder | *Cognitive and Affective Neurosciences Laboratory, Department of Psychology, Tilburg University, The Netherlands*

20.2.2009

Stem cell replacement therapy for Parkinson s disease and regeneration

Stem cell replacement therapy for Parkinson s disease and regeneration - Farmacology room, 2nd floor, FMUC, 16:00
Ernest Arenas | *Stem Cell Neurobiology Unit, Department of Medical Biochemistry and Biophysics Karolinska Institutet, Stockholm, Sweden*

27.2.2009

Role of the transcription factor Cited2 in cellular functions and embryonic development
José Bragança | *Animal Cell Technology Laboratory, ITQB-UNL/IBET, Oeiras*

March

6.3.2009

Brain tumour diagnosis, prognosis and treatment selection by combined use of in vivo molecular imaging and in vivo and ex vivo metabolic average profiles and genomic data. FP6 eTUMOUR project
Bernardo Celda | *Department of Chemistry, Universitat de Valencia, Spain*

12.3.2009

Molecular Mechanism of Insulin Resistance in Obesity and Diabetes
Young Bum-Kim | *Dept Endocrinology, Harvard Medical School, Boston*

20.3.2009

Role of EGFR and HER2 in breast cancer
Fernando Schmitt | *Medicine Faculty of the Porto University - IPATIMUP - Porto - Portugal*

25.3.2009

Real-time imaging of gene expression in living cells
José Rino | *BioImaging Unit - IMM - Int. Medicina Molecular - Lisbon, Portugal*

27.3.2009

The role of immune signals on adult neurogenesis
Fernando Pitossi | *Fundación Instituto Leloir - Buenos Aires, Argentina*

April

3.4.2009

The retinal toxicity of an antiepileptic drug blocking the GABA-transaminase: GABA excitotoxicity or taurine deficiency?
Serge Picaud | *Institut de la Vision – INSERM - Université Pierre et Marie Curie - Paris*

17.4.2009

Oral delivery of therapeutic proteins: How far we are?
Bruno Sarmento | *Faculdade Farmácia - Universidade do Porto*

24.4.2009

The SNARE-complex in fast neurotransmitter release
Jakob B. Sorensen
Dept. Neuroscience and Pharmacology Faculty of Health Sciences - University of Copenhagen - Denmark

May

8.5.2009

Mitochondrial thioredoxin and glutathione systems
Dean Jones | *Department Medicine - Emory University, Atlanta, USA*

8.5.2009

The pregnancy environment - the foundation of health
Mark Nijland | *Department of Obstretics and Ginecology - University of Texas - Health Science Center - San Antonio, Texas - USA*

15.5.2009

Thinking outside the box in diabetes
Maria Paula Macedo - *Departamento de Fisiologia - Universidade Nova de Lisboa*

15.5.2009

Endocannabinoid signaling as an ancient and widespread feed-back signal
István Katona | *Institute of Experimental Medicine, Budapest, Hungary*

18.5.2009

Mn porphyrins suppress oxidative stress injuries through redox-based pathways
Inês Batinic-Haberle | *Depatment of Radiation Oncology - Duke University Medical School, Durham, USA*

22.5.2009

From Molecules to Systems: Deciphering the Molecular Basis of Neurodegeneration
Tiago Outeiro | *Celular and Molecular Neuroscience Unit - IMM, Lisbon*

29.5.2009

Molecular features of the Bone marrow microenvironment in normalcy and in disease
Sérgio Dias | *Centro de Investigação de Patologia Molecular - Instituto Português de Oncologia, Lisboa*

5.6.2009

The peroxisome-mitochondria connection: news and views on organelle dynamics and dysfunction
Michael Schrader | *Centro de Biologia Celular & Dept. Biologia - Universidade de Aveiro,Portugal*

July

2.7.2009

Studies on Lipid Metabolism in Muscle Cells and Brown Adipocyte Differentiation: Mitochondrial Metabolism
Daniel Espinoza | *Joslin Diabetes Center / BIDMC / Boston, MA*

October

27.10.2009

Transcription factors in neurovascular unit protection
Giavanni Mann | *School of Medicine, - King s College, London, UK*

9.10.2009

Specialized Nuclear Export of mRNA Encoding Secretory and Mitochondrial Proteins
Alexander Palazzo | *Dept. of Biochemistry - Univ. Toronto - Canada*

16.10.2009

Modulation of angiogenesis and inflammation. Recent in vivo advances
Raquel Soares | *Dept of Biochemistry -Medical Faculty - University of Porto*

November

2.11.2009

Metabolic Engineering of Corynebacterium glutamicum for the Production of Amino Acids
Elmar Heinzle | *Applied Biochemistry - Biomedical Engineering - University of Saarland - Germany*

6.11.2009

Talks in Free Radical Biology

What are free radicals (RR)
Rafael Radi | *Center for Free Radical and Biomedical Reserach - Faculdad de Medicina, Universidad de la Republica Montevideo, Uruguay*

Mechanisms and Biological Consequences of Protein Tyrosine Nitration (SB)
Silvina Bartesaghi | *Center for Free Radical and Biomedical Reserach - Faculdad de Medicina, Universidad de la Republica Montevideo, Uruguay*

19.11.2009

Mechanisms of the neuroprotection of cannabinoids in Alzheimers disease pathology
Maria L. de Ceballo | *Dept. of Cellular Molecular and Development Neuroscience and CIBERNED, instituto Cajal, CSIC, Madird, Spain*

20.11.2009

The Nitrate-Nitrite-Nitric oxide Pathway in Health and Disease
Jon Lundberg | *Dept Physiology & Pharmacology - Karolinska Institutet, Stockholm, Sweden*

27.11.2009

Unconventional mechanisms of mitochondrial dysfunction
Massimo Zeviani | *National Neurological Institute - "C. Besta", Italy*

December

4.12.2009

Structural sources of robustness in biochemical reaction networks

Guy Schinar | *Dept. Chemical Engineering and - Dept. Mathematics, Ohio State University - Columbus, USA*

11.12.2009

The energy crisis - what can bacteria do for us?
Eliora Run | *Department of Molecular Microbiology and Biotechnology - Faculty of life Sciences, Tel Aviv University, Israel*

10.12.2009

TT viruses – the still elusive human pathogens
Ethel-Michele de Villiers | *German Cancer Research Center, Division of Characterization of Tumor viruses, Heidelberg, Germany*

17.12.2009

Epileptogénese e anti-epileptogénese
Esper A. Cavalheiro | *Neurologia Experimental - Departamento de Neurologia e Neurocirurgia - Universidade Federal de São Paulo, Brasil*

Thesis concludes in 2009

Adriana Oliveira dos Santos

Targeted gene silencing therapy in small cell lung cancer Title of the thesis: Targeted gene silencing therapy in small cell lung cancer

Supervisor: Conceição P. Lima

Ana Margarida Meireles de Sousa,

Genetic study and molecular dissection of novel microtubule regulators in Drosophila

July 24, 2009

Supervisor: Hiro Okhura

Co-Supervisor: Ana Cristina Carvalho Rego

Bruno Miguel Alves Fernandes do Gago

Nitrite in Nitric Oxide Biology in the Stomach: Role of polyphenols and ethanol.

Novembro 2009

Supervisor: João Laranjinha, Rui M. Barbosa

Chantal Ana Vicência Fernandes

Osmotic and Thermal adaptation in ancient thermophilic bacteria. Glucosylglycerate and mannosylglucosylglycerate

July 24, 2009

Supervisor: Milton Costa

Co-supervisor: Nuno Empadinhas

Giana de Paula Cognato

Avaliação do sistema purinérgico de ratos jovens e adultos após indução de epilépsia e sua interacção com parâmetros comportamentais

12 Maio de 2009

Supervisor: Carla Bonan

Co-supervisor: Rodrigo A. Cunha

João Miguel das Neves Duarte

Beneficial effects of caffeine consumption on diabetes-induced alterations in the hippocampus

12 Fevereiro 2009

Supervisor: Rui A. Carvalho

Co-supervisor: Rodrigo A. Cunha

Paula Margarida Gomes Canas

Neuroprotection by adenosine receptors in aged rats – role of neuroinflammation

2-3 Novembro 2009

Supervisor: Rodrigo A. Cunha

Pedro Miguel Brás de Macedo Coelho

Global Tolerance of Biochemical Systems and its Design Implications

September 7, 2009

Supervisor: Armindo Salvador, Michael Savageau, Winchil Vaz

Rui Jorge Gonçalves Pereira Nobre

Human Papillomavirus and Cervical Cancer: novel phylogenetic and viral pathogenesis concepts. Faculdade de Farmácia da Universidade de Coimbra.

December 10, 2009

Supervisor: Teresa Martins

Sandra Catarina Gomes Amaral

Diabetes, Aging and Male Reproductive Function

May 20, 2009

Supervisor:João Ramalho-Santos

Sandra Manuela Domingues dos Santos

Proteomic analysis of the interactome of glutamate receptors of the AMPA type: Contactin associated protein 1 as a regulator of AMPA receptors

September 15, 2009

Supervisor: Ana Luísa Carvalho

Co-supervisor: Carlos B. Duarte

Susana Isabel Elias Alarico

Relevance of mannosylglycerate and trehalose accumulation in the osmoadaptation of Thermus thermophilus

March 30, 2009

Supervisor: Milton Costa

Teresa Cardoso Delgado

Insulin Resistance and Diabetes: Insights from Magnetic Resonance Studies of Hepatic Glucose and Lipid Metabolism

March 19, 2009

Supervisor: Carlos F. G.C. Geraldes and John G. Jones

Vera Marisa Freitas Costa

Role of catecholamines and reactive oxygen species in the mechanism of oxidative stress-induced heart disease: in vitro studies using freshly isolated rat cardiomyocites

June 23, 2009

Supervisors: Fernando Remião and Rui A. Carvalho

Master Thesis

Ana Cristina Oliveira Brett

Huntington's disease – neuropathological mechanisms and therapeutical advances

December 17, 2009

Supervisor: Ana Cristina Carvalho Rego

Ana Catarina Ribeiro da Graça Fonseca

Neuroprotective effects of statins in an *in vitro* model of Alzheimer's disease

Maio 2009

Supervisor: Cláudia MF Pereira

Co-supervisor:Paulo Santos

Ana Cláudia Saraiva Ribeiro

Cell therapy in brain neurodegenerative movement disorders – a clinical perspective

December 9, 2009

Supervisor: Ana Cristina Carvalho Rego

Ana Maria Pereira da Silva

Evaluation of Liver de novo Lipogenesis in an Animal Model by ^{2}H NMR Isotopomer Analysis

September 21, 2009

Supervisor: Rui A. Carvalho

Ana Maria Sequeira Cardoso

Biophysical Characterization of Gemini-based Lipoplexes with Different Levels of Biological Activity

July 2009

Supervisors: Maria Amália da Silva Jurado and Maria da Conceição Pedroso de Lima

Ana Rita Bento

The effect of methamphetamine on subventricular zone neurogenesis: cell death, proliferation and differentiation

July 17, 2009

Supervisors: João Malva

Ana Sofia Lopes Coelho

Apo2l/Trail as new therapeutic approach in myeloid neoplasias

September 17, 2009

Orientador: Ana Bela Sarmento Ribeiro.

Ana Sofia Tremoceiro Lourenço

Characterization of proteomic profiles of Multiple Sclerosis using multivariate analysis

Supervisor: Carlos B. Duarte

Ângela Mara das Neves

Screening e identificação de inibidores naturais da produção de NO e da activação do NF-kB, induzidas pela Interleucina-1beta em condrócitos humanos. Implicações numa potencial utilidade terapêutica em doenças artríticas

June 18, 2009

Supervisor: Alexandrina Ferreira Mendes, Carlos Cavaleiro

Carlos Adriano Albuquerque Andrade de Matos

Post-translational modifications of ataxin-3, the protein involved in Machado-Joseph disease: Evidences for phosphorylation and sumyolation

October 2, 2009

Supervisor: Ana Luísa Carvalho

Co-supervisor: Sandra de Macedo-Ribeiro

Daniela Ribeiro Pinheiro

Quantifying *de novo lipogenesis* with ^{1}H and ^{2}H NMR

Supervisor: Madalena Caldeira

Dina Alexandra Cosme Figueiredo

Citogenética Clássica e FISH em Diagnóstico Pré e Pós-Natal

July 22, 2009

Supervisor: João Ramalho-Santos

Filipa Alexandra Santos Curado

Effect of adenosine in the interaction of macrophages with *Candida albicans*

September 16, 2009

Supervisor: Teresa Maria Fonseca de Oliveira Gonçalves

Filipa Sofia Liborio Carvalho

Mitochondrial and Cellular Toxicity of Two Triterpenoid Derivatives

July 16, 2009

Supervisors: Paulo J. Oliveira, Antonio Moreno

Flávio Fortes Ramos Sousa

Efeito da sequestração de intermediários na eficácia funcional de ciclos metabólicos de transferência de grupos

September 17, 2009

Supervisor: Armindo Salvador, Maria João Moreno

Helena Carvalheiro

Study of the mechanism of temozolomide-induced cytotoxicity in glioma cells.

July 16, 2009

Supervisor: M. Celeste Lopes, Anália do Carmo

Hugo Alexandre Louro Filipe

Quantitative modeling of passive permeation through the blood-brain barrier

July 2009

Supervisor: Maria João Moreno, Armindo Salvador

Joana Balça Pinheiro da Costa e Silva

Efeito da expressão da família miR34 na radiossensibilidade de linhas celulares de cancro do pulmão.

October 22, 2009

Supervisor: Ana Bela Sarmento Ribeiro

Joana Filipa Coelho Fernandes.

Role of Ca^{2+}-Permeable AMPA receptors in excitotoxicity: contribution of GluR4 subunit phosphorylation and characterization of the OGD-induced cell death

Supervisors: Armanda E. Santos, Carlos B. Duarte

João Demétrio Gonçalves Boto Martins
Cell Toxicology of Organotins
October 2009
Supervisors: Maria Amália da Silva Jurado

João Filipe da Costa Martins
Effects of 3,4-methylenedioxymethamphetamine administration in rat electroretinogram
May 14, 2009
Supervisors: Cláudia Cavadas and A. Francisco Ambrósio

Ludgero Canário Tavares
Metabolic Profiling of Prostate Cancer Biopsies by ^{1}H HR-MAS
September 21, 2009
Supervisors: Rui A. Carvalho, Carmen Alpoim

Maria Martins Viegas Nascimento
Alterações do metabolismo energético e a susceptibilidade à morte celular de cíbridos da doença de Huntington
April 28, 2009
Supervisor: Ana Cristina Carvalho Rego

Marília Henriques Cordeiro
A Mitocôndria Testicular como novo Modelo Toxicológico para avaliar Correctamente o efeito de Poluentes Ambientais na Reprodução Masculina
September 21, 2009
Supervisor: João Ramalho-Santos
Co-supervisor: John G. Jones

Michele Curcio
Excitotoxic stimulation-induced changes in the ubiquitin-proteasome system in cultured hippocampal neurons
Supervisor: Carlos B. Duarte

Nuno Gabriel Machado
Does sub-chronic administration of doxorubicin to wistar rats result in lung apoptotic signaling and oxidative stress?
July 17, 2009
Supervisors: Paulo J. Oliveira, Rui A. Carvalho

Nuno Miguel de Jesus Machado
Characterization of an Animal Model of Early Alzheimer Disease
September 18, 2009
Supervisors: Rui A. Carvalho, Rodrigo A. Cunha

Patricia Henriques Domingues
Patterns of protein expression in central nervous system tumours
September 9, 2009.
Orientador: M. Celeste Lopes

Pedro Alexandre Mesquita dos Santos Martins
WikiModels: A RESTful Server for collaborative development of Biochemical model
September 10, 2009
Supervisor: Armindo Salvador, Penousal Machado

Pedro Joaquim Fernandes da Conceição Cruz
Engenharia Estrutural de Peptídeos
Supervisors: Rui Brito

Raquel Patrícia Gomes Silvestre Vinhas
Proteases de Pólenes: Relevância em Doenças Alérgicas
Supervisors: Paula Veríssimo

Ricardo Jorge Fernandes Marques
Avaliação dos efeitos do sildenafil na função mitocondrial com ênfase na isquémia-reperfusão: mecanismos moleculares da acção farmacológica e toxicológica
July 22, 2009
Supervisors: José B.A. Custódio, Maria Augusta Fernandes

Rita Sofia Gonçalves Silva
Caracterização de sub-populações de espermatozóides humanos
July 22, 2009
Supervisor: João Ramalho-Santos

Rui Filipe Ramos Figueiredo
A presença de Legionella spp., não detectável por cultivo, num sistema de distribuição de água potável
July, 2009
Supervisor: António Veríssimo

Rui Gonçalo Batista Mamede da Cruz
Aminopeptidase N de Arabidopsis thaliana: Expressão e Caracterização
Supervisors: Paula Veríssimo

Sofia Baptista
The effect of methamphetamine on dentate gyrus neurogenesis: role of neuropeptide Y".
July 21, 2009
Supervisors: João Malva

Susana Patrícia da Silva Pereira
In vivo effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on heart and liver mitochondrial bioenergetics and ATP-sensitive potassium channels
July 23, 2009
Supervisors: Paulo J. Oliveira, Antonio J. Moreno

Tiago Costa Capote
Mecanismos mitocondriais da acção farmacológica e toxicológica da carbamazepina, homocisteína e homocisteína tiolactona
July 29, 2009
Supervisors: José B.A. Custódio

Vasco de Casimiro Silveirinha
Control by A_{2A} receptors of dopamine uptake in rat striatum and pre-frontal cortex
19 Setembro 2009
Supervisors: Rodrigo A. Cunha

Vera Mendes

Low molecular weight biomarkers in Multiple Sclerosis – Development and validation of a multivariate analysis workflow

Supervisor: Bruno Manadas

Vera Patrícia Lourenço Gonçalves

Factores genéticos de susceptibilidade para cancro da mama esporádico e de agregação familiar.

July 2, 2009

Supervisor: Teresa Martins

Vera Raquel Grandão Cortez

Regulação da libertação de catecolaminas por activação dos receptores adrenérgicos nas células cromafins humanas

October 15, 2009

Supervisors: Cláudia Cavadas, Joana Rosmaninho-Salgado

Technology Transfer

Translational research and technology transfer have been progressivly developed in CNC leading to a promising interaction with Industry and local authorities. The outcome of this interaction was the participation of CNC as a founding member of ABAP (Association envolving seven Municipal Councils of the Center Region of Portugal) aiming at knowledge based development). The main contribution of CNC for that goal was the creation of technology transfer unit (Biocant) in colaboration with Cantanhede Municipal Council.

This unit became the anchor of Biocant Park a Biotechnology Park that is rapidly growing by atracting new Biotechnology companies.

1. BIOCANT

Biocant is a private, non-profit, innovation centre created by CNCB together with the municipality of Cantanhede for technology transfer in biotechnology. Founded 3 years ago, Biocant has grown to become a reference in the field and the catalyst of Biocant Park, the first Portuguese biotechnology park.

Biocant is organized into seven main functional units with highly qualified teams and state of art equipment: Genomics, Cellular Biology, Molecular Biotechnology, Microbiology, Bioinformatics, System Biology, Tissue Engineering, and Advanced Services. Biocant provides services and R&D activities based on post-genomic platforms such as whole-genome sequencing, DNA chips, proteomics, interactomics and metabolomics.

Several research projects are currently in progress, some in collaboration with national or international research institutions, hospitals and companies.

Throughout the past year Biocant has filed four patent applications and its researchers published papers in journals such as PNAS and JBC. Biocant expects to spin-out its first company by the end of 2008.

2. Companies operating in Biocant Park

At the present 8 companies operate in Biocant Park: Crioestaminal, GeneBox, GenePrediT, GeneLab, Novexem, Hematos, 4Health and Biocant Ventures. Along with Biocant they form a biotech cluster of excellence, bringing together over 100 researchers, in a unique enabling environment. Linking basic and applied research more closely to successful innovation, Biocant paved the way for a new paradigm of economic development in the Center Region of Portugal.

Outreach Programme

The Outreach Programme developed by CNC offers opportunities to develop partnerships with schools and to extend our scientific resources to the community. The programme is designed to engage students in their science studies and potential careers related to the life sciences, and to broaden the public's access to science. The dissemination of scientific information equally contributes to the appreciation of the research activity performed at the CNC. The creation of a Science Communication Office by the CNC is the outcome of the successful outreach programme developed in the past years and the recognition of the importance of an appropriate communication strategy.

Brain Awareness Week, March 15-22

In order to promote contact between students and neuroscientists at school, several activities entitled "Brain curiosities" were planned for 5-17 years old students (close to 1000). Students from secondary schools visited several laboratory facilities ("Open Laboratories") and were allowed to perform techniques currently used in a neuroscience laboratory. In collaboration with the Museum of Science at the University of Coimbra, several activities were promoted, including the colloquium EGAS MONIZ, 60 ANOS DO NOBEL, multidisciplinary conferences ("Talking about Brain and Art!"), with speakers from different areas and open for general public, and an interactive exhibition called "Brain in motion". In the scope of the collaboration between CNC and Instituto de Educação e Cidadania (IEC) several activities took place at primary and secondary schools near IEC, and CNC's researchers participated in public sessions at IEC.

"Ciência Viva" Program, July 06-17

Portuguese and Spanish students from secondary schools participated in this 10 day program during Summer Holidays. Adding to visits to facilities and laboratories, students had the opportunity to run several molecular/cell biology techniques as part of short projects.

Innovation Days, June 18-20

The CNC took part in the 4th edition of Innovation Days. This exhibition was intended to present R&D results in order to facilitate the transfer of technology or research in consortium and to promote successful R&D projects amongst the community.

European Researchers' Night, September 25

Together with the Science Museum of the University of Coimbra, CNC took part for the first time in the organization of the activities of the European Researchers' Night. This event is promoted by the European Commission in order to bring the public closer to the researchers in a non-scientific environment. This initiative allowed people to be closer to researchers and their world. The motto for this Night was "Scientists to the Stage", where the researchers were dared to get on stage and perform before a live audience. Besides participating on the play "Monsieur de Chimpanzé", CNC researchers prepared several hands-on activities to be carried-out by the public during the visit to the Museum. More than 800 people participated in this initiative.

Science and Technology Week, November 21-27

As in previous years, CNC organized activities during the Science and Technology week and the National Day for Scientific Culture in order to promote the direct contact with the public. These activities were intended for high-school and undergraduate students, and the general public who had the opportunity to: attend a public conference on the subject of Bacterial Evolution; a café scientifique on Science Communication; to visit CNC's laboratories on the several open days (four) and listen to the investigators talk about their research. We hopefully contributed to the public understanding of the science being carried out in Portugal, by which scientists and how. CNC's researchers also participated in the activities promoted by the Science Museum of the University of Coimbra, namely the Multidisciplinary Conferences targeted for high-school students. Approximately 250 people participated in these activities.

Core Facilities

ANIMAL HOUSE

Head of Unit: Alexandre Pires / *Graduate in Agricultural Engineering and Animal Production*

Head of Facility since 2006

Staff: Carmen Semião (caretaker), Fátima Graça (assistant technician); Maria Eugénia Campos (assistant technician)

The Animal House is a shared resource that provides services in laboratory animal experimentation and husbrandy, for all CNC and FMUC scientists using animals in their research.

The present facility has a capacity to house about 3000 animals (rats/mice). This facility offers the following services: complete husbandry, including feeding, watering, daily cage changing, as well as routine procurement, inventory and care. In 2007, the facility started to provide specialized animal services, namely: breeding and housing of transgenic/knockout strains of mice as well as wild type colonies, production of rats/mice embryos and litters and maintenance of athymic nude mice.

Animal Room – IVC cages (type II)

The Animal House contains a barrier maintained facility, with 8 positive pressurised rooms, which are kept at 22ºC with a relative humidity of 55%. The rodents are breed in individually ventilated cages and a 12-hour light-dark cycle is maintained with an automatic timer. The facility has an animal identification system and software to monitor animal records.

Laminar flow chamber

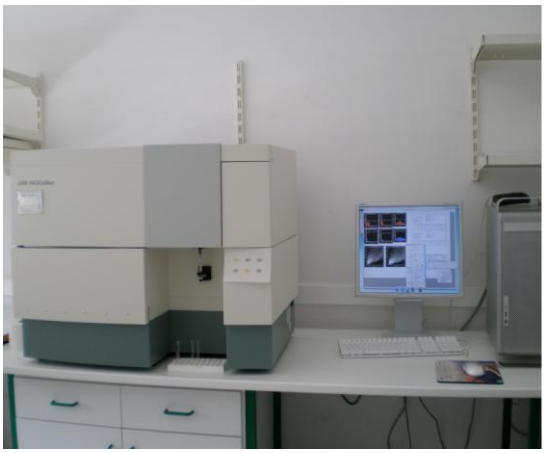
FLOW CYTOMETRY UNIT

Head of Unit: Isabel Nunes Correia | *PhD in Biochemistry Technology (2007) at University of Coimbra*

Head of Facility since 2007

The Flow cytometry Unit provides technical support on flow cytometry both to CNC and external researchers. Currently, it is equipped with a FACSCalibur cell analyser and a separate computer and software to enable researchers to fully analyse their flow cytometry data. For researchers wishing to use flow cytometry in their studies, the unit provides assistance in planning projects, choosing fluorochromes, analyzing experimental results and presenting data.

The Unit organizes annual flow cytometry seminars with the purpose to initiate new users and make this powerful technology known to all researchers, endeavouring to deepen CNC research. Even though the unit has started to operate recently, several CNC research groups are already taking advantage of this facility, performing apoptose, receptor expression and siRNAs intracellular delivery studies, among others.



FACSCalibur cell analyzer

MICROSCOPY UNIT

Head of Unit: Luísa Cortes | *PhD in Enzymology (2006) at University of Coimbra*

Head of Facility since 2007

The Microscopy Unit provides technical support on the investigation made using Light Microscopy. Besides managing the resources, the unit assists in planning microscopy oriented projects, analysing experimental results, processing acquired images and presenting data.

Presently, the unit manages a laser scanning confocal microscope (Zeiss LSM 510 Meta), a P.A.L.M. laser microdissecting microscope, a single cell calcium imaging system, 2 widefield systems and other brightfield microscopes. The systems are prepared for advanced applications which include live cell imaging and single cell calcium measurements, enabling the researchers of imaging dynamic events and molecular interactions.

P.A.L.M. laser microdissecting microscope

The P.A.L.M. laser dissecting microscope is a perfect tool for the isolation of different cell populations within a sample, allowing its full characterization. Using this technology, collaboration has been established, with the service of Anatomical Pathology from the FMUC, with the aim of studying the differences of gene expression between tumour cells at diverse stages.

Laser scanning confocal microscope

MASS SPECTROSCOPY UNIT

Head of Unit: Bruno Manadas | *Post-Doc, PhD in Cellular Biology (2008) at University of Coimbra*

Head of Facility since 2008

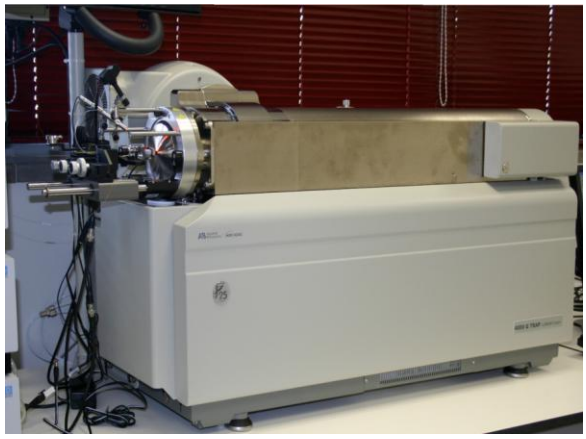
Staff: Vera Mendes (technician)

The Mass Spectrometry Unit is specialized in identification and quantification of proteins from simple and complex samples; identification and quantification of post-translational modifications, and identification and quantification of metabolites. The Unit is also involved in the identification of biomarkers through proteomics and metabolomics techniques with the purpose of developing new prognosis and diagnosis methods, in collaboration with other R&D units at CNC, Biocant, and external partners.

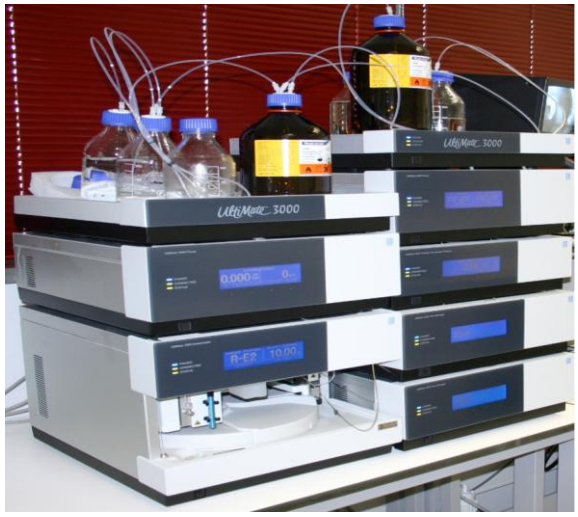
Presently, the Mass Spectrometry Unit is equipped with state of the art technology, namely: a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex), hybrid triple quadrupole/ion-trap mass spectrometer with capacity of MS^3, a two-dimensional liquid chromatography system Ultimate 3000 (Dionex/LCPackings), a ExQuest (Bio-Rad) – image acquisition and spot picking robot and a data processing station (connected to two data acquisition stations). The unit also contains several software packages for data processing, including PDQuest and ProteomeWeaver for 2D gel analysis, Protein Pilot and PEAKS for protein identification, post-translational modifications and de novo sequencing.

By combining the high resolving power of the LC system with the structure elucidation from the mass spectrometer, the Mass Spectrometry Unit is able to identify peptides, metabolites, drugs, pesticides, among others, from complex mixtures.

The Unit integrates the National Mass Spectrometry Network (RNEM).



4000 QTRAP mass spectrometer



Bidimensional chromatography modular system coupled to the 4000 QTRAP spectrometer

NMR SPECTROSCOPY UNIT

Head of Unit: Prof. Carlos Geraldes | *PhD in Inorganic Chemistry (1976) at Oxford University, UK*

Head of Facility since 2008

Staff: John Jones (Investigator)

The Nuclear Magnetic Resonance Spectroscopy Laboratory provides technical support on analysis of liquid and semi-solid samples by Nuclear Magnetic Resonance (NMR) Spectroscopy and Electron Spin Resonance (EPR) Spectroscopy.

The Unit currently stands with a 600 MHz NMR Spectrometer (Varian VNMR 600), a narrow bore 500 MHz NMR Spectrometer (Varian Unity 500), a 20 MHz NMR relaxometer (Bruker mq20) and an X-band EPR Spectrometer (Bruker ESP 300 E).

The state-of-the art equipment comprise unique package of features that can provide information for NMR structural studies, metabolic studies in ex-vivo biosamples and biopsies. The unit also performs 1D, most 2D and some 3D NMR experiments on small-to-medium sized molecules and characterizes aqueous or non-aqueous samples, like paramagnetic and diamagnetic solutions, and biological tissues. Determine the quality control of various samples of industrial interest, such as water contents in oils, study small paramagnetic complexes and paramagnetic metalloproteins, and execute spin label and spin trap research, are also main areas of significance in our Unit.

This Unit integrates the Portuguese Nuclear Magnetic Resonance Network (PTNMR).

Varian 600 NMR Spectrometer

Services

Laboratory of Biochemical Genetics

Coordinators: Catarina Resende Oliveira, Manuela Grazina

Team: Cândida Mendes, Carla Veríssimo, João Pratas, Maria João Santos, Marta Simões

Mitochondrial Respiratory Chain (MRC) and Krebs cycle enzymes

There were studied 108 subjects suspected of Mitochondrial Cytopathy, corresponding to the analysis of 134 samples (some patients had 2 or more tissues analysed), including 86 lymphocytes isolated of peripheral blood, 40 muscular biopsies, 7 liver and 1 heart samples. A MRC deficiency was detected in 41 patients.

The analysis of fumarase activity in lymphocytes isolated from blood was performed in 10 control samples and the first two patients of fumarase deficiency were diagnosed in Portugal.

Mitochondrial DNA (mtDNA) and nuclear genome studies

Molecular differential analysis of mitochondrial cytopathies, as a highthrouput screening, has been performed by sequencing analysis, of 11 mtDNA regions, covering a total of 424 mtDNA sequence variations that include 31 confirmed pathogenic mutations associated to MRC associated diseases. We have continued to screen deletions by flanking PCR of 6 hot-spot regions.

We have received 389 samples from 353 patients suspected of Mitochondrial Cytopathy, that **represent an increase of 89,8%, compared to year 2008**, for DNA extraction, including blood (312), muscle (40), liver (7) and heart (1) tissues, comprising a total of 1304 and 1081 PCR reactions for point mutations and deletions analysis, respectively. Deletions have been detected in 4 samples and a total of 514 (159 different) known mtDNA sequence variations, 2 rearrangements and 39 novel variants have been detected in 68/89 samples analysed of 68/89 patients investigated. Further 61 PCR-RFLP analyses were performed to validate point mutations in 29 samples of 26 patients.

We have implemented mtDNA copy number assays for depletion screening, with the collaboration of Prof. Lee-Jun Wong (Baylor College of Medicine, Houston, Texas, USA). We investigated 23 samples of 15 patients, including blood (3), muscle (9), liver (9) and heart (2) tissues, comprising a total of 186 real time PCR reactions. We have confirmed diagnosis of the first 3 cases of mtDNA depletion in Portugal.

The genetic screening of mitochondrial gama polymerase POLG genes was initiated, by screening. We have analysed 6 samples of patients with liver plus neurological dysfunction, comprising 23 PCR reactions per sample (total of 138 PCR reactions), and 46 sequencing reactions per sample (total of 276 sequencing reactions). We have found 19 sequence variations, 18 located in intronic regions (11 in POLG1 and 7 in POLG2) and one previously described as pathogenic (Q1236H) in Alpers syndrome that requires further confirmation by a different technical approach. There are already 74 samples already waiting for analysis, limited by available personnel and equipment.

Amino Acid Analysis

Our laboratory received 478 samples (395 - plasma, 67 - urine and 16 - cerebrospinal fluid) of physiological fluids for amino acid analysis. The patients investigated (children, adolescents adults) were categorized in three clinical conditions: (1) selective screening of metabolic disorder, characterized by either primary or secondary abnormalities in the amino acid profile (2) amino acid profile changes secondary to proximal renal tubular or hepatic dysfunction of any origin; (3) nutritional evaluation of patients with protein restrictive diets. The majority of samples are from children, although less frequently, adults and adolescents are also monitored. Amino acids analysis is a very important approach in early metabolic disorder diagnosis, and frequently helps to prevent mental retardation or even death.

Laboratory of Molecular Genetics of Cardiopathies

Coordinators: Catarina Resende Oliveira, Isabel Marques Carreira

Team: Ana Cristina Santos

Mutation screening of the genes MYH7, MYBPC3, TNNT2, TNNI3 and MYL2 in Hypertrophic and Dilated Cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is a primary disorder of the myocardium classically characterized by unexplained left ventricular hypertrophy (LVH) and distinct histopathologic features of myocyte disarray and interstitial fibrosis. It has a prevalence of 1:500-1000.

Genetic studies established the paradigm that HCM is a disease of the sarcomere, caused by dominant mutations in genes encoding components of the contractile apparatus, including cardiac b-myosin heavy chain (MYH7), cardiac myosin binding protein C (MYBPC3), cardiac troponin T (TNNT2), cardiac troponin I (TNNI3) and essential myosin light chains (MYL2).

The clinical course of HCM is highly variable. The most serious consequences are heart failure and sudden cardiac death. It is the leading cause of sudden death in competitive athletes. Understanding the genetic basis of HCM provides the opportunity for gene- based diagnosis.

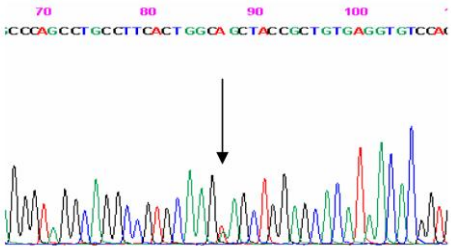


Fig. 1 – Sequencing pattern of MYBPC3 gene (exon 6 – S236G mutation)

Individuals with clinical or imagiological criteria of HCM were referred for the evaluation by sequencing (Fig.1) of the 5 most common genes (56 exons) (Fig. 2) In the year of 2009 we evaluated in the laboratory 62 cases (30 females and 32 males): 22 of which were index patients and 40 were follow up families (parents, siblings, grandparents, uncles and nephews). A total of 1170 exons were sequenced.

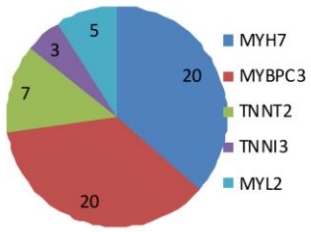


Fig. 2- Number of exons studied in each gene

In this period of time, six sequence variations considered pathogenic were identified (five on the MYBPC3 gene and one of the TNNT2 gene) (Fig. 3). One alteration found for the MYBPC3 gene, is not referred in the Familial Hypertrophic Cardiomyopathy mutation Database or Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff. Family studies in this particular case are still underway to establish whether it is a de novo of familial mutation. There were also identified ten additional sequence variations that could be polymorphic in our population (five on the MYH7 gene, two on the MYBPC3 gene, one on the TNNT2 gene, one on TNNI3 gene and one on MYL2 gene). In order to ascertain whether these are polymorphic or not a population study is under way.

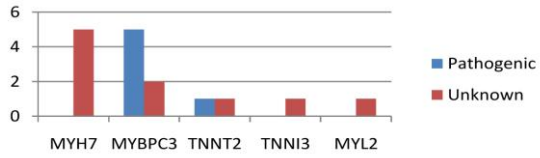


Fig. 3 – Number of mutations identified

In 2009 the number of sequenced exons has shown a 10% increase (2008 – 1097; 2009 – 1170) in relation to the previous year (Fig.4). All patients are followed up in genetic and cardiology consultations.

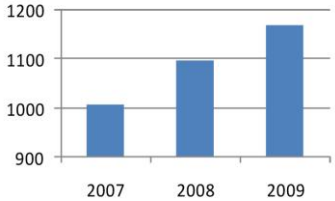


Fig. 4 – Number of sequenced exons from 2007 to 2009

Neuro-Ophthalmology Genetics Laboratory

Coordinators: Maria do Rosário Almeida

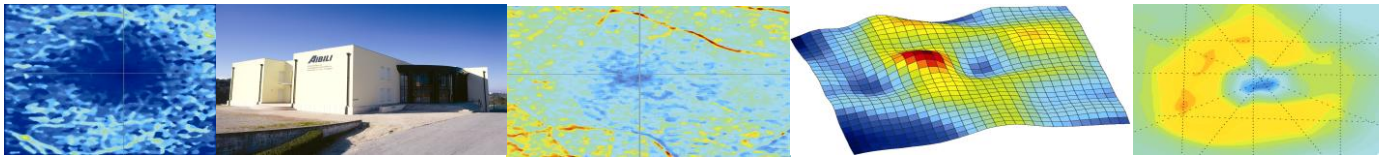
Team: Maria do Rosário Almeida, Maria Helena Ribeiro, Ana Cristina Santos

Molecular testing of Neurodegenerative and Vision related genetic diseases

The aim of the group was to wide the range of genetic tests available in the Neuroscience area in particularly in neurodegenerative disorders such as: Frontotemporal Dementia, Familial Alzheimer Disease and Parkinson Disease. To achieve this, a close functional interaction between the laboratory and the clinicians at the Neurology Department of HUC has been established in order to improve the patient's diagnostics, follow-up and management. Since genetic diagnostic tests are playing an increasingly important role in clinical practice, the clinical referrals have increased in many specialities within medicine. In Neurology, its clinical applicability not only contributes to an accurate diagnosis but also to identify the relatives at high risk to develop the disease, in the context of formal genetic counselling. During 2009, ninety three referrals have been sent to our laboratory with the clinical diagnosis of Alzheimer Disease (9 cases), Parkinson Disease (63 cases) and Frontotemporal dementia (21 cases). The molecular strategy used to perform the molecular diagnosis, involved the mutation search of the genes associated with these disorders using different techniques such as: PCR, RLFP, direct sequencing and dosage. Another challenge that faced this group was the increasing demand for genetic services within the ophthalmology field, which is also one main interest of the Institute of Biomedical Research in Light and Image (IBILI). Therefore, the implementation of molecular genetic tests to vision inherited diseases such as: Nanophthalmia also took place and mutations in *MFRP* gene have been identified for the most of the cases.

At the same time, the group aimed to house both research and diagnostic activity which is fundamental to establish not only the new research findings that are of relevance in a clinical setting, but also to find out the best way to move these quickly from a research setting to diagnostic service in a timely and efficient manner. Therefore, research Projects have been outline and submitted to get financial support.

association for innovation and biomedical research on light and image



PORTUGUESE FOUNDATION FOR SCIENCE AND TECHNOLOGY (FCT)

ASSOCIATE LABORATORY

AIBILI SERVICES

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1. Introduction

AIBILI - Association for Innovation and Biomedical Research on Light and Image is a private non-profit organisation, founded in 1989, established to support technology transfer to industry.

AIBILI is partner of the CNC – Centre of Neuroscience and Cell Biology of the University of Coimbra, as an Associate Laboratory of the Portuguese Foundation for Science and Technology (FCT).

As a complement of CNC laboratory research activities and taking into consideration pharmaceutical industry needs, AIBILI provides clinical trials necessary for effective translational research and physical-chemical testing services to industry.

AIBILI is certified by ISO 9001:2008. Clinical Trials are performed in accordance with ICH Good Clinical Practice Guidelines and the Bioavailability and Pharmacokinetic Studies are also developed in compliance with the OCDE Principles of Good Laboratory Practice.

AIBILI has the following Centres:

- Centre for Clinical Trials (CEC)
- Centre for Bioavailability Studies (CEB)
- Centre of New Technologies for Medicine (CNTM)
- Coimbra Coordinating Centre for Clinical Research (4C)

Other Units:

- Coimbra Ophthalmology Reading Centre (CORC)
- Health Technology Assessment Unit (HTA)

The Administrative Services (SA) is responsible for the management of AIBILI and includes the Quality Management Unit (UGQ) and the Technology Transfer Unit (UTT).

AIBILI is located at the Health Campus of Coimbra University since 1994 and has 15.296 sq. feet with state-of-the-art equipment. Regarding human resources we have 7 investigators, 12 technicians, 5 study coordinators and 3 administrative full time. Also collaborating regularly with AIBILI are 49 investigators, 5 technicians for diagnostic procedures and 7 nurses. Therefore AIBILI has a total of 56 active researchers.

2. Areas of Expertise / Research / Staff

2.1. Centre for Clinical Trials

The Centre for Clinical Trials (CEC) performs randomized clinical trials with special emphasis on Ophthalmology and Cardiology.

It is the purpose of the Centre for Clinical Trials to work with the Pharmaceutical Industry and to function as liaison between the Drug and Medical Device Industry and the Health Services.

CEC has dedicated facilities and the most modern ophthalmological equipment. Its permanent staff includes one Ophthalmologist, one Pharmacist, five experienced Study Coordinators, six Technicians for Diagnostic Procedures, four Nurses, one Laboratory Technician and two Administrative Secretaries.

The professional organisation of the Centre for Clinical Trials with a Manual of SOP (Standardized Operating Procedures) and its convenient location, next to the University Hospital of Coimbra and its Department of Ophthalmology are a guarantee that the deadlines are successfully met and in compliance with the ICH Good Clinical Practice Guidelines. The Centre for Clinical Trials is certified by ISO 9001 to perform clinical trials, thus guaranteeing the continual improvement of its work.

CEC is also certified as a "Site of Excellence" by the EVI.CT.SE Network (European Vision Institute. Clinical Trials. Sites of Excellence), that is a clinical trial center in ophthalmology that complies with ICH GCP Guidelines with written SOPs, has the necessary equipment and personnel to perform clinical trials and has proven expertise and scientific publications in this area.

Areas of Expertise

- Characterisation and evaluation of the most recent methods to study the initial stages of diabetic retinopathy.
- Evaluation of new methodologies for multimodal mapping of the macula.
- Studies of the diseases of the choroid and retina and especially of their blood circulation, particularly in age-related macular degeneration.
- Correlation between structure-function with psychophysics tests and study the early signs of the disease.
- Testing new methods of early diagnosis and characterisation of macular edema and retinal vascular pathology.
- Evaluation of new drugs to treat glaucoma. Development of methods to correlate clinical indicators of disease progression, particularly regarding optic nerve degeneration and the mechanisms of the actions of drugs being tested.
- Evaluation of the quality of cataract microsurgery, testing new drugs for surgery procedures.

Research

Ongoing Clinical Trials

Clinical Trials in Ophthalmology

Macular Edema after CRVO

- *A six-month, phase 3, multicenter, masked, randomized, sham-controlled trial (with six-month open-label extension) to assess the safety and efficacy of 700ug and 350ug Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) applicator system in the treatment of patients with macular edema following central retinal vein occlusion or branch retinal vein occlusion*

Diabetic Macular Edema

- *Reduction in the occurence of center-threatening diabetic macular edema*
- *The effect of Ruboxistaurin on clinically significant macular edema in patients with diabetes Mellitus, as assessed by optical coherence tomography*
- *A randomized, double-masked, parallel group, multi-center, dose-finding comparison of the safety and efficacy of ASI-001A 0.5 ug/day and ASI-001B 0.2 ug/day fluocinolone acetonide intravitreal inserts to sham injection in subjects with diabetic macular edema*
- *A 3-year, phase 3, multicenter, masked, randomized, sham-controlled trial to assess the safety and efficacy of 700 µg and 350µg Dexamethasone Posterior Segment Drug Delivery System (DEX PS DDS) applicator system in the treatment of patients with diabetic macular edema*
- *A phase 2/3 randomized, controlled, double-masked, multi-center, comparative dose-finding trial, in parallel groups, to compare the safety and efficacy of intravitreous injections of 0.3, 0.03 or 0.003mg Pagaptanib Sodium (Macugen®), given as often as every 6 weeks for 3 years, to sham injections, in subjects with diabetic macular edema (DME) involving the center of the macula*
- *Observational study to assess Genotypes/Phenotypes correlations in type-2 diabetic retinopathy*

Glaucoma

- *A five-year, multicenter, open-label study to evaluate the safety of once-daily evening instillation of travoprost 0,004% eyedrops (Travatran®) in subjects with open-angle glaucoma or ocular hypertension*
- *Study of the efficacy and safety of Travatan® therapy compared with Cosopt® therapy in patients with open-angle glaucoma or ocular hypertension*
- *A phase 3 Prospective, Randomized, Double-Masked, 12-week, parallel group study evaluating the efficacy and safety of Latanoprost and Timolol in pediatric subjects with glaucoma*

Age-Related Macular Degeneration

- *Early Markers of choroidal neovascularization (CNV) in fellow eyes of patients with age-related macular degeneration (AMD) and CNV in one eye*
- *A phase IV, long-term, open-label, multicenter extension study to evaluate the safety and tolerability of ranibizumab in patients with subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD)*
- *A randomized, double-masked, active controlled, phase 3 study of the efficacy, safety, and tolerability of repeated doses of intravitreal VEGF Trop-Eye in subjects with neovascular age-related*
- *A multicenter, masked, randomized, sham-controlled, paired-eye comparison, 12-month (plus 12-month extension) study to evaluate the safety and effects on Retinal Structure and Visual Function of Brimonidine tartrate posterior segment drug delivery system (Brimonidine Tartrate PS DDS) applicator system in patients with Geographic atrophy from Age-related Macular Degeneration*
- *A 102-week, open label, multicenter trial to investigate the efficacy of macugen for the preservation of visual function in subjects with neovascular age-related macular degeneration (AMD) and to assess the benefit of treating early choroidal neovascularization (CNV)*
- *A phase 3, randomized, double-masked, parallel-assignment study of intravitreal bevasiranib sodium, administered every 8 or 12 weeks as maintenance therapy following three injections of Lucentis® compared with Lucentis® monotherapy every 4 weeks in patients with exsudative age-related macular degeneration (AMD)*
- *A 6-month, single-masked, multicenter, randomized, controlled study to assess the safety and efficacy of 700µg Dexamethasone Posterior Segment Drug Delivery System applicator system as adjunctive therapy to Lucentis® compared with Lucentis® alone in the treatment of patients with choroidal neovascularization secondary to age-related macular degeneration*

Cataract

- *Efficacy and safety assessment of intracameral T2380 (fixed combination of lidocaine, phenylephrine and tropicamide) for anaesthesia and mydriasis in phacoemulsification cataract surgery*
- *A multicenter, investigator-masked, parallel-group, randomized, study of the efficacy and safety of Indomethacin 0,1% eyedrops compared with Kerorolac 0,5% eyedrops in ocular Inflammation after cataract surgery*

Retinal Toxicity

- *Long term (3 years) ophthalmic safety and cardiac efficacy and safety of ivabradine administered at the therapeutic recommended doses (2.5/5/7.5 mg b.i.d.) on top of anti anginal background therapy, to patients with chronic stable angina pectoris. An international, double-blind placebo controlled study.*

- *(GA) secondary to age-related macular degeneration (AMD)*

Retinitis Pigmentosa

- *An exploratory, multicenter, patient-masked, dose-escalation, paired-eye comparison, sham-controlled, 6-Month (plus 6-month extension) study to evaluate the safety and effects on visual function of 100ug, 200 ug, and 400 ug Brimonidine Tartrate Posterior Segment Drug Delivery System (Brimonidine Tartrate PS DDS) applicator system in patients with retinitis pigmentosa.*

Uveitis

- *An 8-week, multicenter, masked, randomized trial (with an 18-week masked extension) to assess the safety and efficacy of 700 ug and 350 ug dexamethasone posterior segment drug delivery system (DEX PS DDS) applicator system compared with sham DEX PS DDS applicator system in the treatment of non-infectious ocular inflammation of the posterior segment in patients with intermediate uveitis*

Geographic Atrophy

- *The safety and efficacy of AL-8309B ophthalmic solution for the treatment of geographic atrophy*

Multiple Sclerosis

- *A 12-month double-blind, randomized, multicenter, active-controlled, parallel-group study comparing the efficacy and afety of 0.5mg and 1.25mg fingolimod (FTY720) administered orally once daily versus interferon β-1a (Avonex®) administered i.m. Once weekly in patients with relapsing-remitting multiple sclerosis*
- *An extension of the double-blind, randomized, placebo-controlled, parrallel-group, multicenter study evaluating safety, tolerability and effect on MRI lesion parameters of FTY720 vs placebo in patients with relapsing multiple sclerosis*

Parkinson's Disease

- *A phase III, double-blind, placebo-controlled extension trial to investigate the long-term efficacy and safety of low (50 mg/day) and high (100 mg/day) dose safinamide, as add-on therapy in subjects with early idiopathic Parkinson's disease treated with a stable dose of a single dopamine agonist*

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2.2. Centre for Bioavailability Studies

The Centre for Bioavailability Studies (CEB) is a qualified resource, skilled to collaborate with Pharmaceutical Industry in all the different phases of drug development.

The main area of activity has been the performance of Bioavailability/Bioequivalence Studies in human healthy volunteers to assess the efficacy and security of drugs. CEB can be responsible for the elaboration of protocols and other documents needed for the studies execution, the organization of all documents to regulatory authorities submission and the development of specific analytical methods for the drugs quantification.

CEB has broaden its competences to perform clinical trials (phases I to III), which is now one of the most relevant areas of activity, with special emphasis on Neurology and Endocrinology. The proximity of the University Hospital of Coimbra and a strong relation with national pharmaceutical industry are key-points of this development.

Regarding human resources, the team includes a coordinator, a study director and four laboratory technicians/study-coordinators. Multidisciplinary medical doctors, pharmacists and nurses also collaborate in the clinical trials performed by CEB.

CEB is equipped with the most up-to-date and suitably calibrated equipment for the development and validation of analytical methods in order to ensure precision and quality of the results presented.

All CEB's activities are performed according to Good Laboratory Practices (certification since 1999 by INFARMED for the performance of Bioavailability/Bioequivalence and Pharmacokinetic Studies), Good Clinical Practices and ISO 9001 Guidelines (certification since 2004 for the performance of Clinical Trials, Bioavailability/Bioequivalence Studies and Drug Dosages).

Areas of Expertise

- Studies of absolute bioavailability of a drug.
- Elaboration of documentation to submit bioequivalence studies to the regulatory authorities.
- Bioequivalence studies of pharmaceutical products having the same drug in the same formulation or different formulations.
- Development and validation of analytical methods.
- Dosage of drugs in the finished product or during the manufacturing process and in biological matrixes.
- Clinical studies on the variability of different batches of preparation from a single manufacturer.
- Chemical control of raw materials and manufactured products.
- Organisation and scientific coordination of reviews or reports for the introduction of drugs in Portugal and the European Union.

Research

Ongoing Studies

- **Bioavailability/Bioequivalence Studies**
  - Open, randomised and crossover study on the bioequivalence between tablets containing 75 mg of clopidogrel from two different pharmaceutical laboratories

- **Clinical Trials**
  - A 54-week, double-blind, randomized, placebo-controlled, parallel-group study to investigate the effects of rosiglitazone as adjunctive therapy to donepezil on cognition and overall clinical response in APOE e-stratified subjects with mild to moderate alzeheimer's disease (REFLECT-2)
  - A pan-european randomized, parallel group, two-arm placebo-controlled, double-blind multicenter study of Rimonabant 20mg once daily in the treatment of abdominally obese patients with impaired fasting blood glucose with or without other comorbidities
  - A 52-week open-label extension study of the long-term safety and efficacy of rosiglitazone extended-release (RSG XR) as adjunctive therapy to acetylcholinesterase inhibitors in subjects with mild-to-moderate alzheimer´s disease (REFLECT-4)
  - Safety and efficacy of S 38093 and donepezil, during 4 weeks, in patients with mild to moderate Alzheimer's Disease. An international, multi-centre, randomised, double-blind, placebo-controlled, phase II add-on study
  - Efficacy and safety of agomelatine oral administration (25 to 50 mg/day) in elderly patients suffering from Major Depressive Disorder
  - Efficacy and safety of eslicarbazepine acetate (BIA 2-093) as monotheraphy for patients with newly diagnosed partial-onset seizures: a double- blind, double-dummy, randomized, active-controlled, parallel-group, multicenter clinical study
  - Safety and efficacy of eslicarbazepine acetate (ESL) as adjunctive therapy for partial seizures in elderly patients
  - A Phase 3, multi-center, randomized, double-blind, placebo-controlled 26-week trial to evaluate the efficacy and safety of Dimebon in patients with moderate-to-severe alzheimer's disease

- ***Drug Dosages Studies***
  - Dosage of repaglinide in plasma samples from the BIA-91067-115 clinical trial
  - Dosage of S- and R-warfarin in plasma samples from the BIA-91067-116 clinical trial

- ***Scientific Report***
  - Therapeutic advantage report of injectable somazine 1000 mg/ 4 ml

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2.3. Coimbra Coordinating Centre for Clinical Research

In order to meet needs for coordination and management of clinical trials, AIBILI has created the Coimbra Coordinating Centre for Clinical Research (4C) which provides planning and coordination of clinical trials at national and international level. This unit is particularly relevant for Investigator-Driven Clinical Trials (IDCT), as it has the necessary infrastructure to plan, organize, manage and monitor clinical trials.

Within the European Union the 4C will address the coordination of the following:

- EVI.CT.SE Network - European Network of Clinical Sites of Excellence in Ophthalmology
- planning and management of investigator-driven clinical trials (IDCT)
- education and training
- regulatory and legal issues

For the investigator-driven clinical trials (IDCT) the 4C is prepared to provide:

- all the necessary documents for the submission of IDCT
- submission of IDCT in Portugal and follow-up
- coordination and implementation of the IDCT within the Clinical Sites
- monitoring of IDCT
- Data management
- Final report

Services

- Management of investigator-driven clinical trials (IDCT)
- Education and training
- Regulatory and legal issues

Research

Ongoing Studies

- Epidemiological study of the prevalence of Age-Related Macular Degeneration in Portugal
- Prospective, randomized, open label phase II study to assess efficacy and safety of Macugen® (pegaptanib 0.3 mg intravitreal injections) plus panretinal photocoagulation (PRP) and PRP (monotherapy) in the treatment of patients with high risk proliferative diabetic retinopathy
- Prospective, randomized, multicenter, open label phase II study to access efficacy and safety of Lucentis® monotherapy (ranibizumab 0.5 mg intravitreal injections) compared with Lucentis® plus panretinal photocoagulation (PRP) and PRP (monotherapy) in the treatment of patients with high risk proliferative diabetic retinopathy

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3. Publications

1. Barry P., Gardner S., Seal D., Gettinby G., Lees F., Peterson M., Revie C., ESCRS Endophthalmitis Study Group: *Clinical observations associated with proven and unproven cases in the ESCRS study of prophylaxis of postoperative endophthalmitis after cataract surgery*. J. Cataract Refract. Surg. 2009 Sep; 35(9):1523-31, 1531.e1.

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8. Nunes S., Pires I., Rosa A., Duarte L., Bernardes R., Cunha-Vaz J.: *Microaneurysm Turnover Is a Biomarker for Diabetic Retinopathy Progression to Clinically Significant Macular Edema: Findings for Type 2 Diabetics with Nonproliferative Retinopathy*. Opthalmologica 2009; 223(5):292-297.

9. Rosa, A., Murta, J.N., Quadrado, M.J., Tavares, C., Lobo, C., Van Velze, R., Castanheira-Dinis, A.: *Femtosecond laser versus mechanical microkeratomes for flap creation in laser in situ keratomileusis and effect of postoperative measurement interval on estimated femtosecond flap thickness*. J. Cataract Refract. Surg. 2009; 833-838.

10. Rosa, A., Pires, I., Silva, R., Santos, A.R., Nunes, S., Silva, M.P., Cachulo, M.L., Faria de Abreu, J.R., Cunha-Vaz, J.G.: *Distrofia Foveomacular Viteliforme do Adulto e Tomografia de Coerência Óptica*. Oftalmologia 2009; 33:33-43.

Tavares R., Coelho D., Macário M.C., Torres A., Quadrado M.J., Murta J.: *Evaluation of Treatment with cysteamine eyedrops for cystinosis with confocal microscopy*. Cornea 2009 Sep;28(8):938

Funding

Introduction

In 2009 funding of "Laboratório Associado – Centro de Neurociências e Biologia Celular" ascended the amount of 4 569 008,85€.

The main financing contribution was made by "Fundação para a Ciência e Tecnologia (FCT)", concerning global institution programs and national projects, namely amount of 4 221,340,90€ distribuited as follows:

Plurianual 2009:	2 184 847,50€
Projects:	1 225 564,48€
Infrastructures:	319 798,82€
Science Program:	430 159,82€
Doctoral Program:	50 470,28€
Integration Fellowship:	10.500,00€

The related items supported the main part of Center for Neuroscience and Cell Biology costs during 2009.

Besides Center for Neuroscience is financed by other national and international agencies. In 2009 Center for Neuroscience received the amount of 39 880,50€ concerning other national projects and 88 271,59€ concerning international projects.

In the following are listed FCT ongoing projects as well as other national and international projects.

The amount of other resting funds, which are not dicriminated ascends a value of

219 515,86€.

Note: Financing values are based on expenditure values 2009

ONGOING PROJECTS

Title	Financing Agency	Duration	Budget (CNC)	Expenditure 2009
National Projects:				
Diagnóstico precose de Doença de Alzheimer: avaliação de critérios de classificação recente e exploração de novos instrumentos de estudo Coordinator: Sandra Cardoso	FCT Refª : PIC/IC/83206/2007	01/01/2009 to 31/12/2011	20.280,00	14.819,79
Avanço na área de entrega de fármacos: terapias combinadas no tratamento do cancro da mama e leucemia (a rede Onco Target Nano Med) Coordinator: Maria da Conceição Lima	FCT Refª: NANO/NMed-At/0042/2007	01/07/2009 to 30/06/2011	72.069,00	6.415,79
Rede Nacional de Espectrometria de Massa Coordinator: Euclides Manuel Vieira Pires	FCT Refª: REDE/1506/REM/2005	01/01/2009 to 31/12/2011	138.960,42	43.418,67
Caracterização de alterações genéticas em gliomas humanos por arrays de polimorfismos de necleótido único (SNP): correlação com as características clínicas e biológicas e citogenéticas da doença Coordinator: Catarina Isabel N. R. de Oliveira	FCT Refª: PIC/IC/83108/2007	05/01/2009 to 04/01/2012	150.440,00	8.912,02
Acção protectora de polifenóis do vinho tinto na inflamação e disfunção do endotélio vascular: Implicações na prevenção da aterosclerose Coordinator: Leonor Martins de Almeida	FCT Refª: PPCDT/AGR/59919/2004	02/05/2005 to 30/06/2009	64.280,00	11.012,46
Manipulação de DNA em solução e interfaces Coordinator: Sérgio Paulo de Magalhães Simões Participants: Faculdade de Ciências e Tecnologia	FCT Refª: PPCDT/QUI/58689/2004	01/07/2005 to 30/06/2009	4.440,00	1.988,30
Estudo dos possíveis factores ambientais e moleculares que levam ao desenvolvimento de diabetes tipo 2 e obesidade em Portugal Coordinator: Eugénia Maria Lourenço de Carvalho	FCT Refª: POCI/SAU-MO/57598/2004	15/10/2005 to 31/10/2009	90.250,00	16.059,75
Desenvolvimento de novos compostos de Vanádio. Sua aplicação como agentes antidiabéticos e anticancerígenos Coordinator: Maria Margarida C. Almiro e Castro Participants: Inst. Sup. Técnico; Inst. de Ciências e Tecnologias Agrárias e Agro-Alimentares (ICETA)	FCT Refª: PPCDT/QUI/56949/2004	01/10/2005 to 30/08/2009	29.500,00	14.791,20

Alterações nas vias fisiológicas e mecanismos moleculares reguladores da homeostase energética na obesidade e síndrome metabólico: identificação de novas estratégias e alvos terapeuticos Coordinator: Carlos Manuel Marques Palmeira	FCT Refª: PTDC/SAU-OSM/72443/2006	01/09/2007 to 31/08/2010	156.000,00	45.347,96
Effecto das purinas no desenvolvimento do hipocampo: Consequências para o establecimento de circuitos relacionados com aprendizagem e memória Coordinator: Rodrigo Pinto Cunha	FCT Refª: PTDC/SAU-NEU/74318/2006	01/07/2007 to 30/09/2010	94.439,00	35.807,31
Regulação dos receptores AMPA pela hiperglicémia na retina Coordinator: Francisco Ambrósio Participants: Faculdade de Medicina da Universidade de Coimbra (FMUC)	FCT Refª: PTDC/SAU-NEU/71228/2006	01/06/2007 to 31/05/2010	40.064,00	14.012,83
Desenvolvimento de novas estratégias para terapia anti-tumoral baseadas na utilização do peptídeo permeante S4(13)-PV com o objectivo de potenciar a entrega intracelular de ácidos nucleicos e proteínas com actividade terapêutica Coordinator: : Mª da Conceição M. Pedroso de Lima	FCT Refª: PTDC/BIO/65627/2006	01/05/2007 to 30/04/2010	136.000,00	52.686,32
Nanostructured photoluminescent rare-earth nonotubes and microporous silicates Coordinator: Carlos F. Gusmão Campos Geraldes Participants: Universidade de Aveiro	FCT Refª: PTDC/CTM/73243/2006	01/12/2007 to 30/11/2010	14.544,00	4.561,03
Contribuição de subunidades dos receptores N-metil-D-aspartato na disfunção neuronal na doença de Alzheimer Coordinator: Ana Cristina Rego	FCT Refª: PTDC/SAU-NEU/71675/2006	01/09/2007 to 31/08/2010	99.944,00	24.035,61
Silenciamento da doença de Machado-Joseph: interferencia de RNA para a ataxina-3 mediada por vectores lentivirais Coordinator: Luis de Almeida	FCT Refª: PTDC/SAU-FCF/70384/2006	01/07/2007 to 30/06/2010	170.000,00	60.803,57
Alterações do metabolismo da glicose e lipído por agentes imunossupressores: implicações no diagnóstico e tratamento da diabetes pós-transplante Coordinator: John Jones	FCT Refª: PTDC/SAU-OSM/65140/2006	01/10/2007 to 30/09/2010	152.223,00	49.757,86

Alterações na Microglia e Neurónios do Hipocampo Induzidas por Metanfetamina: Papel das Citocinas Pró-inflamatórias e do Neuropeptídeo y Coordinator: Ana Paula Silva Martins Participants: AIBILI; Faculdade de Farmácia; IBILI;	FCT Refª: PTDC/SAU-FCF/67053/2006	01/05/2007 to 30/04/2010	88.000,00	21.337,32
Interacção entre a nicotina e a cafeína no núcleo estriado. Relevância na doença de Parkinson Coordinator: Rodrigo Pinto dos Santos Antunes da Cunha	FCT Refª: PTDC/SAU-NEU/81064/2006	01/05/2007 to 30/04/2010	94.378,00	12.063,80
Mecanismos de plasticidade sináptica e de neuroprotecção pelo BDNF no hipocampo: inibição da neurodegemeração vs. regeneração. Coordinator: Carlos Jorge A. Bandeira Duarte	FCT Refª: PTDC/SAU-FCF/72283/2006	01/05/2007 to 30/04/2010	136.000,00	40.636,12
Células estaminais da região subventricular na reparação cerebral em epilepsia do lobo temporal. Coordinator: João José Oliveira Malva	FCT Refª: PTDC/SAU-NEU/68465/2006	01/05/2007 to 30/04/2010	148.828,00	56.165,44
Papel do ATP extracelular e caracterização dos receptores purinérgicos envolvidos na resitência da Candida albicans à resposta immune de macrógafos Coordinator: Teresa Maria Fonseca de Oliveira Gonçalves	FCT Refª: PTDC/SAU-FCF/81436/2006	01/06/2007 to 31/08/2010	78.936,00	20.563,57
Novos Mecanismos Mitocondriais Para a Toxicidade Cardioselectiva da Doxorubicina Coordinator: Paulo Jorge Gouveia Simões da Silva Oliveira	FCT Refª: PTDC/SAU-OSM/64084/2006	15/09/2007 to 13/03/2010	115.800,00	46.556,31
Influência do Estado de Diferenciação Celular na Apoptose Induzida por Isoproterenol em Células Ventriculares Embrionárias H9c2-Vias de Sinalização Envolvidas Coordinator: Paulo Jorge Gouveia Simões da Silva Oliveira	FCT Refª: PTDC/QUI/64358/2006	01/11/2007 to 31/04/2010	85.000,00	33.897,89
Modelação Quantitativa da Difusão Passiva Trans-Citótica de Moléculas Anfifílicas através da Barreira Hemato-Encefálica Coordinator: Armindo José A. da Silva Salvador Participants: Faculdade de Ciências e Tecnologia da Universidade de Coimbra; Instituto de Tecnologia Química e Biológica	FCT Refª: PTDC/SAU-FCF/69072/2006	01/07/2007 to 30/06/2010	18.720,00	5.926,62

Papel da Células Dendriticas na Leishmaniose: estudos de sinalização intracelular na infecção pelo parasita Leismania infantum virulento ou atenuado Coordinator: Maria Teresa de Teixeira Cruz Participants: Instituto de Biologia Molecular e Celular	FCT Refª: PTDC/SAU-FCF/67351/2006	16/08/2007 to 31/12/2009	25.059,00	8.716,82
Função da cortactina no tráfego celular dos receptores do glutamato do tipo do tipo AMPA Coordinator: Ana Luísa Monteiro de Carvalho	FCT Refª: PTDC/BIA-BCM/71789/2006	01/04/2008 to 31/03/2011	89.000,00	38.577,55
Acções troficas dos factores neurotróficos: dependência da coactivação de receptores A2A da adenosina. Coordinator: Emilia Conceição Pedrosa Duarte Participants: Instituto de Medicina Molecular; Faculdade de Farmácia da Universidade de Lisboa	FCT Refª: PTDC/SAU-NEU/64126/2006	01/07/2007 to 30/06/2010	29.907,00	8.548,61
Neuroprotecção pela insulina e IGF-1 na diabetes associada à doença de Huntington Coordinator: Ana Cristina Carvalho Rego	FCT Refª: PTDC/SAU-FCF/66421/2006	22/08/2007 to 21/08/2010	124.000,00	44.295,71
Elucidação de Mecanismos patológicos associados a forma juvenil da lipofuscinose ceróide neuronal: do modelo de levedura para sistemas mais complexos. Coordinator: João António Nave Laranjinha Participante: Universidade do Minho	FCT Refª: PTDC/SAU-NEU/70161/2006	01/07/2007 to 30/06/2010	25.000,00	14.787,13
Estabilidade conformacional de proteínases aspárticas com importância biotecnológica e médica - O unfolding/refolding de proteínas diméricas e monoméricas. Coordinator: Marlene Barros	FCT Refª: PPCDT/QUI/60791/2004	01/01/2007 to 04/03/2009	9.480,00	1.726,45
Clivagem dos transportadores vesiculares do glutamato (VGLUT) e do GABA (VGAT) em condições de excitotoxicidade: identificação dos locais de clivagem e implicações funcionais Coordinator: Carlos Duarte	FCT Refª: PTDC/SAU-NEU/65846/2006	17/04/2007 to 16/04/2010	115.256,00	38.741,08
Neuropeptídeo Y na retina: porquê? E para quê? Coordinator: Cláudia Cavadas	FCT Refª: PTDC/SAU-NEU/73119/2006	01/05/2007 to 30/04/2010	123.668,00	51.864,10

P-found: computação GRID e armazenamento distribuido de dados de simulações de dobragem de proteínas. Coordinator: Rui Brito Participants: Univ. Minho, Faculdade Ciências Coimbra, Faculdade Ciências Tecnologia Univ. Coimbra, Critical Software	FCT Refª: GRID/GRI/81809/2006	01/06/2007 to 31/05/2010	27.545,00	12.637,48
Estudo de processos de bioluminescência. Coordinator: Rui Brito Participants: ADDF	FCT Refª: PTDC/FIS/73578/2006	01/07/2007 to 30/06/2010	50.928,00	13.990,38
Novas funções biológicas de compostos fenólicos do vinho: regulação celular e acção anti-inflamatória via formação de óxido nítrico a partir de nitrito contido na dieta. Coordinator: João Laranjinha	FCT Refª: PTDC/AGR-ALI/71262/2006	15/05/2007 to 14/05/2010	123.478,00	37.413,10
Papel e mecanismos moleculares do receptor CD36 na fagocitose de célulasapoptóticas: implicações para a aterosclerose Coordinator: Otília Vieira	FCT Refª: PTDC/SAU-MII/66285/2006	01/09/2007 to 31/08/2010	159.936,00	55.987,71
Actividade metabólica e viabilidade do condrócito em enxertos osteocartilagíneos humanos criopreservados.Coordinator: Celeste Lopes	FCT Refª: PTDC/SAU-OSM/67936/2006	01/09/2007 to 31/08/2010	32.648,83	14.174,66
Design, synthesis and biological assessment of multifunctional compounds as anti-Alzheimer drugs Coordinator: Paula Agostinho Participantes: Faculdade de Farmácia Univ. Lisboa	FCT Refª: PTDC/SAU-NEU/64151/2006	01/08/2007 to 31/07/2010	12.740,00	852,82
Modulação das vias metabólicas envolvidas no stress oxidativo mitocondrial em condições de hiperglicémia: sua relevância na prevenção da diabetes. Coordinator: Carlos Palmeira	FCT Refª: PTDC/QUI/72826/2006	01/01/2008 to 31/03/2010	36.000,00	14.871,52
Searching for high level rules in protein folding and unfolding: from amyloid diseases to protein structure prediction Coordinator: Rui Brito Participants: Universidade do Minho	FCT Refª: PTDC/BIA-PRO/72838/2006	01/01/2008 to 31/12/2010	39.556,00	14.401,43

Nanoestruturas endereçadas para imagem molecular médica multimodal. Coordinator: Carlos Geraldes Participants:Universidade do Minho, Faculdade de Medicina Universidade de Coimbra	FCT Refª: PTDC/QUI/70063/2006	01/01/2008 to 31/12/2010	32.352,00	4.913,29
Nanoquimica de compósitos magnéticos/ luminescentes para aplicações de diagnóstico médico in vitro Coordinator: António Guiomar Participants: Universidade de Aveiro	FCT Refª: PTDC/QUI/67712/2006	01/01/2008 to 31/12/2010	15.300,00	5.747,57
Reconstrução e análise sistémica da rede reaccional de espécies reactivas de oxigénio, azoto e enxofre em sistemas fisiológicos representativos. Coordinator: Armindo Salvador Participants: Fundação da Faculdade de Ciências, Universitat de Lleida	FCT Refª: PTDC/QUI/70523/2006	01/01/2008 to 31/12/2010	162.752,00	43.658,06
BIOINK - Aprendizagem incremental de Kernel Machines para análise de dados em bioinformática Coordinator: Paula Verissimo Participants: Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Instituto Superior de Engenharia de Coimbra	FCT Refª: PTDC/EIA/71770/2006	01/01/2008 to 31/12/2010	4.200,00	0,00
Proteases de Polens, relevância nas doenças alérgicas. Coordinator: Paula Verissimo	FCT Refª: PTDC/SAU-ESA/72571/2006	01/05/2008 to 30/04/2011	199.850,00	36.944,59
AspectGrid: Aspectos Grid para Aplicações Científicas Coordinator: Rui Brito	FCT Refª: GRID/GRI/81880/2006	01/07/2007 to 30/06/2010	10.446,00	0,00
Programa MIT Coordinator: Catarina Oliveira, Lino Ferreira	FCT Refª: MIT-Portugal	01/09/2006 to 31/08/2011	854.145,02	121.136,88
Sub – Total FCT				**1.225.564,48**
Studies of the molecular and cellular actions of Eslicarbazepine Acetate (BIA 2-093) as compared to other anti-epileptic drugs Coordinator: Caetana Carvalho, Inês Araújo	BIAL	01/04/2008 to 31/03/2010	145.000,00	34.143,62

Histamine in the neural and cancerstem cell niche: a role inglioblastoma ontogeny. Coordinator: Liliana Bernardino e Fabienne Agasse	Fundação Calouste Gulbenkian Refª: 96542	10/11/2008 to 09/11/2011	50.000,00	5.736,88
Sub – Total Outros				**39.880,50**
Total National Projects				**1.265.444,98**
International Projects:				
Noninvasive measurement of hepatic glycogen kinetics in Type 1 diabetics. Coordinator: John Griffth Jones	JDRF Refª: 1-2006-74	01/05/2006 to 29/02/2009	217.313,00	153,47
EMIL Coordinator: Carlos Geraldes	EMIL Ref.ª EMIL: LSHC-CT-2004-503569	01/07/2005 to 30/06/2009	43.646,87	21.377,02
Dissecting mechanisms of neuronal dysfunction in wound healing in diabetes type 1 Coordinator: Eugenia Carvalho	EFSD/JDRF	01/01/2009 to 31/12/2010	100.000,00	65.114,63
BNOX – The role of reactive oxygen species in B cell tolerization and immune memory Coordinator: Margarida Carneiro	Marie Curie Actions - 239422 Ref .ª: FP7- PEOPLE– ERG-2008	01/06/2009 to 31/05/2012	45.000,00	1.478,61
Transplantation of magnetic – labelled vascular cells and cardiomyocytes isolated from human embryonic stem cells in a bioactive injectable gel for myocardium regeneration after infarct Coordinator: Lino Ferreira	Marie Curie Actions – 230929 Refª: FP7-PEOPLE-2007-4-3-IRG	01/04/2009 to 31/03/2013	100.000,00	147,86
Total International Projects				**88.271,59**
TOTAL				**1.353.716,57**

List of Staff and Research Students | General List

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Amílcar Falcão	(Full Prof., FFUC)	80
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Ledo	(Assistant Inv., CNC)	100
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Silva Martins	(Assistant Inv., FMUC)	Collaborator
Anabela Maduro Almeida	(Assistant Prof., Univ. Vasco Gama)	80
Anabela P. Rolo	(Assistant Inv., CNC)	100
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Ângelo R. Tomé	(Assistant Prof., FCTUC)	70
António F. Ambrósio	(Assistant Inv., FMUC)	Collaborator
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Armanda E. Santos	(Assistant Prof., FFUC)	60
Armando Cristóvão	(Assistant Prof., FCTUC)	70
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Attila Köfalvi	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Carlos G. Geraldes	(Full Prof., FCTUC)	80
Carlos Manuel Matias	(Assistant Inv., FCTUC)	60
Carlos Faro	(Associate Prof., FCTUC)	80
Carlos M. Palmeira	(Associate Prof., FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	80
Célia M. Antunes	(Assistant Prof., FCTUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	60
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Elsa Henriques	(Assistant Inv., FCTUC)	100
Emília P. Duarte	(Associate Prof., FCTUC)	80
Euclides Pires	(Associate Prof., FCTUC)	60
Eugénia Carvalho	(Assistant Inv., CNC)	100
Fabienne Agasse	(Assistant Inv., CNC)	100
Geanne M. Cunha	(Assistant Prof., Brasil)	30
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	Collaborator
Henrique Faneca	(Assistant Inv., CNC)	100
Henrique Bernardo Silva	(Assistant Inv., CNC)	100

Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Araújo	(Assistant Inv., CNC)	100
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Ivana Jarak	(Assistant Inv., CNC)	100
Jean-Pierre Oses	(Professor, Brasil)	30
João Laranjinha	(Associate Prof., FFUC)	60
João Nuno Moreira	(Assistant Prof., FFUC)	60
João O. Malva	(Principal Inv., FMUC)	100
João Ramalho Santos	(Associate Prof., FCTUC)	80
John Griffith Jones	(Principal Inv., CNC)	100
José Custódio	(Associate Prof., FCTUC)	80
Leonor Almeida	(Full Prof., FFUC)	60
Lino Ferreira	(Assistant Inv., CNC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Luís M. Rosário	(Associate Prof., FCTUC)	80
Luís Pereira Almeida	(Assistant Prof., FFUC)	80
Mª Amália Jurado	(Assistant Prof., FCTUC)	60
Mª Carmen Alpoim	(Associate Prof., FCTUC)	60
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Conceição Venâncio Egas	(Investigator, FCTUC)	100
Mª Emilia O. Quinta Ferreira	(Associate Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Mª Isabel J. Santana	(Associate Prof., FMUC)	80
Mª Luisa D. Ramos	(Investigator, FCTUC)	80
Mª Madalena Caldeira Santos	(Associate Prof., FCTUC)	80
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª Sancha Santos	(Investigator, FCTUC)	80
Mª Teresa Cruz Rosete	(Assistant Prof., FFUC)	8
Mª Teresa Girão da Cruz	(Assistant Inv., CNC)	100
Marília Rocha	(Investigator, HUC)	60
Marlene Maria Tourais Barros	(Assistant Prof., FCTUC)	60
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	50
Paula G. Agostinho	(Investigator, FMUC)	80
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Paulo Santos	(Assistant Prof., FCTUC)	80
Ramiro Almeida	(Assistant Inv., CNC)	20

Renata Silva	(Assistant Inv., CNC)	100
Ricardo Reis	(Associate Prof., Brasil)	70
Rodrigo A. Cunha	(Associate Prof., FMUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	60
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui M. M. Brito	(Associate Prof., FCTUC)	50
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Assistant Inv., FMUC)	60
Sérgio Simões	(Assistant Prof., FFUC)	80
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Dinis	(Associate Prof., FFUC)	60
Teresa Gonçalves	(Assistant Prof., FMUC)	40
Teresa Maria C. Martins	(Assistant Investigator, IPO)	80
Tiago Quininha Faria	(Assistant Inv., CNC)	100
Vitor Manuel C. Madeira	(Full Prof., FCTUC)	80

Post-Doc Members

Ana Isabel Duarte	100
Ana Luísa Cardoso	100
Ana Rita Álvaro	100
Anália do Carmo	100
Bharathi Pandurangan	100
Bruno O. Manadas	100
Carla Nunes	100
Chakkaravarthi Saravanan	100
Clévio Nóbrega	100
Daniela Pochmann	40
Dora Pedroso	100
Elisabete Baptista Ferreiro	100
Ermelindo Leal	100
Giovannia Araujo de Lima Pereira	40
Joana Cardoso da Costa	100
Joana Salgado	100
João Gonçalo Silva Frade	100
João Miguel Neves Duarte	100
Licinia J. Simões	100
Liliana Bernardino	100
Luis Miguel Estronca	100
Manuel Garrido	100
Manuella P. Kaster	100

Margarida Vaz Caldeira	100
Mª Teresa Cunha Oliveira	100
Paula Sofia S. Lacerda	Collaborator
Rosa M. B. Matos Resende	100
Sara Xapelli	100
Susana Isabel E. Alarico	100
Tatiana R. Rosenstock	100
Vilma A. Oliveira	100

PhD Students

Adriana Santos	100
Alexandra Rosa	100
Alexandre S. Rodrigues	75
Ana Burgeiro	100
Ana C. Fortuna	100
Ana Carolina Moreira	100
Ana Catarina R. Graça Fonseca	100
Ana Catarina H. Oliveira	100
Ana Cristina R. Silva	100
Ana Filipe Branco	100
Ana Francisca Soares	100
Ana Isabel Serralheiro	100
Ana Luísa N. Gomes Nobre	100
Ana Luísa Vital	100
Ana Maló de Abreu	100
Ana Paula Ardais	40
Ana Paula Marques de Sousa	100
Ana Patricia S. Gomes	100
Ana Patrícia Simões	100
Ana Rafael	100
Ana Raquel Esteves	100
Ana Raquel M. Soares	100
Ana Rita A. Santos	100
Ana Santos Carvalho	100
Ana Sofia V. Cunha	100
Ana Sofia Rodrigues	100
Ana Sofia Bento Baptista	50
Ana Tellechea	100
Ana Teresa I. Varela	100
Ana Teresa Rufino	100
Ana Teresa Simões	100
Andrea Lobo	100

Anaísa Pires	100
André F. Martins	100
Ângela Inácio	100
António Sales Mano	100
Bárbara Rocha	100
Beatriz Lacerda de Sousa	100
Bruno Carreira	100
Bruno Miguel das Neves	100
Camile Woitiski	100
Cândida S. Gonçalves da Silva	85
Carla Sofia G. Silva	100
Carlos Adriano Matos	100
Carlos Manuel Melo	100
Carlos José Vieira Simões	80
Carlos Samuel M. Boto	100
Carlos Rodrigues	100
Carolina Coelho	50
Cassilda Pereira	100
Catarina Sofia H. Jesus	100
Cátia Diogo	100
Cátia Marques	100
Clarissa S. Schitine	70
Cláudia Sofia Alves Pereira	100
Cristina Carvalho	100
Cristiana Paulo	100
Cristina Barosa	100
Daniela Cipreste Vaz	100
Daniela M. Arduíno	100
Diana Margarida Carvalho	100
Elisabete Oliveira Augusto	100
Filipe Coreta Gomes	40
Filipe Duarte	100
Filomena Grilo da Silva	100
Francisca Soares	100
Gabriel Costa	100
Gianna Paula Cognato	70
Gonçalo Pereira	100
Gracina Tributo	60
Graciano da Silva Leal	100
Helena Carvalheiro	100
Helena Leitão	100
Helena Sofia Domingues	10
Helena Vazão	100
Hugo Prazeres	100
Inês Crespo	100

Inês Violante	10
Igor Clemente Tiago	100
Ilídio Martins	100
Inês Biscaia Barbosa	100
Inês Vasconcelos Miranda Santos	100
Isabel Maria Santos Onofre	100
Ivan Viegas	100
Joana Filipa C. Fernandes	100
Joana Ferreira	100
Joana I. Real	100
Joana Paixão	100
Joana Santos Barbosa	10
João André Duarte	25
João Monteiro	100
João Carlos R. Gomes	100
João Teixeira	100
João Teodoro	100
João Trigueiro Costa	100
José Mário Tenera Morgado	100
Liana Moura	100
Ligia Gomes da Silva	100
Lígia Maria Ferreira	100
Liliana Mendonça	100
Luís André A. França	100
Luís Ribeiro	100
Magda Santana	100
Márcio José C. Ribeiro	100
Márcio José Abreu M. Rodrigues	90
Marco António P. Matos	100
Mª Alexandra B. Amaral	100
Mª Francisca Eiriz	100
Mª Inês Morte	100
Mª Isabel Nascimento Ferreira	100
Mª João Rodrigues Pereira	50
Maria Nunes Pereira	100
Marco Alves	100
Mariana Freitas	100
Mariana Ponte C. Ribeiro	100
Mariana Vagos Ribeiro	100
Marília Henriques Cordeiro	100
Mário Laço	100
Marta Daniela Passadouro Caetano	100
Marta Isabel D. Mota Vieira	100

Marta Isabel Rodrigues Baptista	100
Marta Viegas da Silva	100
Michelle Stumpf Viegas	100
Nélio Gonçalves	100
Pablo Devessa Peleteiro	70
Pablo Pandolfo	75
Paula M. Canas	100
Paula Mota	100
Paulo Jorge Fernandes Rodrigues dos Santos	25
Paulo Gameiro	100
Pedro Manuel Batista Branco	100
Pedro Coxito	100
Pedro Manuel V. Garção	100
Pedro Miguel Brás M. Coelho	10
Pedro Réu Carvalho	10
Pedro Miguel Costa	100
Raquel Ferreira	100
Renata Gomes	100
Renata Santos Tavares	100
Renato Xavier C. Santos	100
Ricardo Santos	100
Rita Perfeito	100
Rui Nobre	100
Rui Oliveira Costa	100
Rui Sanches	100
Rui Vasco Simões	100
Sandra Catarina G. Amaral	100
Sandra Filipa T. Varum	100
Sandra Isabel F. Mota	100
Sandra Marina A. Santos	100
Sandro Pereira	100
Samira C. Ferreira	100
Sara C. Figueiredo	100
Sara Gonçalves	100
Sara Tavares M. Lima	100
Sara Trabulo	100
Sezin Aday	100
Sofia Grade	100
Sónia Correia	100
Sónia Duarte	100
Sueli Cristina Marques	100
Susana Carvalho Rosa	100
Susana Cardoso	100
Susana Ribeiro Louros	100

Tatiana Catarino	100
Teresa Serafim	100
Tiago Alfaro	20
Tiago Alves	100
Vera Lúcia G. Francisco	100
Vera Moura	100
Vera Patricia Gonçalves	100
Vitor Gonçalo Silva C. Mendes	100

MSc Students

Ana Branco M. Tiago	100
Ana Mª Sequeira Cardoso	100
Ana Metelo	100
Ana Catarina M. Ferreira	50
Ana Catarina Oliveira	50
Ana Rita Bento	100
Ana Rita Gonçalves	100
Ana Isabel Plácido Fernandes	100
Ana Patrícia Marques	100
Ana Silva	100
Ana Sofia L. Coelho	100
Andreia Esteves Sousa	100
Carla Sofia Alexandre	100
Carla Mª N. Lopes	80
Carla Patrícia R. Paiva	100
Carolina Noronha	50
Catarina Morais	100
Cláudia Vanessa Moniz	100
Daniel Ramos Andrade	20
Daniela Pinheiro	100
David G. Dias	100
Diana F. Gomes Pimentel	100
Diana Moreira	50
Diana Silva	100
Diogo Comprido	100
Diogo Martins-Branco	20
Dulce Marisa ferreira Bento	100
Fátima Martins	100
Filipa Alexandra S. Curado	70
Filipa Carvalho	100
Filipa Raquel Lebre	100
Flávio Fortes R. Sousa	75

Gabriel Paiva	100
Henrique Carvalho	100
Hugo Aragão	100
Hugo Alves Figueiredo	100
Inês Cardoso	100
Inês Santarino	100
Isaura Vanessa Martins	100
Joana Balsa C. Silva	100
Joana Barra	100
Joana Serôdia	100
Liliana Correia	100
Luana Naia	100
Lucília Silva	100
Maria Joana G. Pinto	100
Mª João R. Ferreira Ribeiro	100
Mariana Vagos Ribeiro	100
Miguel Bajouco	50
Nuno Gabriel Machado	100
Patrícia Araújo Sousa	20
Patricia Henriques Domingues	100
Patrícia Soares Rebelo	100
Patrícia Sofia A. Morais	100
Pedro Alexandre Martins	100
Raquel Vinhas	100
Rui Filipe Carvalho	100
Rui Cruz	100
Rui Pedro Lopes	60
Sandrine Machado	10
Sara Varela Amaral	100
Sílvia Catarina F. Gomes	100
Steve François dos Santos Carvalho	100
Tiago Alexandre Sousa Santos	100
Vera Grandão Cortez	100
Vitor Hugo R. Cabral	25
Zaida Catarina Almeida	100

Grant Technician

Alexandra Sofia T. Silva Moura	17
Anabela Simões	100
Ankit Goenka	25
Gonçalo Nuno Neto	70
Joana Pedro	100

José Manuel Moreno Silvestre	50
Katia Mesquita	100
Ludgero Tavares	100
Manuel Joaquim G. Matos	100
Miguel Maria Ferreira Lino	5
Nuno Miguel Machado	100
Pedro Cruz	100
Susana Pereira	100
Tiago Francisco Santos Ferreira	100

Undergraduate Students

Branca M. Silva	20
Diana dos Santos Mota	10
Diana Isabel Guedes Rodrigues	100
Estela Filipa S.A Alves	100
Filipa Isabel C. Baptista	10
Joana Mararida N. Gaspar	10
João Filipe C. Martins	10
João Silva	100
Nelson Cunha	10
Sabina Chiello	100

SERVICE STAFF

		Time % at CNC
Sandra Manuela Domingues dos Santos	(Graduate Technician, CNC)	100
Ana Margarida Ferreira	(Graduate Technician, CNC)	100
António José Azinhaga Teles Grilo	(Graduate Technician, CNC)	100
Cândida Elsa Frias Mendes	(Graduate Technician, CNC)	100
Carla Margarida dos Santos Veríssimo	(Graduate Technician, CNC)	100
Emeric Wasielewski	(Graduate Technician, CNC)	100
João Miguel Pratas	(Graduate Technician, CNC)	100
Luciana C. Albuquerque Pinto	(Graduate Technician, CNC)	100
Luís Miguel M. Vidal Oliveira	(Graduate Technician, CNC)	100
Maria Helena Garruncho	(Graduate Technician, CNC)	Collaborator
Maria João Ferreira Canas dos Santos	(Graduate Technician, CNC)	100
Marta Sofia Marques Simões	(Graduate Technician, CNC)	100
Teresa Proença	(Graduate Technician, CNC)	Collaborator
Vera Mendes	(Graduate Technician, CNC)	100

TECHNICAL STAFF

		Time % at CNC
Alexandre Simão Vieira Pires	(Graduate Technician, CNC)	100
Cármen Lídia Graça Semeão	(Graduate Technician, CNC)	100
Diana dos Santos Simões Graça	(Technician, CNC)	100
Fátima Cristina dos S. Carvalho Graça	(Technician, CNC)	100
Filomena Maria F. Pereira dos Santos	(Technician, CNC)	100
Isabel Conceição Calado Esteves Costa	(Technician, CNC)	100
Isabel Nunes Correia	(PhD, Graduate Technician, CNC)	100
Isabel Dantas Fernandes	(Graduate Technician, CNC)	100
Luisa Leitão Cortes	(PhD, Graduate Technician, CNC)	100
Maria do Céu Mendes Gomes	(Technician, CNC)	100
Maria Isabel Gonçalves	(Technician, CNC)	100
Maria Eugénia A. Silva Lopes Campos	(Technician, CNC)	100
Virginia Maria R. Ferreira Fonseca	(Technician, CNC)	100
Maria da Rosário da Costa Faro	(Graduate Technician, CNC)	100
Odete Pereira Cabanelas	(Graduate Technician, CNC)	100

ADMINISTRATIVE STAFF

		Time % at CNC
Carla Lopes Rodrigues	(Administrative Assistant, CNC)	100
Catarina Alexandra Ferreira Gomes	(Graduate Administrative, CNC)	100
Elisabete Cosmos dos Santos Machado	(Graduate Administrative, CNC)	100
Heidi Maria da Silva Lopes Gonçalves	(Graduate Administrative, CNC)	100
Lia da Costa Jordão Aparício Lopes	(Graduate Administrative, CNC)	100
Rosa Alexandra Folhas Fernandes	(Graduate Administrative, CNC)	100
Sandra Cristina Santos Luís	(Graduate Administrative, CNC)	100
Sílvia Marisa Esteves de Sousa	(Graduate Administrative, CNC)	100
Susana Adelaide Rocha da Silva	(Administrative Assistant, CNC)	100
Tatiana de Azevedo Paula	(Graduate Administrative, CNC)	100

Research Staff and Students | Research Área

Neuroscience and Disease

Catarina Resende Oliveira, MD, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Ana Cristina Rego	(Assistant Prof., FMUC)	60
Ana Luísa Carvalho	(Assistant Prof., FCTUC)	80
Ana Paula Silva Martins	(Assistant Inv., FMUC)	Collaborator
Ângelo Tomé	(Assistant Prof., FCTUC)	70
António F. Ambrósio	(Investigator, FMUC)	Collaborator
Armanda E. Santos	(Assistant Prof., FFUC)	60
Armando Cristóvão	(Assistant Prof., FCTUC)	70
Arsélio P. Carvalho	(Full Prof., FCTUC)	80
Attila Köfalvi	(Assistant Inv., CNC)	100
Caetana Carvalho	(Full Prof., FCTUC)	80
Carlos B. Duarte	(Associate Prof., FCTUC)	80
Catarina R. Oliveira	(Full Prof., FMUC)	80
Cláudia Cavadas	(Assistant Prof., FFUC)	80
Cláudia M. F. Pereira	(Investigator, FMUC)	60
Emília P. Duarte	(Associate Prof., FCTUC)	80
Fabienne Agasse	(Assistant Inv., CNC)	100
Geanne M. Cunha	(Associate Prof., Brasil)	30
Henrique Bernardo Silva	(Assistant Inv., CNC)	100
Ildete Luísa Ferreira	(Assistant Inv., CNC)	100
Inês Araújo	(Assistant Inv., CNC)	100
Isabel Maria Marques Carreira	(Assistant Prof., FMUC)	60
Jean-Pierre Oses	(Professor, Brasil)	30
João O. Malva	(Principal Inv., FMUC)	100
Lisiane O. Porciúncula	(Assistant Prof., Brasil)	30
Mª Isabel J. Santana	(Associate Prof., FMUC)	80
Mª Manuela Monteiro Grazina	(Assistant Prof., FMUC)	60
Paula G. Agostinho	(Investigator, FMUC)	80
Paula Isabel Moreira	(Assistant Prof., FMUC)	60
Paulo Santos	(Assistant Prof., FCTUC)	80
Ramiro Almeida	(Assistant Inv., CNC)	20
Ricardo Reis	(Associate Prof., Brasil)	70
Rodrigo A. Cunha	(Associate Prof., FMUC)	80
Sandra Isabel M. Cardoso	(Assistant Prof., FMUC)	60
Sandra Maria R. Carvalho Bós	(Investigator, FMUC)	60

Post-Doc Members

Ana Isabel Duarte	100
Ana Rita Álvaro	100
Daniela Pochmann	40
Elisabete Baptista Ferreiro	100
Joana Salgado	100
Liliana Bernardino	100
Manuella P. Kaster	100
Margarida Alexandra Vaz Caldeira	100
Mª Teresa Cunha Oliveira	100
Rosa M. B. Matos Resende	100
Sara Xapelli	100
Tatiana R. Rosenstock	100

PhD Students

Alexandra Rosa	100
Alexandre S. Rodrigues	75
Ana Catarina H. Oliveira	100
Ana Catarina Ribeiro G. Fonseca	100
Ana Cristina R. Silva	100
Ana Patrícia Simões	100
Ana Raquel Esteves	100
Ana Rita A. Santos	100
Ana Santos Carvalho	100
Andrea Lobo	100
Bruno Carreira	100
Carla Sofia G. Silva	100
Carlos Adriano Matos	100
Clarissa S. Schitine	70
Cristina Carvalho	100
Daniela M. Arduíno	100
Elisabete Oliveira Augusto	100
Gabriel Costa	100
Gianna Paula Cognato	70
Graciano da Silva Leal	100
Helena Sofia Azevedo Domingues	10
Joana Ferreira	100
Joana Filipa C. Fernandes	100
Joana Santos Barbosa	10

João Rodrigues Gomes	100
João Trigueiro Costa	100
Magda Santana	100
Márcio José C. Ribeiro	100
Marco António P. Matos	100
Mª Francisca Eiriz	100
Mª Inês Morte	100
Mário Laço	100
Marta Isabel D. Mota Vieira	100
Pablo Devessa Peleteiro	70
Pablo Pandolfo	75
Paula M. Canas	100
Pedro Manuel V. Garção	100
Pedro Réu Carvalho	10
Raquel Ferreira	100
Renato Santos	100
Rita Perfeito	100
Rui Oliveira Costa	100
Rui Sanches	100
Samira C. Ferreira	100
Sandra Isabel F. Mota	100
Sílvia Viana Silva	40
Sofia Grade	100
Sónia Correia	100
Sueli Cristina Marques	100
Susana Cardoso	100
Susana Ribeiro Louros	100
Tatiana Catarino	100
Tiago Alfaro	20

MSc Students

Ana Isabel Plácido Fernades	100
Ana Patrícia Marques	100
Ana Rita Bento	100
Carla Mª N. Lopes	80
Carolina Noronha	50
Daniel Ramos Andrade	100
Diana Gomes Pimentel	100
Diogo Martins-Branco	20
Diogo Oliveira Comprido	100
Isaura Vanessa Martins	100

Luana Naia	100
Maria Joana G. Pinto	100
Mª João R. Ferreira Ribeiro	100
Miguel Bajouco	50
Patrício Araújo Sousa	20
Patrícia C. Soares Rebelo	100
Sandrine Machado	10
Sílvia Catarina F. Gomes	100
Steve François S. Carvalho	100
Tiago Alexandre Sousa Santos	100
Vera Grandão Cortez	100

Undergraduate Students

Filipa Isabel C. Baptista	100
Joana Margarida N. Gaspar	100
João Filipe C. Martins	100

Grant Technician

Diana Isabel Gudes Rodrigues	100
Nuno Miguel Machado	100

Molecular Biotechnology and Health

Euclides Pires, PhD, Coordinator

Members holding PhD		**Time % at CNC**
André Xavier C. Negrão Valente	(Assistant Inv., CNC)	100
Armindo J. Alves S. Salvador	(Assistant Inv., CNC)	100
Carlos Faro	(Associate Prof., FCTUC)	80
Elsa Henriques	(Investigator, FCTUC)	100
Euclides Pires	(Associate Prof., FCTUC)	60
Henrique Faneca	(Assistant Inv., CNC)	100
João Nuno Moreira	(Assistant Prof., FFUC)	60
Lino Ferreira	(Assistant Inv., CNC)	100
Luís Pereira Almeida	Assistant Prof., FFUC)	80
Mª Conceição Pedroso de Lima	(Full Prof., FCTUC)	80
Mª Conceição Venâncio Egas	(Investigator, FCTUC)	100
Marlene Maria Tourais Barros	(Assistant Prof., FCTUC)	60
Olga Maria F. Borges Ribeiro	(Assistant Prof., FFUC)	50
Paula Veríssimo Pires	(Assistant Prof., FCTUC)	60
Renata Dias da Silva	(Assistant Inv., CNC)	100
Rui M. M. Brito	(Associate Prof., FCTUC)	50
Sérgio Simões	(Assistant Prof., FFUC)	80
Tiago Quininha Faria	(Assistant Inv., CNC)	100

Post-Doc Members	
Ana Luísa Cardoso	100
Bharathi Pandurangan	100
Chkkaravarthi Pandurangan	100
Clévio Nóbrega	100
Dora Pedroso	100
Manuel Garrido	100
Paula Sofia S. Lacerda	5

PhD Students	
Adriana Santos	100
Ana Teresa Simões	100
Cândida S. Gonçalves da Silva	85
Carlos José Vieira Simões	80

Carlos Samuel M. Boto	100
Catarina Sofia H. Jesus	100
Cristiana Paulo	100
Daniela Cipreste Vaz	100
Helena Vazão	100
Inês Vasconcelos Miranda Santos	100
Isabel Maria Santos Onofre	100
Lígia Maria Ferreira	100
Ligia Gomes da Silva	100
Liliana Mendonça	100
Maria Nunes Pereira	100
Mª Isabel Nascimento Ferreira	100
Marta Daniela Passadouro Caetano	100
Nélio Gonçalves	100
Pedro Manuel Batista Branco	100
Pedro Miguel Brás M. Coelho	10
Pedro Miguel Costa	100
Renata Gomes	100
Sara Trabulo	100
Sezin Aday	100
Sónia Duarte	100
Vera Moura	100

MSc Students

Cláudia Vanessa Moniz	100
Dulce Marisa Ferreira Bento	100
Filipa Raquel Maia F. Lebre	100
Flávio Fortes R. Sousa	75
Inês Cardoso	100
Joana Serôdia	100
Pedro Alexandre Martins	100
Raquel Vinhas	100
Rui Cruz	100
Sara Varela Amaral	100
Zaida Catarina L. Almeida	100

Grant Technician

Alexandra Sofia Silva Moura	17
Ankit Goenka	25
Gonçalo Nuno Neto	70
José Manuel P. Moreno Silvestre	50
Miguel Maria Ferreira Lino	5
Pedro Cruz	100
Tiago Francisco Santos Ferreira	100

Cell and Molecular Toxicology

Leonor Almeida, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Amílcar Falcão	(Full Prof., FFUC)	80
Ana Ledo	(Assistant Inv., CNC)	100
Anabela Maduro de Almeida	(Assistant Prof., Univ. Vasco Gama)	80
Anabela P. Rolo	(Assistant Inv., CNC)	100
Carlos M. Palmeira	(Associate Prof., FCTUC)	80
Gilberto Alves	(Assistant Prof., Univ Beira Int.)	Collaborator
João Laranjinha	(Associate Prof., FFUC)	60
José Custódio	(Associate Prof., FCTUC)	80
Leonor Almeida	(Full Prof., FFUC)	60
Mª Carmen Alpoim	(Associate Prof., FCTUC)	60
Mª Amália Jurado	(Assistant Prof., FCTUC)	60
Maria S. Santos	(Investigator, FCTUC)	80
Marília Rocha	(Investigator, HUC)	60
Paulo J. Oliveira	(Assistant Inv., CNC)	100
Rui Barbosa	(Assistant Prof., FFUC)	60
Rui A. Carvalho	(Assistant Prof., FCTUC)	60
Teresa Dinis	(Associate Prof., FFUC)	60

Post-Doc Members	
Carla Nunes	100
João Gonçalo Oliveira Frade	100
João Miguel Neves Duarte	100
Vilma Sardão Oliveira	100

PhD Students	
Ana Burgeiro	100
Ana C. Fortuna	100
Ana Carolina Moreira	100
Ana Filipe Branco	100
Ana Francisca Soares	100
Ana Isabel A. Serralheiro	100
Ana Maló de Abreu	100
Ana Patricia S. Gomes	100
Ana Rafael	100
Ana Teresa I. Varela	100

Anaísa Pires	100
António Sales Mano	100
Bárbara Rocha	100
Camile Woitiski	100
Carlos Rodrigues	100
Cassilda Pereira	100
Cátia Diogo	100
Cátia Marques	100
Cláudia Sofia Alves Pereira	100
Filipe Duarte	100
Filomena Grilo da Silva	100
Francisca Soares	100
Gonçalo Pereira	100
Graciana Tributo	60
Ilídio Martins	100
Inês Biscaia Barbosa	100
Joana Paixão	100
João Monteiro	100
João Teodoro	100
Márcio José M. Rodrigues	90
Marco Aurélio Alves	100
Mariana Ponte Cardoso Ribeiro	100
Mariana Vagos Ribeiro	100
Paulo Gameiro Guerreiro	100
Ricardo Santos	100
Rui Vasco P. Simões	100
Sandra Marina A. Santos	100
Sandro Pereira	100
Sara Gonçalves	100
Teresa Serafim	100
Tiago Alves	100

MSc Students

Ana Mª Sequeira Cardoso	100
Ana Silva	100
Carla Sofia O. Alexandre	100
Catarina Morais	100
Fátima Martins	100
Filipa Carvalho	100
Hugo Aragão	100
Mariana Vagos Ribeiro	100
Nuno Gabriel Machado	100

Undergraduate Students

Estela Filipa Alves	100

Grant Technician

Anabela Simões	100
Joana Mª Ramalho A. Sousa	100
Ludgero Canário Tavares	100
Manuel Joaquim G. Matos	100
Susana Pereira	100

Microbiology

Milton Costa, PhD, Coordinator

Members holding PhD		**Time % at CNC**
António Manuel Veríssimo Pires	(Assistant Prof., FCTUC)	80
Mª Fernanda P. N. Gomes Nobre	(Investigator, FCTUC)	80
Milton Simões da Costa	(Full Prof., FCTUC)	80
Nuno Miguel Silva Empadinhas	(Assistant Inv., CNC)	100
Teresa Gonçalves	(Assistant Prof., FMUC)	40
Post-Doc Members		
Joana Cardoso da Costa		100
Susana Isabel E. Alarico		100
PhD Students		
Ana Luísa N. Gomes Nobre		100
Ana Sofia V. Cunha		100
Carolina Coelho		50
Igor Clemente Tiago		100
Luis André A. França		100
Vitor Gonçalo Silva C. Mendes		100
MSc Students		
Gabriel Paiva		100
Ana Branco M. Tiago		100
Ana Catarina M. Ferreira		50
Filipa Alexandre S. Curado		70
Vitor Hugo R. Cabral		25
Undergraduate Students		
Branca Silva		20
Diana dos Santos Mota		10
Nelson Alexandre C. Cunha		10

Biophysics and Biomedical NMR

Carlos Geraldes, PhD, Coordinator

Members holding PhD		**Time % at CNC**
Carlos G. Geraldes	(Full Prof., FCTUC)	80
Célia M. Antunes	(Assistant Prof., FCTUC)	80
Ivana Jarak	(Assistant Inv., CNC)	100
John Griffith Jones	(Principal Inv., CNC)	100
Luís M. Rosário	(Associate Prof., FCTUC)	80
Mª Luisa D. Ramos	(Investigator, FCTUC)	80
Mª Madalena Caldeira Santos	(Associate Prof., FCTUC)	80
Mª Margarida Catalão Castro	(Assistant Prof., FCTUC)	80
Rosa M. Santos	(Assistant Prof., FCTUC)	60
Post-Doc Members		
Giovannia Araujo de Lima Pereira		40
Licinia J. Simões		100
PhD Students		
André Martins		100
Cristina Barosa		100
Filipe Coreta Gomes		40
Helena Leitão		100
Hugo Prazeres		100
Inês Violante		10
Ivan Viegas		100
Joana I. Real		100
João André Duarte		25
João Teixeira		100
Pedro Coxito		100
Sara Figueiredo		100
MSc Students		
Ana Rita Gonçalves		100
Ana Metelo		100
Andreia Raquel Sousa		100
Daniela Pinheiro		100
David Gaspar Dias		100
Henrique Carvalho		100

Hugo Figueiredo	100
Joana Barra	100
Rui Silva Carvalho	100
Rui Pedro Lopes	60

Cell and Development Biology

Mª Celeste Lopes, PhD, João Ramalho Santos, PhD, Coordinators

Members holding PhD		**Time % at CNC**
Alexandrina F. Mendes	(Assistant Prof., FFUC)	80
Ana Bela Sarmento Ribeiro	(Assistant Prof., FMUC)	40
Artur Augusto Paiva	(Graduate Technician, HUC)	50
Eugénia Carvalho	(Assistant Inv., CNC)	100
João Ramalho Santos	(Associate Prof., FCTUC)	80
Mª Celeste Lopes	(Full Prof., FFUC)	80
Mª Margarida Souto-Carneiro	(Assistant Inv., CNC)	100
Mª Otilia Vieira	(Assistant Inv., CNC)	100
Mª Teresa Cruz Rosete	(Assistant Prof., FFUC)	80
Sukalyan Chaterjee	(Principal Inv., CNC)	100
Teresa Maria C. Martins	(Assistant Inv., IPO)	80

Post-Doc Members	
Anália do Carmo	100
Ermelindo Leal	100
Luis Miguel Estronca	100

PhD Students	
Ana Luísa Vital	100
Ana Paula Marques de Sousa	100
Ana Raquel M. Soares	100
Ana Sofia Rodrigues	100
Ana Tellechea	100
Ana Teresa Rufino	100
Ângela Inácio	100
Beatriz Lacerda de Sousa	100
Bruno Miguel das Neves	100
Carlos Manuel Melo	100
Diana Margarida Carvalho	100
Helena Carvalheiro	100
Inês Crespo	100
José Mário Tenera Morgado	100
Liane Moura	100
Mª Alexandra B. Amaral	100

Mª João R. Pereira	50
Mariana Freitas	100
Marília Henriques Cordeiro	100
Marta Isabel Rodrigues Baptista	100
Marta Viegas da Silva	100
Michelle Stumpf Viegas	100
Paula Mota	100
Paulo Jorge R. dos Santos	25
Sandra Catarina G. Amaral	100
Sandra Filipa T. Varum	100
Sara Tavares M. Lima	100
Susana Carvalho Rosa	100
Renata Santos Tavares	100
Rui Nobre	100
Vera Lúcia G. Francisco	20

MSc Students

Ana Catarina Oliveira	50
Ana Sofia L. Coelho	100
Carla Patrícia R. Paiva	100
Diana Moreira	100
Joana Balça C. Silva	100
Inês Santarina	100
Liliana Correia	100
Lucília Silva	100
Patricia Henriques Domingues	100
Patrícia Sofia A. Morais	100

Grant Technician

Joana Pedro	100
Katia Mesquita	100

Undergraduate Students

João Silva	100
Sabina Chielo	100

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