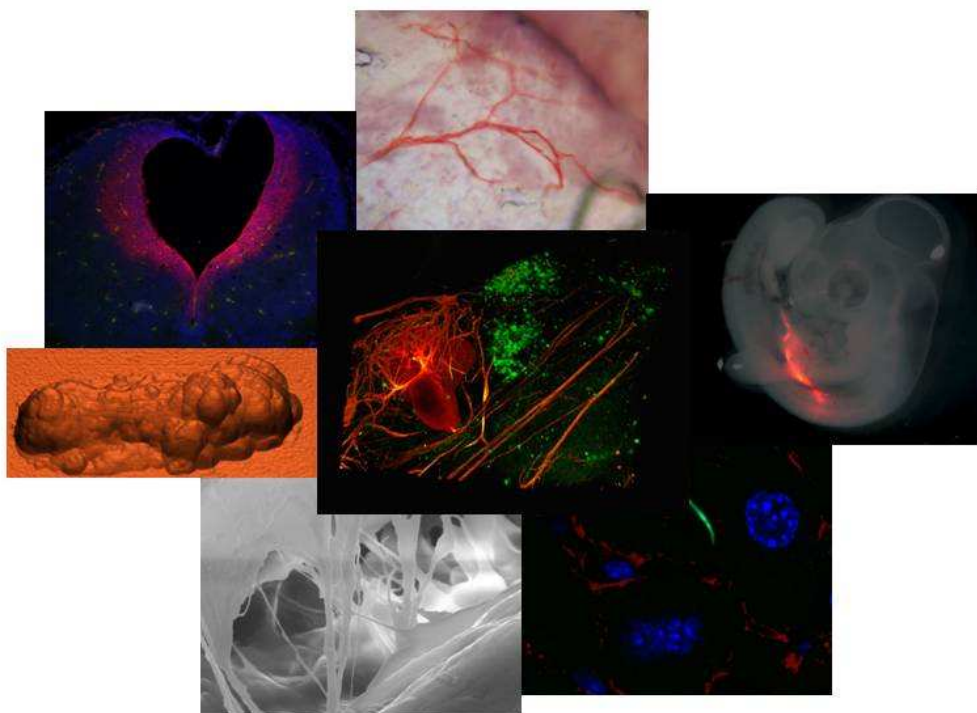
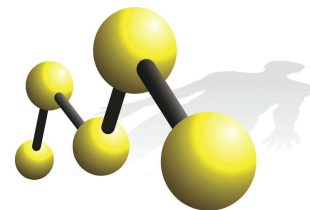


# INSTITUTO DE MEDICINA MOLECULAR



## *2009 Activity Report*



INSTITUTO DE  
MEDICINA MOLECULAR



**TABLE OF CONTENTS**

<b>Structure and Organization</b>	<b>5</b>
<b>2009 Activities Highlights</b>	<b>7</b>
<b>Research Funding</b>	<b>9</b>
<b>IMM Indicators</b>	<b>13</b>
<b>Publications in International Journals in 2009</b>	<b>15</b>
<b>Seminars and Workshops</b>	<b>28</b>
<b>IMM International PhD Programme</b>	<b>40</b>
<b>Partnerships</b>	<b>45</b>
<b>RESEARCH</b>	<b>47</b>
<b>Cell and Developmental Biology programme</b>	<b>49</b>
Cell Biology Unit	51
Chromatin Biology Unit	55
Developmental Biology Unit	58
Tissue Morphogenesis and Repair Unit	63
Microvascular Biology and Inflammation Unit	68
Angiogenesis Unit	74
Biomembranes Unit	77
Physical Biochemistry Unit	83
Cell Biology of the Immune System Unit	90
Cancer Biology Unit	94
Clinical and Translational Oncology Research Unit	99
Embryonic Development of Vertebrates Unit	103
Gene Expression & Biophysics Unit	106
<b>Immunology &amp; Infectious Diseases Programme</b>	<b>109</b>
Clinical Immunology Unit	111
Rheumatology Research Unit	119
Viral Pathogenesis Unit	129
Molecular Microbiology and Infection Unit	131
Malaria Unit	135
Cellular Immunology Unit	141
Molecular Immunology Unit	147
Nutrition and Metabolism Unit	152
Immunobiology Unit	164
Molecular Parasitology Unit	168
Mycobacteria-Host Interactions Unit	171
Retroviruses Unit	176
Parasite Molecular Genetics Unit	180
<b>Neurosciences</b>	<b>183</b>
Neurosciences Unit	185
Neuromuscular Unit	198
Autonomic Nervous System Unit	207
Neurological Clinical Research Unit	214
Cell and Molecular Neuroscience Unit	246

#### **4 | 2009 IMM Activity Report**

##### **FACILITIES AND SUPPORT**

Management Unit	253
Communication and Training Unit	255
Information Systems Unit	261
Lab Management Unit	263
BioImaging Unit	265
Flow Cytometry Unit	267
Animal Facility	270
Zebrafish Facility	271
Histology Service	272

##### **COMPANIES**

GenoMed	275
TechnoPhage	281

## Structure and Organization

Research and development in the Biomedical Sciences plays a central role in generating knowledge and in the application of this knowledge to improving the quality of life. *The mission of the Institute of Molecular Medicine (IMM) is to foster basic, clinical and translational biomedical research* with the aim of contributing to a better understanding of disease mechanisms, developing novel predictive tests, improving diagnostics tools and developing new therapeutic approaches.

Located on the campus of the Faculty of Medicine of the University of Lisbon, IMM is a Laboratory Associated to the National Ministry of Science and Higher Education. IMM is mainly supported by national public funds and European Union funds. The research expenditure includes additional funds obtained from peer reviewed competitive grants, private donations and industrial partnerships.

Created in December 2001, IMM results from the association of 5 former research units from the Faculty of Medicine of the University of Lisbon: the Biology and Molecular Pathology Centre, the Lisbon Neurosciences Centre, the Microcirculation and Vascular Pathobiology Centre, the Gastroenterology Centre and the Nutrition and Metabolism Centre. The Institute is a Private, Non-profit Association.

### IMM Associate Members

Universidade de Lisboa  
Faculdade de Medicina da Universidade de Lisboa  
Hospital de Santa Maria

Fundação da Universidade de Lisboa  
Associação para a Investigação e o Desenvolvimento da Faculdade de Medicina  
Fundação Oriente

### Board of Trustees

The Board of Trustees is composed by representatives of associate members and meets at least once per year to analyse the scientific and finance report and to approve the plan of activities and budget for the next year.

### Board of Directors

The Board of Directors is responsible for the management of the Institute according to the plans approved by the Trustees. The Board of Directors is elected by the Trustees.

João Lobo Antunes, MD, PhD (President)  
Maria Carmo-Fonseca, MD, PhD (Executive Director)  
Joaquim Alexandre Ribeiro, MD, PhD (Vice-President)

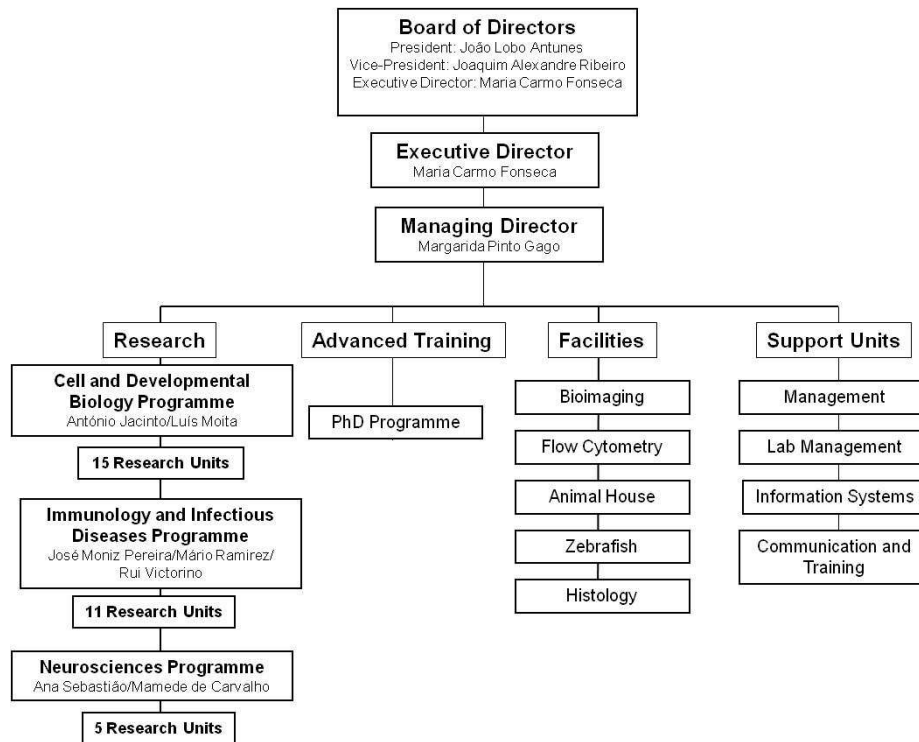
*Patrícia da Cunha (Secretary)*

### Administration Board

The Administration Board is composed by the Directors, the Research Programme Coordinators, and the Directors of the *Faculdade de Medicina* and *Hospital de Santa Maria*. It meets at least twice a year to define scientific strategies and actions, administrative organization plans and budgetary priorities.

### Council of Scientists

The Council of Scientists is composed by all Principal Investigators. It meets at least once per year to discuss the scientific strategies and plan of actions proposed by the Board of Directors. This Council elects two representatives to participate in the meetings of the Board of Trustees.



### Scientific Advisory Committees

Undertake periodic evaluations to the IMM specific programmes and include international experts of scientific fields analysed.

#### *Immunology and Infectious Diseases Programme – 2009*

Anne O’Garra, National Institute for Medical Research, UK  
 Alain Fischer, Hôpital Necker Enfants Malade, Paris, France  
 William Paul, National Institute of Allergy and Infectious Diseases, NIH, USA  
 Philippe Sansonetti, Institut Pasteur, France  
 Antonio Freitas, Institut Pasteur, France

#### *Neurosciences Programme – 2005*

Hanna Damasio (Chair), University of Southern California, USA  
 Charles Warlow, Western General Hospital Edinburgh, UK  
 Michael Swash, Royal London Hospital, UK  
 S. Grillner, Karolinska Institutet, Stockholm, Sweden  
 K.M. Spyer, University College of London; UK

#### *Cell and Developmental Biology Programme – 2006*

Philip Ingham, University of Sheffield/Institute of Molecular and Cell Biology, Singapore  
 Frank Grosveld, Erasmus University, Rotterdam, the Netherlands  
 Outi Hovatta, Karolinska Institutet, Stockholm, Sweden  
 Iain Mattaj, European Molecular Biology Laboratory, Heidelberg, Germany  
 Axel Pries, Department of Physiology, Charité-Berlin, Germany

## 2009 IMM Activities Highlights

### Productivity

- **187** papers published in peer-reviewed journals;
  - **13** publications in journals with an impact factor higher than 10
  - **36** publications in journals with an impact factor between 5 - 10
- **337** and **235** communications in international and national conferences, respectively;
- **294** seminars/invited lectures were given;
- **54** conferences were organized.

The number of citations for IMM papers (according to the Web of Science) has been increasing and IMM now has an average of **10.56** citations per paper.

Year	Publications JIF > 10	Publications JIF 5-10
2005	1	11
2006	8	20
2007	6	19
2008	3	20
2009	13	36

### Prizes

#### **Bial Award in Clinical Medicine (edition 2008)**

Fonseca, J.E., Canhão, H., Santos, M.J., Mourão, A.F., Sousa, E., Caetano-Lopes, J., Moura, R., Weinmann, P., Pereira da Silva, J.A., Cunha-Branco, J., Viana-Queiroz, M.

#### **Grunenthal Award in Pain Research (edition 2008)**

Tavares, I., Ribeiro, M. and Castanho M.A.R.B.

#### **Competition Idea To Product ®, Austin, Texas, USA - 2<sup>nd</sup> place**

Graça, L., Monteiro, M., Cristina, D.

#### **Calouste Gulbenkian Foundation Programme to Stimulate Creativity and Quality on Scientific Research**

Domingues, M.

#### **Pfizer Award in Clinical Research**

Silva-Santos, B.

### Research Funding

#### Research Funding started in 2009

- **36** Research Grants
  - 30 Public (18 FCT; 10 EC; 1 FEDER; 1 EMBO) and 6 Private
- **15** Protocols/Donations
- **3** Awards

### Partnerships

- Centro Académico de Medicina de Lisboa
- Harvard Medical School – Portugal programme
- Health Cluster Portugal
- INSERM – IMM Partnership
- Programa de Formação Médica Avançada, PFMA.

### IMM PhD Programme

#### PhD Programme in numbers

**94** Total nº of Students (December 31, 2009)

**17** New students in 2009 (22 – 2008; 19 – 2007; 20 - 2006)

**85** Total nº of Students with fellowships from Fundação para a Ciência e Tecnologia

Other PhD fellowships: 1 Axa Research Fund, 1 Marie Curie Initial Training Network, 2 MD Resident (PFMA-FCT), 1 IMM.

### Outreach

- New website
- Launch of internal weekly newsletter.
- 4-5 media appearances per week – constant media presence
- New dissemination system, internal and external (>1700 new contacts)
- School visits program fully operational (15 visits; >students)
- Co-organizers of the EC project Researchers' Night “ Cientistas Ao Palco” (13.000 visitors).

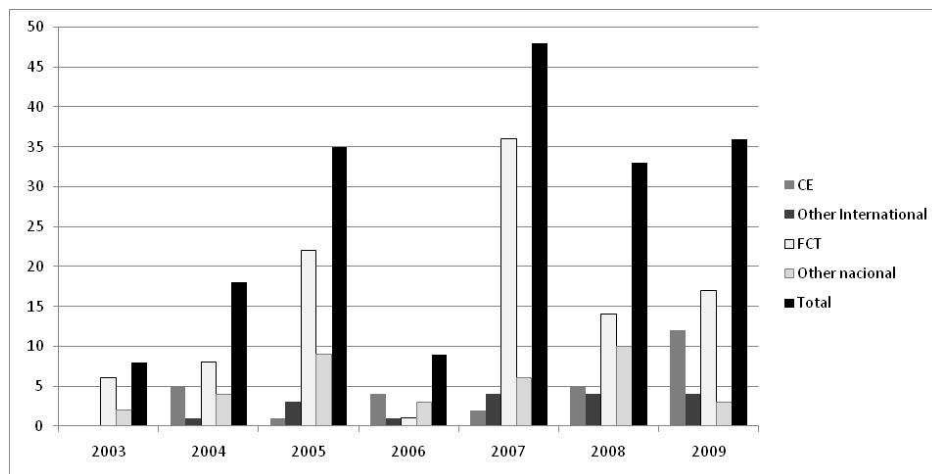


## Research Funding

### Number of ongoing research projects in 2009

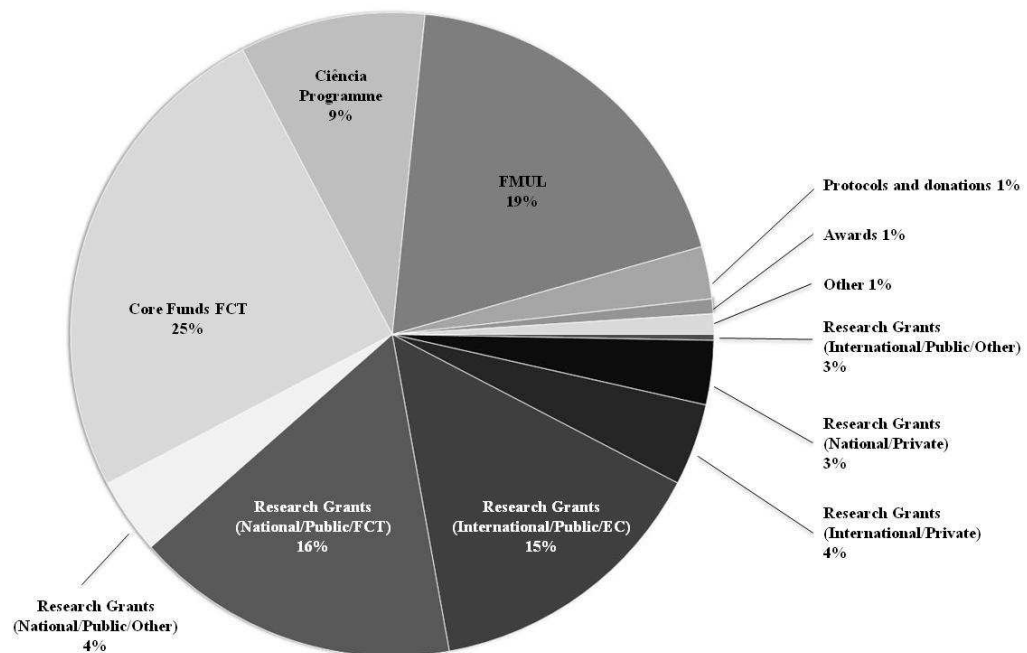
- **115 Research grants**
  - 59 Public National
  - 2 Public International
  - 27 Public European Commission
  - 17 Private National
  - 10 Private International

### Number of research grants by start date



### Expenditure in 2009 by Funding Source

Total expenditure: 9,450,192 €



## Grants and protocols that started in 2009

*Research Grants (36)*

Funding Agency	Reference	Title	PI at IMM	Duration	IMM Budget
<i>Public</i>					
EC	Pep2Brain	Selected peptides as drug candidates directed to pain and neurodegeneration	Miguel Castanho	48	248,037.00
EC	PeptideBasedHydrogel	Toward the development of peptide-based hydrogels for tissue regeneration applications with inherent antibacterial activity	Miguel Castanho	24	235,102.89
EC	People 220318 - Cyclotide mechanism	Elucidating the mechanism of action of cyclotides: ultra-stable proteins from plants	Miguel Castanho	24	222,590.10
EC	NEURASYN		Tiago Outeiro	48	180,687.00
EC	CAREPNEUMO - 223111	Combating Antibiotics Resistant Pneumococci by Novel Strategies Based on in vivo and in vitro Host – Pathogen Interactions	Mário Ramirez	36	149,930.00
EC	People IEF 2008 GA235864	The function and dynamic of cholesterol during Plasmodium liver stage infection	Ghislain Cabal	24	148,048.66
EC	Dengue Virus Capsid	The role of lipid membranes in dengue virus assembly	Nuno Santos	24	137,311.75
EC	EVIMalaR	Towards the establishment of a permanent European Virtual Institute dedicated to Malaria Research	Maria Mota	60	102,888.00
EC	Cientistas ao Palco	Setting the Stage	Marta Agostinho	7	12,179.30
EC	CORMRA	Development of carbon monoxide releasing molecules for the treatment of rheumatoid arthritis (COMRA)	João Eurico Fonseca	36	3,500.00
EMBO	EMBO Installation Grant	Installation Grant	Tiago Outeiro	60	159,905.00
FCT	PTDC/BIA-MIC/66412/2006	Bacteriophage genome delivery to gram-positive bacteria	Carlos São José	36	197,148.00
FCT	PIC/IC/82760/2007	Slow and Rapid Progression Parkinson's Disease: Molecular Determinants and Biomarkers	Tiago Outeiro	36	195,000.00
FCT	PIC/IC/82712/2007	Lymphoid tissues in HIV-2 infected patients (PIC/IC/82712/2007)	Ana Espada Sousa	36	195,000.00
FCT	PIC/IC/83062/2007	The differential effect of WT and V30M Transthyretin in the hepatic microenvironment and vasculature response	Rui José Rodrigues Perdigoto	36	191,700.00
FCT	PIC/IC/82821/2007	Prognostic Signatures in Colorectal Cancer (PSCC)	Luís Costa	36	183,600.00
FCT	PIC/IC/83065/2007	Implications for therapy and disease presentation of the changes triggered by the use of the pneumococcal 7-valent conjugate vaccine	Mário Ramirez	36	180,000.00
FCT	PIC/IC/82991/2007	Immunological basis for the association of hypersomnic sleep disorders and cardiovascular disease	Luís Moita	36	170,795.00
FCT	PIC/IC/83214/2007	Does malaria increase susceptibility to tuberculosis? The role of malaria-pigment induced immune-suppression.	Thomas Hanscheid	36	150,000.00
FCT	PIC/IC/82920/2007	Cardiovascular risk and inflammation-contribution to the etiopathogenesis of atherosclerosis and cardiovascular disease	João Eurico da Fonseca	36	150,000.00
FCT	PIC/IC/83193/2007	Neuropsychological predictors of the outcome in non-demented subjects with cognitive complaints	João Barata	36	134,000.00
FCT	PIC/IC/83023/2007	Clinical implications of activation of PI3K/Akt signaling pathway in primary adult acute lymphoblastic leukemia	João Lacerda	36	134,000.00
FCT	PIC/IC/82895/2007	Invariant Natural Killer T Cells in	Luis Graça	36	

		Allergic Asthma and Tolerogenic Therapy.			125,934.00
FCT	PIC/IC/82765/2007	Respiratory Rehabilitation in Amyotrophic Lateral Sclerosis: clinical and biochemical impact	Mamede de Carvalho	36	100,632.00
FCT	PIC/IC/83068/2007	Long-term impact of thymectomy performed in early infancy (PIC/IC/83068/2007)	Rui Victorino	36	75,000.00
FCT	PIC/IC/83084/2007	Development and validation of vaso-occlusion early predictors in a mendelian model of vascular disease	Ângelo Calado	36	65,010.00
FCT	PIC/IC/82796/2007	Neuropsychological predictors of the outcome in non-demented subjects with cognitive complaints	Alexandre Mendonça	36	58,741.00
FCT	PIC/IC/83243/2007	Autonomic nervous system in recurrent renal stone disease	Isabel Rocha	24	45,000.00
FCT	FCT/CAPEs-2009	Bilateral agreement	Nuno Santos	24	10,000.00
FEDER	Sudoe	Rede de Imunoterapia	Luis Graça	16	115,218.17
Private	UK Parkinson Disease Society		Tiago Outeiro	36	52,991.05
ADIR	IRIS/ADIR	Determination of metabolic, structural and biomechanical bone factors that explain osteoporotic fracture risk	João Eurico Fonseca	36	20,001.00
AXA	Research Fund		Tiago Outeiro	36	120,000.00
FCG	Doenças Tropicais Negligenciadas		Luísa Figueiredo	36	55,778.00
Fund. Século XXI	Genomed - Fármaco-Genética		M. Carmo-Fonseca	3	27,500.00
GE	GEHC Pilot		Mário Rosa	17	23,760.00

FCT – Fundação para a Ciência e Tecnologia  
 EC – European Commission  
 GE – GE Healthcare and Biosciences  
 FCG – Fundação Calouste Gulbenkian

*Protocols and Donations (15)*

<b>Funding agency</b>	<b>Project reference</b>	<b>PI at IMM</b>	<b>IMM Budget</b>
AMC Medical Research B.V.	Support to research	João Eurico Fonseca	27,712.00
FCT	BII-2008 - Bolsas Integração Investigação FCT 2008	Margarida Pinto Gago	46,368.00
Fundação Calouste Gulbenkian	Support to PhD thesis research, PFMA	Luís Moita	25,000.00
Fundação das Universidades	CRUP A-6/08	Miguel Castanho	4,500.00
Gilead	Sponsor - HIV Meeting	Ana Espada Sousa	5,000
Merck Sharp & Dohme	Sponsor - HIV Meeting	Ana Espada Sousa	5,000
GlaxoSmithKline	Sponsor - Curso em Doenças Movimento	Joaquim Ferreira	15,000.00
Roche Farmaceutica Quimica	Sponsorship	João Eurico Fonseca	2,500.00
Soc Portuguesa Neurociências	Support to research	Tiago Outeiro	15,000.00
Soc Portuguesa Pneumologia	PSP Genetics	Sofia Oliveira	6,000.00
Wyeth Lederle Portugal Lda	ArtriteWyeth	João Eurico Fonseca	21,942.00
Ciência Viva-Agência Nacional	CiênciaViva2009	Margarida Pinto Gago	2,261.00
Mundinter	Sponsorship	Mamede Carvalho	1,500.00
British Council	Support to research	Gunnar Mair	1,000.00
MCI BENELUX SA	Support to meetings	José Ferro	1,000.00
CNRS	Travel support	Carlos São José	185.49

*Awards (3)*

<b>Funding agency</b>	<b>Researcher</b>	<b>IMM Budget</b>
CSL Behring	Luis Graça	20,000.00
Sociedade Portuguesa de Oncologia	Isabel Monteiro Grilo	10,000.00
BIAL	João Eurico Fonseca	50,000.00

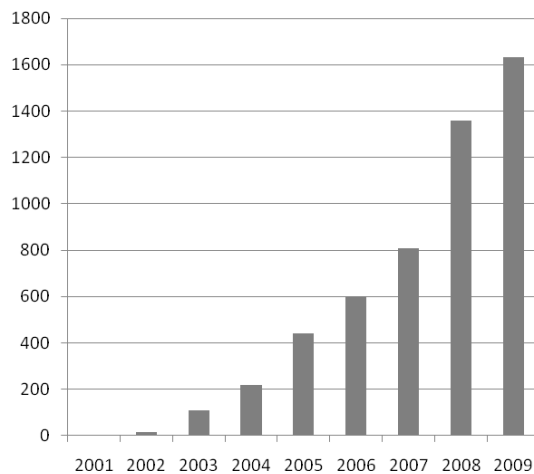
## IMM Indicators

Publications International Journals	187
Book/Book Chapters International	24
Communications in International Conferences	337
Communications in National Conferences	235
Seminars and Invited Lectures	294
Organization of Conferences	54
Prizes	40
PhD Thesis	11
Master Thesis	47
<b><i>People</i></b>	
PhD-holders	158
Non PhD-holders	290

## Citation Report

Source: Web of Science, March 10<sup>th</sup>, 2010

### *N° of citations in each year*



N° of articles: **527**

Average Citations per Item: **10.56**

Search Criteria: AD=(Inst Med Mol SAME Portugal)  
OR AD=(Mol Med Inst SAME Portugal) OR  
AD=(Inst Mol Med SAME Portugal). Refined by:  
[excluding] Document Type=(CORRECTION OR  
MEETING ABSTRACT) Timespan=2001-2009.  
Databases = SCI-EXPANDED, SSCI, A&HCI, CPCI-  
S, CPCI-SSH, IC, CCR-EXPANDED.

### 10 Most Cited Articles 2001 -2009

Grossman, Z., Meier-Schellersheim, M., Sousa, A.E., Victorino, R.M. and Paul W.E. (2002) CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat. Med.* 8: 319-321 (**Times cited: 230**)(Journal IF: 27.553)

Di Fonzo, A., Rohe, C.F., Ferreira, R.J., Chien, H.F., Vacca, L., Stocchi, F., Guedes, L., Fabrizio, E., Manfredi, M., Vanacore, N., Goldwurm, S., Breedveld, G., Sampaio, C., Meco, G., Barbosa, E., Oostra, B.A. and Bonifati, V. (2005) A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet* 365: 412-415 (**Times cited: 190**)(Journal IF: 28.409)

Sousa, A.E., Carneiro, J., Meier-Schellersheim, M., Grossman, Z., and Victorino, R.M. (2002) CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J. Immunol.* 169: 3400-3406 (**Times cited: 182**)(Journal IF: 6.000)

Ribeiro, J.A., Sebastião, A.M. and De Mendonça, A. (2002) Adenosine receptors in the nervous system: pathophysiological implications. *Prog. Neurobiol.* 68, 377-392 (**Times cited: 162**)(Journal IF: 9.130)

Duarte, A., Hirashima, M., Benedito, R., Trindade, A., Diniz, P., Bekman, E., Costa, L., Henrique, D. and Rossant, J. (2004) Dosage-sensitive requirement for mouse D114 in artery development. *Gene Dev.* 18: 2474-2478 (**Times cited: 140**)(Journal IF: 13.623)

Carmo-Fonseca, M. (2002) The contribution of nuclear compartmentalization to gene regulation. *Cell* 108:1-20 (**Times cited: 118**)(Journal IF: 31.253)

Emre, M., Aarsland, D., Brown, R., Bum, D.J., Duyckaerts, C., Mizuno, Y., Broe, G.A., Cummings, J., Dickson, D.W., Gauthier, S., Goldman, J., Goetz, C., Korczyn, A., Lees, A., Levy, R., Litvan, I., McKeith, I., Olanow, W., Poewe, W., Quinn, N., Sampaio, C., Tolosa, E. and Dubois, B. (2007) Clinical diagnostic criteria for dementia associated with Parkinson's disease. *Movement. Dis.* 22: 1689-1707 (**Times cited: 101**)(Journal IF: 3.898)

Stamm, S., Riethoven, J.J., Le Texier, V., Gopalakrishnan, C., Kumanduri, V., Tang, Y.S., Barbosa-Morais, N.L. and Thanaraj, T.A. (2006) ASD: a bioinformatics resource on alternative splicing *Nucleic Acids Res.* 34 (Special Issue): D46-D55 (**Times cited: 91**)(Journal IF: 6.878)

Santos, N.C., Figueira-Coelho, J., Martins-Silva, J. and Saldanha, C. (2003) Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspect. *Biochem. Pharmacol.* 65: 1035-1041 (**Times cited: 89**)(Journal IF: 4.838)

Bonifati, V., Rohe, C.F., Breedveld, G.J., Fabrizio, E., De Mari, M., Tassorelli, C., Tavella, A., Marconi, R., Nicholl, D.J., Chien, H.F., Fincati, E., Abbruzzese, G., Marini, P., De Gaetano, A., Horstink, M.W., Maat-Kievit, J.A., Sampaio, C., Antonini, A., Stocchi, F., Montagna, P., Toni, V., Guidi, M., Dalla Libera, A., Tinazzi, M., De Pandis, F., Fabbrini, G., Goldwurm, S., de Klein, A., Barbosa, E., Lopiano, L., Martignoni, E., Lamberti, P., Vanacore, N., Meco, G. and Oostra, B.A. (2005) Early-onset parkinsonism associated with PINK1 mutations - Frequency, genotypes, and phenotypes. *Neurology* 65: 87-95 (**Times cited: 79**)(Journal IF: 7.043)

### Publications in International Journals in 2009

Abdulghani, S., Caetano-Lopes, J., Canhão, H. and Fonseca, J.E. (2009) Biomechanical effects of inflammatory diseases on bone-rheumatoid arthritis as a paradigm. *Autoimmun. Rev.* 8: 668-671 (Journal IF: 5.371)

Abellan van Kan, G., André, E., Bischoff Ferrari, H.A., Boirie, Y., Onder, G., Pahor, M., Ritz, P., Rolland, Y., Sampaio, C., Studenski, S., Visser, M., Vellas, B. (2009) Carla Task Force on Sarcopenia: propositions for clinical trials. *J. Nutr. Health Aging* 13: 700-707 (Journal IF: 2.321)

Abranches, E., Silva, M., Pradier, L., Schulz, H., Hummel, O., Henrique, D. and Bekman, E. (2009) Neural differentiation of embryonic stem cells “in vitro”: a road map to neurogenesis in the embryo. *PLoS ONE* 4: e6286 (Journal IF: NA)

Aguiar, S.I., Pinto, F.R., Nunes, S., Serrano, I., Melo-Cristino, J., Sá-Leão, R., Ramirez, M. and de Lencastre, H. (2010) Increase of Denmark14-230 clone as a cause of pneumococcal infection in Portugal within a background of diverse serotype 19A lineages. *J. Clin. Microbiol.* 48: 101-108 (*Epub October 28, 2009*)(Journal IF: 3.945)

Albuquerque, S.S., Carret, C., Grosso, A.R., Tarun, A.S., Peng, X., Kappe, S.H., Prudêncio, M and Mota, M.M. (2009) Host cell transcriptional profiling during malaria liver stage infection reveals a coordinated and sequential set of biological events. *BMC Genomics* 10: 270 (Journal IF: 3.926)

Andrieu, S., Coley, N., Aisen, P., Carrillo, M.C., Dekosky, S., Durga, J., Fillit, H., Frisoni, G.B., Froelich, L., Gauthier, S., Jones, R., Jönsson, L., Khachaturian, Z., Morris, J.C., Orgogozo, J.M., Ousset, P.J., Robert, P., Salmon, E., Sampaio, C., Verhey, F., Wilcock, G. and Vellas, B. (2009) Methodological issues in primary prevention trials for neurodegenerative dementia. *J. Alzheimers Dis.* 16: 235-270 (Journal IF: 5.01)

Azevedo, R.I.\*, Soares, M.V.D.\*, Barata, J.T., Tendeiro, R., Serra-Caetano, A., Victorino, R.M.M. and Sousa, A.E. (2009) IL-7 sustains CD31 expression in human naive CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. *Blood* 113: 2999-3007 \*Joint first authors (Journal IF: 10.432)

Aziz, N.A., Jurgens, C.K., Landwehrmeyer, G.B., van Roon-Mom, W.M., van Ommen, G.J., Stijnen, T., Roos, R.A., Orth, M., Handley, O.J., Schwenke, C., Ho, A., Wild, E.J., Tabrizi, S.J. and Landwehrmeyer, G.B. (2009) EHDN Registry Study Group. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. *Neurology* 73:1280-1285 (Journal IF: 7.043)

Baum, J., Papenfuss, A.T., Mair, G.R., Janse, C.J., Waters, A.P., Cowman, A.F., Crabb, B.S. and de Konig-Ward T. (2009) Molecular genetics and comparative genomics suggests RNAi is not functional in malaria parasites. *Nucleic Acid Res.* 37: 3788-3798 (Journal IF 6.968)

Benisty, S., Gouw, A.A., Porcher, R., Madureira, S., Hernandez, K., Poggesi, A., van der Flier, W.M., Van Straaten, E.C., Verdelho, A., Ferro, J., Pantoni, L., Inzitari, D., Barkhof, F., Fazekas, F. and Chabriat, H.; LADIS Study group (2009) Location of lacunar infarcts correlates with cognition in a sample of non-disabled subjects with age-related white-matter changes: the LADIS study. *J. Neurol. Neurosurg. Psychiatry* 80: 478-483 (Journal IF: 4.622)

Bentes, C., Costa, J., Pires, J. and Paiva, T. (2009) Dream Recall and Seizures in Patients with Temporal Lobe Epilepsy. *J. Neurol. Sci.* 285(S1): S264-265 (Journal IF: 2.359)

Blahak, C., Baezner, H., Pantoni, L., Poggesi, A., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J.M., Langhorne, P., O'Brien, J., Visser, M.C., Wahlund, L.O., Waldemar, G., Wallin, A., Inzitari, D., Hennerici, M.G.; LADIS Study Group (2009) Deep frontal and periventricular age related white matter changes but not basal ganglia and infratentorial hyperintensities are associated with falls: cross sectional results from the LADIS study. *J. Neurol. Neurosurg. Psychiatry* 80: 608-613 (Journal IF: 4.622)

Boussier, M.G., Amarenco, P., Chamorro, A., Fisher, M., Ford, I., Fox, K., Hennerici, M.G.,

Mattle, H.P., Rothwell, P.M.; PERFORM Study Investigators (2009) Rationale and design of a randomized, double-blind, parallel-group study of terutroban 30 mg/day versus aspirin 100 mg/day in stroke patients: the prevention of cerebrovascular and cardiovascular events of ischemic origin with terutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study. *Cerebrovasc. Dis.* 27: 509-518 (Journal IF: 3.041)

Cadima-Couto, I., Freitas-Vieira, A., Nowarski, R., Britan-Rosich, E., Kotler, M. and Goncalves J. (2009) Ubiquitin-fusion as a strategy to modulate protein half-life: A3G antiviral activity revisited. *Virology* 393: 286-294 (Journal IF: 4.2)

Caetano-Lopes, J., Canhão, H. and Fonseca, J.E. (2009) Osteoimmunology – the immune regulation of bone. *Autoimmun. Rev.* 8: 250-255 (Journal IF: 5.371)

Caetano-Lopes, J., Nery, A.M., Henriques, R., Canhão, H., Duarte, J., Amaral, P.M., Vale, M., Moura, R.A., Pereira, P.A., Weinmann, P., Abdulghani, S., Souto-Carneiro, M., Rego, P., Monteiro, J., Sakagushi, S., Queiroz, M.V., Konttinen, Y.T., Graça, L., Vaz, M.F. and Fonseca, J.E. (2009) Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. *Clin. Exp. Rheumatol.* 27: 475-482 (Journal IF: 2.366)

Campos, I., Geiger, J.A., Santos, A.C., Carlos, V. and Jacinto, A. (2010) Genetic screen in *Drosophila melanogaster* uncovers a novel set of genes required for embryonic epithelial repair. *Genetics* 184: 129-140 (*Epub November 2, 2009*)(Journal IF: 4.002)

Cansado, P., Ravasco, P. and Camilo, M. (2009) Hospital undernutrition in the elderly: a longitudinal study. *J. Nutr. Health Aging* 13: 159-164. (Journal IF: 2.321)

Cardoso, B.A.\*, Martins, L.R.\*, Santos, C.I.\*, Nadler, L.M., Boussiotis, V.A., Cardoso, A.A. and Barata, J.T. (2009) Interleukin-4 stimulates proliferation and growth of T-cell acute lymphoblastic leukemia cells by activating mTOR signaling. *Leukemia* 23: 206-208 \*Co-first authors (Journal IF: 8.634)

Carlos, T.S., Young, D.F., Schneider, M., Simas, J.P. and Randall, R.E. (2009) Parainfluenza virus 5 genomes are located in viral cytoplasmic bodies whilst the virus dismantles the interferon-induced anti-viral state of cells. *J. Gen. Virol.* 90: 2147-2156 (Journal IF: 3.092)

Carrolo, M., Pinto, F.R., Melo-Cristino, J. and Ramirez, M. (2009) Pherotypes are driving genetic differentiation within *Streptococcus pneumoniae*. *BMC Microbiol.* 9: 191 (Journal IF: 2.877)

Carvalho, F.A., de Almeida, J.P., Freitas-Santos, T. and Saldanha, C. (2009) Modulation of erythrocyte acetylcholinesterase activity and its association with G protein-band 3 interactions. *J. Membr. Biol.* 228: 89-97 (Journal IF: 2.320)

Casimiro, S., Guise, T.A. and Chirgwin, J. (2009) The critical role of the bone microenvironment in cancer metastases. *Mol. Cel. Endoc.* 310: 71-81 (Journal IF: 3.611)

Castro-Caldas, A., Nunes, M.V., Maestú, F., Ortiz, T., Simões, R., Fernandes, R., La Guia, E., Garcia E. and Gonçalves, M. (2009) Learning orthography in adulthood: a magnetoencephalographic study. *J. Neuropsychol.* 3(Pt1): 17-30 (Journal IF: NA)

Cavaleiro, R., Baptista, A.P., Foxall, R.B., Victorino, M. and Sousa, A.E. (2009) Dendritic cell differentiation and maturation in the presence of HIV type 2 envelope. *AIDS Res. Hum Retroviruses* 25: 425-431 (Journal IF: 2.024)

Cavaleiro, R., Baptista, A.P., Soares, R.S., Tendeiro, R., Foxall, R.B., Gomes, P., Victorino, M. and Sousa, A.E. (2009) Major Depletion of Plasmacytoid Dendritic Cells in HIV-2 Infection, an Attenuated Form of HIV Disease. *PLoS Pathog* 5: e1000667 (Journal IF: 9.125)

CLOTS Trials Collaboration, Dennis, M., Sandercock, P.A., Reid, J., Graham, C., Murray, G., Venables, G., Rudd, A. and Bowler, G. (2009) Effectiveness of thigh-length graduated compression stockings to reduce the risk of deep vein thrombosis after stroke (CLOTS trial 1): a multicentre, randomised controlled trial. *Lancet* 373: 1958-1965 (Journal IF: 28.409)

Connolly, S.J., Ezekowitz, M.D., Yusuf, S., Eikelboom, J., Oldgren, J., Parekh, A., Pogue, J.,



- Reilly, P.A., Themeles, E., Varrone, J., Wang, S., Alings, M., Xavier, D., Zhu, J., Diaz, R., Lewis, B.S., Darius, H., Diener, H.C., Joyner, C.D., Wallentin, L.; RE-LY Steering Committee and Investigators (2009) Dabigatran versus warfarin in patients with atrial fibrillation. *N. Engl. J. Med.* 361: 1139-1151 (Journal IF: 50.017)
- Correia, D.V., d'Orey, F., Cardoso, B.A., Lança, T., Grosso, A.R., deBarros, A., Martins, L.R., Barata, J.T. and Silva-Santos, B. (2009) Highly active microbial phosphoantigen induces rapid yet sustained MEK/ Erk- and PI-3K/ Akt-mediated signal transduction in anti-tumor human gd T cells. *PLoS ONE*: e5657 (Journal IF: NA)
- Cortez-Pinto, H. and Machado, M.V. (2009) Uncoupling proteins and non-alcoholic fatty liver disease. *J. Hepatol.* 50: 857-860 (Journal IF: 7.056)
- Costa, L. and Major, P.P. (2009) Effect of bisphosphonates on pain and quality of life in patients with bone metastases. *Nat. Clin. Prac. Oncol.* 6: 163-174. (Journal IF: 9.113)
- Coutinho, J.M., Ferro, J.M., Canhão, P., Barinagarrementeria, F., Cantú, C., Boussier, M.G. and Stam, J. (2009) Cerebral venous and sinus thrombosis in women. *Stroke* 40: 2356-2361 (Journal IF: 6.499)
- Cristóvão-Ferreira, S., Vaz, S.H., Ribeiro, J.A. and Sebastião, A.M. (2009) Adenosine A2A receptors, through PKA activation, restrain PKC inhibition of GAT-1-mediated GABA uptake by nerve terminals. *J. Neurochem.* 109: 336-347 (Journal IF: 4.500)
- da Costa, G., Gomes, R., Correia, C.F., Freire, A., Monteiro, E., Martins, A., Barroso, E., Coelho, A.V., Outeiro, T.F., Ponces Freire, A., Cordeiro, C. (2009) Identification and quantitative analysis of human transthyretin variants in human serum by Fourier transform ion-cyclotron resonance mass spectrometry. *Amyloid.* 16: 201-207 (Journal IF: 1,714)
- de Almeida, J.P., Freitas-Santos, T. and Saldanha, C. (2009) Fibrinogen-dependent signaling in microvascular erythrocyte function: implications on nitric oxide efflux. *J. Membr. Biol.* 231: 47-53 (Journal IF: 2.320)
- de Almeida, J.P.L., Carvalho, F.A., Silva-Herdade, A.S., Santos-Freitas, T. and Saldanha C. (2009) Redox thiol status plays a central role in the mobilization and metabolism of nitric oxide in human red blood cells. *Cell Biol. Int.* 33: 268-275 (Journal IF: 1.619)
- de Carvalho M, Swash M (2009). An interesting case: a woman, her butcher and a Japanese name. *Pract. Neurol.* 9: 284-288 (Journal IF: NA)
- de Carvalho, M. (2009) Natural history of young-adult amyotrophic lateral sclerosis. *Neurology* 73: 649 (Journal IF: 7.043)
- de Carvalho, M. and Swash, M. (2009) Stratifying disease stages with different progression rates determined by electrophysiological tests in patients with amyotrophic lateral sclerosis. *Muscle Nerve* 40: 318 (Journal IF: 2.574)
- de Carvalho, M. and Swash, M. (2009) What is really new in progressive muscle atrophy? *Arch. Neurol.* 66: 1448-1429 (Journal IF: 5.874)
- de Carvalho, M., Costa, J., Pinto, S. and Pinto, A. (2009) Percutaneous nocturnal oximetry in amyotrophic lateral sclerosis: periodical desaturation. *Amyotr. Lat. Scler.* 10: 154-161 (Journal IF: 1.823)
- de Carvalho, M., Pinto, S. and Swash, M. (2009) Motor unit changes in thoracic paraspinal muscles in ALS. *Muscle Nerve* 39: 83-86. (Journal IF: 2.574)
- de Carvalho, M., Pinto, S., Swash, M. (2009) Association between paraspinal muscles and diaphragm denervation. *Amyotroph. Lateral Scler.* 15: 1-4 (Journal IF: 1.823)
- de Carvalho, M., Swash, M. (2009) Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. *Amyotr. Lat. Scler.* 10: 53-57 (Journal IF: 1.823)
- Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho, L. (2009) L-Arginine and L-Nitroarginine Methyl ester effects on vasomotion in isolated rabbit eyes. *Ophthalmic Res.* 43: 113-121 (Journal IF: 1.317)

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho L. (2010) Amlodipine effects on vasomotion in rabbit external ophthalmic artery. *Graefes Arch. Clin. Exp. Ophthalmol.* 248: 213-221 (*Epub November 17, 2009*)(Journal IF: 1.770)

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho, L. (2009) Endothelin-1 effects on spontaneous oscillations in choroidal arterioles. *Acta Ophthalmol.* (*Epub August 6, 2009*)(Journal IF: 2.138)

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho, L. (2009) Intrinsic vasomotricity and adrenergic effects in a model of isolated rabbit eye. *Acta Ophthalmol.* 87: 443-449 (Journal IF: 2.138)

Di Fonzo, A., Fabrizio, E., Thomas, A., Fincati, E., Marconi, R., Tinazzi, M., Breedveld, G.J., Simons, E.J., Chien, H.F., Ferreira, J.J., Horstink, M.W., Abbruzzese, G., Borroni, B., Cossu, G., Dalla Libera, A., Fabbri, G., Guidi, M., De Mari, M., Lopiano, L., Martignoni, E., Marini, P., Onofri, M., Padovani, A., Stocchi, F., Toni, V., Sampaio, C., Barbosa, E.R., Meco, G.; Italian Parkinson Genetics Network, Oostra, B.A. and Bonifati, V. (2009) GIGYF2 mutations are not a frequent cause of familial Parkinson's disease. *Parkinsonism Relat Disord.* 15: 703-705 (Journal IF: 1.907)

Domingues, M.M., Castanho, M.A.R.B. and Santos, N.C. (2009) rBPI<sub>21</sub> promotes lipopolysaccharide aggregation and exerts its antimicrobial effects by (hemi)fusion of PG-containing membranes. *PLoS ONE* 4: e8385 (Journal IF: NA)

Domingues, M.M., Lopes, S.C.D.N., Santos, N.C., Quintas, A. and Castanho, M.A.R.B. (2009) Fold-unfold transitions in the selectivity and mechanism of action of the N-terminal fragment of the Bactericidal/Permeability-Increasing protein (rBPI<sub>21</sub>). *Biophys. J.* 96: 987-996 (Journal IF: 4.683)

Dworkin, R.H., Turk, D.C., McDermott, M.P., Peirce-Sandner, S., Burke, L.B., Cowan, P., Farrar, J.T., Hertz, S., Raja, S.N., Rappaport, B.A., Rauschkolb, C. and Sampaio, C. (2009) Interpreting the clinical importance of group differences in chronic pain clinical trials: IMMPACT recommendations. *Pain* 146: 238-424 (Journal IF: 6.030)

Earnshaw, S., Monnet, D.L., Duncan, B., O'Toole, J., Ekdahl, K., Goossens, H., European Antibiotic Awareness Day Technical Advisory Committee, and European Antibiotic Awareness Day Collaborative Group (2009) European Antibiotic Awareness Day, 2008 - the first Europe-wide public information campaign on prudent antibiotic use: methods and survey of activities in participating countries. *Euro. Surveill.* 14: 19280 (Journal IF: NA)

European Heart Rhythm Association (EHRA); Heart Failure Association (HFA); Heart Rhythm Society (HRS); European Society of Emergency Medicine (EuSEM); European Federation of Internal Medicine (EFIM); European Union Geriatric Medicine Society (EUGMS); American Geriatrics Society (AGS); European Neurological Society (ENS); European Federation of Autonomic Societies (EFAS); American Autonomic Society (AAS), Moya, A., Sutton, R., Ammirati, F., Blanc, J.J., Brignole, M., Dahm, J.B., Deharo, J.C., Gajek, J., Gjesdal, K., Krahn, A., Massin, M., Pepi, M., Pezawas, T., Granell, R.R., Sarasin, F., Ungar, A., van Dijk, J.G., Walma, E.P., Wieling, W., Abe, H., Benditt, D.G., Decker, W.W., Grubb, B.P., Kaufmann, H., Morillo, C., Olshansky, B., Parry, S.W., Sheldon, R., Shen, W.K., ESC Committee for Practice Guidelines (CPG), Vahanian, A., Auricchio, A., Bax, J., Ceconi, C., Dean, V., Filippatos, G., Funck-Brentano, C., Hobbs, R., Kearney, P., McDonagh, T., McGregor, K., Popescu, B.A., Reiner, Z., Sechtem, U., Sirnes, P.A., Tendera, M., Vardas, P., Widimsky, P., Auricchio, A., Acarturk, E., Andreotti, F., Asteggiano, R., Bauersfeld, U., Bellou, A., Benetos, A., Brandt, J., Chung, M.K., Cortelli, P., Da Costa, A., Extramiana, F., Ferro, J., Gorenek, B., Hedman, A., Hirsch, R., Kaliska, G., Kenny, R.A., Kjeldsen, K.P., Lampert, R., Mølgard, H., Paju, R., Puodziukynas, A., Raviele, A., Roman, P., Scherer, M., Schondorf, R., Sicari, R., Vanbrabant, P., Wolpert, C., Zamorano, J.L. (2009) Guidelines for the diagnosis and management of syncope (version 2009): the Task Force for the Diagnosis and Management of Syncope of the European Society of Cardiology (ESC). *Eur. Heart J.* 30: 2631-2671 (Journal IF: 8.917)

Evangelista, T., Ferro, J.M., Pereira, P. and de Carvalho, M. (2009) A case of asymptomatic

cytoplasmic body revealed by simvastatin. *Neuromusc. Disord.* 19: 66-68 (Journal IF: 2.932)

Evatt, M.L., Chaudhuri, K.R., Chou, K.L., Cubo, E., Hinson, V., Kompoliti, K., Yang, C., Poewe, W., Rascol, O., Sampaio, C., Stebbins, G.T. and Goetz, C.G. (2009) Dysautonomia rating scales in Parkinson's disease: sialorrhea, dysphagia, and constipation-critique and recommendations by movement disorders task force on rating scales for Parkinson's disease. *Mov. Disord.* 24: 635-646 (Journal IF: 3.898)

Fabrino, D.L., Bleck, C.K.E., Anes, E., Hasilik, A., Melo, R.C.N., Niederweis, M., Griffiths, G. and Gutierrez, M.G. (2009) Role of porins in the susceptibility to nitric oxide-mediated killing of mycobacteria. *Microbes Infect.* 11: 868-875 (Journal IF 3.7)

Fernandes, A., Falcão, A.S., Abranches, E., Bekman, E., Henrique, D., Lanier, L.M. and Brites, D. (2009) Bilirubin as a determinant for altered neurogenesis, neuritogenesis and synaptogenesis. *Dev. Neurobiol.* 69: 568-562 (Journal IF: 2.333)

Ferre, R., Melo, M.N., Correia, A.D., Felia, L., Planas, M., Bardaji, E. AND Castanho, M.A.R.B. (2009) The synergistic effect of multiple membrane actions of the cecropin-melittin antimicrobial hybrid peptide BP100. *Biophys. J.* 96: 1815-1827 (Journal IF: 4.683)

Ferro, J.M., Bacelar-Nicolau, H., Rodrigues, T., Bacelar-Nicolau, L., Canhão, P., Crassard, I., Bousser, M.G., Dutra, A.P., Massaro, A., Mackowiack-Cordioli, M.A., Leys, D., Fontes, J., Stam, J., Barinagarrementeria, F.; ISCVT and VENOPORT investigators (2009) Risk score to predict the outcome of patients with cerebral vein and dural sinus thrombosis. *Cerebrovasc. Dis.* 28: 39-44 (Journal IF: 3.041)

Ferro, J.M., Caeiro, L. and Santos, C. (2009) Poststroke emotional and behavior impairment: a narrative review. *Cerebrovasc. Dis.* 27(Suppl 1): 197-203 (Journal IF: 3.041)

Ferro, J.M., Canhão, P., Stam, J., Bousser, M.G., Barinagarrementeria, F., Massaro, A., Ducrocq, X., Kasner, S.E.; ISCVT Investigators (2009) Delay in the diagnosis of cerebral vein and dural sinus thrombosis: influence on outcome. *Stroke* 40: 3133-3138 (Journal IF: 6.499)

Fior, R. and Henrique, D., (2009) "Notch-Off": a perspective on the termination of Notch signalling. *Int. J. Dev. Biol.* 53: 1379-1384 (Journal IF: 2.359)

Fonseca, A.C., Costa, J., Cordeiro, C., Geraldes, R. and de Sá, J. (2009) Influence of climatic factors in the incidence of multiple sclerosis relapses in a Portuguese population. *Eur. J. Neurol.* 16: 537-539 (Journal IF: 2.732)

Fonseca, A.C., Martins, A.C., Silvestre, A.R., Serrano, M.R., Calado, A., Vilarinho, L. and Evangelista, T. (2009) Mitochondrial changes in patients with polymyositis. *Eur. J. Neurol.* 16 (Suppl. 3): 217 (Journal IF: 2.732)

Fonseca, J.E., Canhão, H., Tavares, N.J., Cruz, M., Branco, J. and Queiroz, M.V. (2009) Persistent low grade synovitis without erosive progression in magnetic resonance imaging of rheumatoid arthritis patients treated with infliximab over 1 year. *Clin. Rheumatol.* 28: 1213-1216 (Journal IF: 1.559)

Fonseca, J.E., Santos, M.J., Canhão, H. and Choy, E. (2009) Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmun. Rev.* 8: 538-542 (Journal IF: 5.371)

Fonseca, L., Aires-da-Silva, F., Santa-Marta, M., Barata, J., Barbas, C.F. 3rd and Goncalves, J. (2009) Programming specificity of lentiviral cell targeting using anti-FITC single-chain antibody. *Human Gene Therapy* 20: 1417-1545 (Journal IF: 5.2)

Fontinha, B.M., Delgado-García, J.M., Madroñal, N., Ribeiro, J.A., Sebastião, A.M. and Gruart, A. (2009) Adenosine A2A receptor modulation of hippocampal CA3-CA1 synapse plasticity during associative learning in behaving mice *Neuropsychopharmacology* 34: 1865-1874 (Journal IF: 6.835)

Foreid, H., Barroso, C., Carvalho, H., Roque, L. and Pimentel, J. (2009) A 22-year-old man with intracranial hypertension and impaired sensation over the perineum and left foot. *Brain Pathol* 19: 735-738 (Journal IF: 5.576)

Francisco, A.P., Bugalho, M., Ramirez, M. and Carriço, J.A. (2009) Global optimal eBURST

analysis of multilocus typing data using a graphic matroid approach. *BMC Bioinformatics* 10: 152 (Journal IF: 3.781)

Frias, M.J., Melo-Cristino, J. and Ramirez, M. (2009) The autolysin LytA contributes to efficient bacteriophage progeny release in *Streptococcus pneumoniae*. *J. Bacteriol.* 191: 5428-5440. (Journal IF: 3.636)

Galrinho, A., Branco, L., Oliveira, M.M., Silva, N., Abreu, J., Santos, S., Leal, A. and Ferreira, R. (2009) Cardiac resynchronization therapy- clinical and echocardiographic characteristics of responders and exceptional responders. *Rev. Port. Cardiol.* 28: 959-969

Galrinho, A., Branco, L.M., Oliveira, M.M., Da Silva, N., Abreu, J., Santos, S., Leal, A. and Ferreira, R.C. (2009) Cardiac resynchronization therapy--clinical and echocardiographic characteristics of responders and exceptional responders. *Rev. Port. Cardiol.* 28: 959-969 (Journal IF: NA)

Garcia-Fernandez, B., Campos, I., Geiger, J., Santos, A.C. and Jacinto, A. (2009) Epithelial resealing. *Int. J. Dev. Biol.* 53: 1549-1556 (Journal IF: 2.359)

Gascoyne, D.M., Long, E., Veiga-Fernandes, H., de Boer, J., Williams, O., Seddon, B., Coles, M., Kioussis, D., Brady, H.J. (2009) The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat. Immunol.* 10: 1118-1124 (Journal IF: 26.218)

Gil-Gouveia, R. and Goadsby, P.J. (2009) Neuropsychiatric side-effects of lidocaine: examples from the treatment of headache and a review. *Cephalalgia* 29: 496-508 (Journal IF: 3.686)

Gomes, C.A., Simões, P.F., Canas, P.M., Quiroz, C., Sebastião, A.M., Ferré, S., Cunha, R.A. and Ribeiro, J.A. (2009) GDNF control of the glutamatergic cortico-striatal pathway requires tonic activation of adenosine A2A Receptors. *J. Neurochem.* 108: 1208-1219 (Journal IF: 4.500)

Gonçalves-Sousa, N., Ribot, J.C., deBarros, A., Correia, D.V., Caramalho, I. and Silva-Santos B. (2010) Inhibition of murine gammadelta lymphocyte expansion and effector function by regulatory alphabeta T cells is cell-contact-dependent and sensitive to GITR modulation. *Eur. J. Immunol.* 40: 61-70 (*Epub October 28, 2009*)(Journal IF: 4.865)

Guerreiro, C.S., Ferreira, P., Tavares, L., Moura Santos, P., Neves, M., Brito, M. and Cravo, M. (2009) Fatty acids, IL-6 and TNF- $\alpha$  polymorphisms: An example of nutrigenetics in Crohn's disease. *Am. J. Gastroenterol.* 104: 2241-2249. (Journal IF: 6.444)

Gutierrez, M.G., Gonzalez, A.P., Anes, E. and Griffiths, G. (2009) Role of lipids in killing mycobacteria by macrophages: evidence for NF-KB dependent and independent killing induced by different lipids. *Cell Microbiol.* 11: 406-420 (Journal IF 5.6)

Hänscheid, T., Frita, R., Längin, M., Kreamsner, P.G. and Grobusch, M.P. (2009) Is flow cytometry better in counting malaria pigment-containing leukocytes compared to microscopy? *Malar. J.* 8: 255 (Journal IF: 2.913)

Hänscheid, T., Längin, M., Codices, V., Luty, A.J., Adegnika, A.A., Kreamsner, P.G. and Grobusch, M.P. (2009) Automated detection of haemozoin-containing monocytes for the diagnosis of malaria in microscopically negative cases during pregnancy. *Acta Trop.* 109: 245-246 (Journal IF: 1.707)

Hänscheid, T., Valadas, E. (2009) Diagnosis of cryptosporidiosis using PCR or auramine O with LED fluorescent microscopy: which end of the stick? *Acta Trop.* 109: 247-248 (Journal IF: 1.707)

Hauser, R.A., Bronzova, J., Sampaio, C., Lang, A.E., Rascol, O., Theeuwes, A., van de Witte, S.V.; Pardoprinox Study Group (2009) Safety and tolerability of pardoprinox, a new partial dopamine agonist, in a randomized, controlled study of patients with advanced Parkinson's disease. *Eur. Neurol.* 62: 40-48 (Journal IF: 1.897)

Henriques, R., Mhlanga, M.M. (2009) PALM and STORM: What hides beyond the Rayleigh limit? *Biotechnol. J.* 4: 846 – 857 (Journal IF: NA)

Henriques, S.T. and Craik D.J. (2010) Cyclotides as templates in drug design. *Drug Discov Today.* 15: 57-64 (*Epub October 28, 2009*)(Journal IF 6.618).

- Henriques, S.T., Pattenden, L. K., Aguilar, M. I. and Castanho M.A.R.B. (2009) The toxicity of prion protein fragment PrP(106-126) is not mediated by membrane permeabilization as shown by M112W substitution. *Biochemistry* 48: 4198-208 (Journal IF: 3.379)
- Henson, S.M., Franzese, O., Macaulay, R., Libri, V., Azevedo, R.I., Kiani-Alikhan, S., Plunkett, F.J., Masters, J.E., Jackson, S., Griffiths, S.J., Pircher, H.P., Soares, M.V. and Akbar, A.N. (2009) KLRG1 signaling induces defective Akt (ser473) phosphorylation and proliferative dysfunction of highly differentiated CD8+ T cells. *Blood* 113: 6619-6628 (Journal IF: 10.9)
- Inzitari, D., Pracucci, G., Poggesi, A., Carlucci, G., Barkhof, F., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J.M., Hennerici, M., Langhorne, P., O'Brien, J., Scheltens, P., Visser, M.C., Wahlund, L.O., Waldemar, G., Wallin, A., Pantoni, L.; LADIS Study Group (2009) Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ* 339: b2477 (Journal IF: 12.827)
- Jokinen, H., Kalska, H., Ylikoski, R., Madureira, S., Verdelho, A., van der Flier, W.M., Scheltens, P., Barkhof, F., Visser, M.C., Fazekas, F., Schmidt, R., O'Brien, J., Waldemar, G., Wallin, A., Chabriat, H., Pantoni, L., Inzitari, D., Erkinjuntti, T.; LADIS group (2009) Longitudinal cognitive decline in subcortical ischemic vascular disease - the LADIS Study. *Cerebrovasc. Dis.* 27: 384-391 (Journal IF: 3.041)
- Jokinen, H., Kalskam H., Ylikoski, R., Madureira, S., Verdelho, A., Gouw, A., Scheltens, P., Barkhof, F., Visser, M.C., Fazekas, F., Schmidt, R., O'Brien, J., Hennerici, M., Baezner, H., Waldemar, G., Wallin, A., Chabriat, H., Pantoni, L., Inzitari, D., Erkinjuntti, T.; LADIS group (2009) MRI-defined subcortical ischemic vascular disease: baseline clinical and neuropsychological findings. The LADIS Study. *Cerebrovasc. Dis.* 27: 336-344 (Journal IF: 3.041)
- Jotta, P.Y., Ganazza, M.A., Silva, A., Viana, M.B., da Silva, M.J., Zambaldi, L.J.G., Barata, J.T., Brandalise, S.R. and Yunes, J.A. (2009) Negative prognostic impact of PTEN mutation in pediatric T-cell acute lymphoblastic leukemia. *Leukemia* 24: 239-242 (*Epub October 15, 2009*)(Journal IF: 8.634)
- Jungbluth, H., Lillis, S., Zhou, H., Abbs, S., Swash, M. and Muntoni, F. (2009) Late onset axial myopathy with cores associated with a novel dominant mutation in skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscul. Disord.* 19: 344-347 (Journal IF: 2.932)
- Justino, G.C., Rodrigues, M., Florêncio, M.H. and Mira, L. (2009) Structure and antioxidant activity of brominated flavonols and flavanones. *J. Mass Spectrom.* 44: 1459-1468 (Journal IF: 3.263)
- Kopecka-Pilarczyk, J. and Correia, A.D. (2009) Biochemical response in gilthead seabream (*Sparus aurata*) to in vivo exposure to pyrene and fluorene. *J. Exp. Mar. Biol. Ecol.* 372: 49-57 (Journal IF: 2.074)
- Kopecka-Pilarczyk, J. and Correia, A.D. (2009) Biochemical response in gilthead seabream (*Sparus aurata*) to in vivo exposure to a mix of selected PAHs. *Ecotox. Environ. Saf.* 72: 1296-1302 (Journal IF: 2.859)
- Kuehnle, M., Rybin, V., Anand, P., Anes, E. and Griffiths, G. (2009) Lipids regulate P2X7 receptor-dependent actin assembly by phagosomes via ADP translocation and ATP synthesis in the phagosome lumen. *J. Cell Science* 122: 499-504 (Journal IF: 6.4)
- Lavazec, C., Moreira, C.K., Mair, G.R., Waters, A.P., Janse, C.J. and Templeton T.J. (2009) Analysis of mutant *Plasmodium berghei* parasites lacking expression of multiple PbCCP genes. *Mol. Biochem. Parasitol.* 163: 1-7 (Journal IF: 2.896)
- Liehl, P., França, A.R., Prudêncio, M., Latz, E., Zaidman-Rémy, A. and Mota, M.M. (2009) Phosphothioate oligodeoxynucleotides inhibit Plasmodium sporozoite gliding motility. *Cell Microbiol.* (*Epub November 12, 2009*)(Journal IF: 5.598)
- Lipton, A., Campbell-Baird, C., Harvey, H., Kim, C., Demers, L. and Costa, L. (2010) Phase I trial of Zoledronic acid + Imatinib mesylate (Gleevec) in patients with bone metastases. *Am. J.*

Clin. Oncol. 33: 75-78 (*E-pub July 31, 2009*)(Journal IF: 1.792)

Machado, M., Ravasco, P., Martins, A., Almeida, M.R., Camilo, M.E. and Cortez-Pinto, H. (2009) Iron homeostasis and H63D mutations in alcoholics with or without liver disease. *World J. Gastroenterol.* 15: 106-111 (Journal IF: 2.081)

Machado, M.V. and Cortez-Pinto, H. (2009) Insulin resistance and steatosis in chronic hepatitis C. *Ann. Hepatol.* 8 (Suppl 1): S67-S75 (Journal IF: NA)

Machado, M.V., Martins, A., Almeida, R., Marques-Vidal, P., Gonçalves, M.S., Camilo, M.E. and Cortez-Pinto, H. (2009) Does the simultaneous tumor necrosis factor receptor 2 and Tumor Necrosis Factor promoter gene polymorphism represent a higher risk for alcoholic liver disease? *Eur. J. Gastroenterol. Hepatol.* 21: 201-205 (Journal IF: 2.080)

Mancuso, M.E., Graça, L., Auerswald, G. and Santagostino, E. (2009) Haemophilia care in children--benefits of early prophylaxis for inhibitor prevention. *Haemophilia Suppl* 1: 8-14 (Journal IF: 2.394)

Marcelino, G., Oliveira, J.M., Ravasco, P. and Marques-Vidal, P. (2009) Weight concerns and weight reduction practices of Portuguese adolescents. *Nutr. Hosp.* 24: 758 (Journal IF: 1.096)

Martins, I.P., Loureiro, C., Ramos, S. and Moreno, T. (2009) Grammatical dissociation during acquired childhood aphasia. *Dev. Med. Child Neurol.* 51: 999-1002 (Journal IF: 2.561)

McVeigh, P., Mair, G.R., Atkinson, L., Ladurner, P., Zamanian, M., Novozhilova, E., Marks, N.J., Day, T.A. and Maule A.G. (2009) Discovery of multiple neuropeptide families in the phylum Platyhelminthes. *Int. J. Parasitol.* 39: 1243-1252 (Journal IF 3.392)

Melo, M.N., Ferre, R. and Castanho, M.A.R.B. (2009) Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat. Rev. Microbiol.* 7: 245-250 (Journal IF: 14.31)

Melo, M.N., Sousa, F.J., Carneiro, F.A., Castanho, M.A.R.B., Valente, A.P., Almeida, F.C., Da Poian, A.T. and Mohana-Borges, R. (2009) Interaction of the Dengue virus fusion peptide with membranes assessed by NMR: The essential role of the envelope protein Trp101 for membrane fusion. *J. Mol. Biol.* 392: 736-746 (Journal IF: 4.146)

Mestre, T. and Ferreira, J.J. (2009) Eslicarbazepine acetate: a new option for the treatment of focal epilepsy. *Expert Opin. Investig. Drugs* 18: 221-229 (Journal IF: 4.058)

Mestre, T., Ferreira, J., Coelho, M.M., Rosa, M. and Sampaio C. (2009) Therapeutic interventions for symptomatic treatment in Huntington's disease. *Cochrane Database Syst Rev.* (3): CD006456 (Journal IF: 5.182)

Mestre, T., Ferreira, J.J. and Jain, S.A (2009) A Multimodal approach to physical therapy in Parkinson's disease: optimizing strategies. *Mov. Disord.* 24: 946 (Journal IF: 3.898)

Mhlanga, M.M., Bratu, D.P., Genovesio, A., Rybarska, A., Chenouard, N., Nehrbass, U. and Olivo-Marin, J.C. (2009) "In vivo" colocalisation of oskar mRNA and trans-acting proteins revealed by quantitative imaging of the *Drosophila* oocyte *PLoSOne* 4: e6241 (Journal IF: NA)

Milho, R., Smith, C.M., Marques, S., Alenquer, M., May, J.S., Gillet, L., Gaspar, M., Efstathiou, S. and Simas, J.P.\*, Stevenson PG\* (2009) "In vivo" Imaging of Murid Herpesvirus-4 infection. *J. Gen. Virol.* 90: 21-32 \*Joint corresponding authors (Journal IF: 3.092)

Mourão, A.F., Amaral, M., Caetano-Lopes, J. and Isenberg, D. (2009) An analysis of joint replacement in patients with systemic lupus erythematosus. *Lupus* 18: 1298-1302 (Journal IF: 2.244)

Mourão, A.F., Caetano-Lopes, J., Costa, P., Canhão, H., Santos, M.J., Pinto, P., Brito, I., Nicola, P., Cavaleiro, J., Teles, J., Sousa, A., Gomes, J.M., Branco, J., da Costa, J.T., Pedro, J.G., de Queiroz M.V. and Fonseca, J.E. (2009) Tumor necrosis factor-alpha -308 genotypes influence inflammatory activity and TNF-alpha serum concentrations in children with juvenile idiopathic arthritis. *J Rheumatol.* 36: 837-842 (Journal IF: 3.151)

Olanow, C.W., Rascol, O., Hauser, R., Feigin, P.D., Jankovic, J., Lang, A., Langston, W.,

- Melamed, E., Poewe, W., Stocchi, F., Tolosa, E.; ADAGIO Study Investigators (2009) A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *N. Engl. J. Med.* 361: 1268-1278 (Journal IF: 50.017)
- Oliveira, M. (2009) Ablation of atrial fibrillation: what is the gold standard strategy? *Rev. Port. Cardiol.* 28 : 1043-1046 (Journal IF: NA)
- Oliveira, M.M. (2009) Effect of dronedarone on cardiovascular events in atrial fibrillation. *Rev. Port. Cardiol.* 28: 345-347 (Journal IF: NA)
- Oliveira, M.M., da Silva, N., Timóteo, A.T., Feliciano, J., Silva, S., Xavier, R., Rocha, I., Silva-Carvalho, L. and Ferreira, R. (2009) Alterations in autonomic response head-up tilt testing in paroxysmal atrial fibrillation patients: a wavelet analysis. *Rev. Port. Cardiol.* 28: 243-257
- Oliveira, M.M., Silva, N., Timóteo, A., Feliciano, J., Silva, S., Xavier, R., Rocha, I., Silva-Carvalho, L. and Ferreira, R. (2009) Effects of stimulation and blockade of the autonomic nervous system on atrial refractoriness in patients with lone paroxysmal atrial fibrillation. *Rev. Port. Cardiol.* 28: 655-670 (Journal IF: NA)
- Oliveira, M.M., Silva, N., Timóteo, A., Feliciano, J., Sousa, L., Santos, S., Silva-Carvalho, L. and Ferreira, R. (2009) Inducibility of atrial fibrillation during electrophysiological evaluation is associated with increase dispersion of atrial refractoriness. *Int. J. Cardiol.* 136: 130-135 (Journal IF: 3.121)
- Ostrowski, M., Carmo, N.B., Krumeich, S., Fanget, I., Raposo, G., Savina, A., Moita, C.F., Schauer, K., Hume, A.N., Freitas, R.P., Goud, B., Benaroch, P., Hacothen, N., Fukuda, M., Desnos, C., Seabra, M.C., Darchen, F., Amigorena, S. and Moita, L.F.\*, Thery, C.\* (2009) Rab27 controls constitutive exosome secretion. *Nat. Cell Biol.* 12: 19-30 \*Corresponding authors (Journal IF: 17.623)
- Outeiro, T.F. and Ferreira, J. (2009) Current and Future Therapeutic Strategies for Parkinson's Disease. *Curr. Pharm. Des.* 15: 3968-3976 (Journal IF: 4.399)
- Outeiro, T.F., Klucken, J., Bercury, K., Tetzlaff, J., Putcha, P., Oliveira, L.M., Quintas, A., McLean, P.J. and Hyman, B.T. (2009) Dopamine-induced conformational changes in alpha-synuclein. *PLoS One* 4: e6906 (Journal IF: NA)
- Pamplona, A., Hanscheid, T., Epiphanyo, S., Mota, M.M. and Vigário, A.M. (2009) Cerebral malaria and the hemolysis/methemoglobin/heme hypothesis: shedding new light on an old disease. *Int. J. Biochem. Cell Biol.* 41: 711-716 (Journal IF: 4.178)
- Parvanova, I., Epiphanyo, S., Fauq, A., Golde, T.E., Prudêncio, M. and Mota, M.M. (2009) A small molecule inhibitor of signal peptide peptidase inhibits Plasmodium development in the liver and decreases malaria severity. *PLoS One* 4: e5078 (Journal IF: NA)
- Pimentel-Santos, F.M., Ligeiro, D., Matos, M., Mourão, A.F., Sousa, E., Pinto, P., Ribeiro, A., Sousa, M., Barcelos, A., Godinho, F., Cruz, M., Fonseca, J.E., Guedes-Pinto, H., Trindade, H., Evans, D.M., Brown, M.A. and Branco, J.C. (2009) Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. *Clin. Exp. Rheumatol.* 27: 800-806 (Journal IF: 2.366)
- Pimentel, J., Barroso, C., Miguéns, J., Firmo, C. and Antunes, J.L. (2009) Papillary glioneuronal tumor – prognostic value of the extension of surgical resection. *Clin. Neuropath.* 28: 287-294 (Journal IF: 1.200)
- Pinto, S., Geraldés, R., Vaz, N., Pinto, A. and de Carvalho, M. (2009) Changes of the phrenic nerve motor response in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 120: 2082-2085 (Journal IF: 2.972)
- Pinto, S., Turkman, A., Pinto, A., Swash, M. and de Carvalho, M. (2009) Predictive value of respiratory tests for respiratory insufficiency in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 120: 941-946 (Journal IF: 2.972)
- Ploemen, I.H., Prudêncio, M., Douradinha, B.G., Ramesar, J., Fonager, J., van Gemert, G.J., Luty, A.J., Hermsen, C.C., Sauerwein, R.W., Baptista, F.G., Mota, M.M., Waters, A.P., Que, I.,

- Lowik, C.W., Khan, S.M., Janse, C.J. and Franke-Fayard, B.M. (2009) Visualisation and quantitative analysis of the rodent malaria liver stage by real time imaging. PLoS One 4: e7881 (Journal IF: NA)
- Polido-Pereira, J., Ferreira, D., Rodrigues, A.M., Nascimento, C., Costa, P., Almeida, M., da Silva, J.E., Simão, C., Stone, R., Ramos, F., Neto, A., da Costa, J.C., Melo-Gomes, J., Gomes-Pedro, J., Viana-Queiroz, M., Canhão, H. and Fonseca, J.E. (2009) Rituximab use in pediatric autoimmune diseases: four case reports. Ann. NY Acad. Sci. 1173: 712-720 (Journal IF: 2.303)
- Pons-Estel, B.A., Sánchez-Guerrero, J., Romero-Díaz, J., Iglesias-Gamarra, A., Bonfa, E., Borba, E.F., Shinjo, S.K., Bernatsky, S., Clarke, A., García, M.A., Marcos, J.C., Duarte, A., Berbotto, G.A., Scherbarth, H., Marques, C.D., Onetti, L., Saurit, V., Souza, A.W., Velozo, E., Catoggio, L.J., Neira, O., Burgos, P.I., Ramirez, L.A., Molina, J.F., De La Torre, I.G., Silvariño, R., Manni, J.A., Durán-Barragán, S., Vilá, L.M., Fortin, P.R., Calvo-Alén, J., Santos, M.J., Portela, M., Esteva-Spinetti, M.H., Weisman, M., Acevedo, E.M., Segami, M.I., Gentiletti, S.B., Roldán, J., Navarro, I., Gonzalez, E., Liu, J.M., Karlson, E.W., Costenbader, K.H., Wolfe, F. and Alarcón, G.S. (2009) Validation of the Spanish, Portuguese and French versions of the Lupus Damage Index questionnaire: data from North and South America, Spain and Portugal. Lupus 18: 1033-1052 (Journal IF: 2.244)
- Pousinha, P.A., Sebastião, A.M and Ribeiro, J.A. (2010). Predominance of adenosine excitatory over inhibitory effects on transmission at the neuromuscular junction of infant rats. J. Pharmacol. Exp. Ther. 332: 153-163 (*Epub September 29, 2009*) (Journal IF: 4.309)
- Prudêncio, M. and Lehmann, M.J. (2009) Illuminating the host - how RNAi screens shed light on host-pathogen interactions. Biotechnol. J. 4: 826-837 (Journal IF: NA)
- Prudêncio, M., Derbyshire, E.T., Marques, C.A., Krishna, S., Mota, M.M. and Staines, H.M. (2009) *Plasmodium berghei*-infection induces volume-regulated anion channel-like activity in human hepatoma cells. Cell Microbiol. 11: 1492-1501 (Journal IF: 5.598)
- Ravasco, P. (2009) Cancer and nutrition: key determinants of Quality of Life. Eur. J. Cancer 45 (Suppl 1): 409 (Journal IF: 4.475)
- Ravasco, P., Aranha, M.M., Borralho, P.M., Moreira da Silva, I.B., Correia, L., Fernandes, A., Rodrigues, C.M.P. and Camilo, M.E. (2010) Colorectal carcinogenesis: interaction between nutrients, histology and NF-kB? Clin. Nutr. 29: 42-46 (*Epub July 1, 2009*)(Journal IF: 3.203)
- Ribeiro, J.A. and Sebastião A.M. (2010). Modulation and MetaModulation of Synapses by Adenosine. Acta Physiol. (in press) (accepted in 2009). (Journal IF: 2.455)
- Ribot, J.C., deBarros, A., Pang, D.J., Neves, J.F., Peperzak, V., Girardi, M., Borst, J., Hayday, A. C., Pennington, D.J. and Silva-Santos, B. (2009) CD27 is a thymic determinant of the balance between IFN-g- and IL-17-producing gd T cell subsets. Nat. Immunol. 10: 427-436 (Journal IF: 26.218)
- Ringel, S. and Swash, M. (2009) Money and Medicine. Neurology. 72: 766-768 (Journal IF: 7.043)
- Rino, J. and Carmo-Fonseca, M. (2009) The spliceosome: a self-organized macromolecular machine in the nucleus? Trends Cell Biol. 19: 357-384 (Journal IF: 13.385)
- Rino, J., Braga, J., Henriques, R. and Carmo-Fonseca, M. (2009) Frontiers in Fluorescence microscopy. Int. J. Dev. Biol. 53: 1569-1579 (Journal IF: 2.359)
- Robinson, M.J., Osorio, F., Rosas, M., Freitas, R., Schweighoffer, E., Groß, O., Verbeek, J.S., Ruland, J., Tybulewicz, V., Brown, G.D., Moita, L.F., Taylor, P.R. and Reis e Sousa, C. (2009) Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. J. Exp. Med. 206: 2037-2051 (Journal IF: 15.612)
- Rocha, S.F., Lopes, S.S., Gossler, A. and Henrique, D. (2009) Dll1 and Dll4 function sequentially in the retina and pV2 domain of the spinal cord to regulate neurogenesis and create cell diversity. Dev. Biology 328: 54-65 (Journal IF: 4.416)
- Rodrigues-Alves, R., Lopes, A., Pereira-Santos, MC., Lopes-Silva, S., Spínola-Santos, A., Costa,



- C., Branco-Ferreira, M., Lidholm, J. and Pereira-Barbosa, M. (2009). Clinical, Anamnestic and Serological Features of Peach Allergy in Portugal. *Int. Arch. Allergy Immunol.* 149: 65-73 (Journal IF: 2.160)
- Rodrigues, L., Filipe, J., Seldon, M.P., Anrather, J., Soares, M.P. and Simas, J.P. (2009) Termination of NF- $\kappa$ B activity via a gherpesvirus protein that assembles an EC<sub>5</sub>S ubiquitin-ligase. *EMBO J.* 28: 1283-1295 (Journal IF: 8.295)
- Ropele, S., Seewann, A., Gouw, A.A., van der Flier, W.M., Schmidt, R., Pantoni, L., Inzitari, D., Erkinjuntti, T., Scheltens, P., Wahlund, L.O., Waldemar, G., Chabriat, H., Ferro, J., Hennerici, M., O'Brien, J., Wallin, A., Langhorne, P., Visser, M.C., Barkhof, F., Fazekas, F.; LADIS study group (2009) Quantitation of brain tissue changes associated with white matter hyperintensities by diffusion-weighted and magnetization transfer imaging: the LADIS (Leukoaraiosis and Disability in the Elderly) study. *J. Magn. Reson. Imaging.* 29: 268-274 (Journal IF: 2.658)
- Ross, O.A., Spanaki, C., Griffith, A., Lin, C.H., Kachergus, J., Haugarvoll, K., Latsoudis, H., Plaitakis, A., Ferreira, J.J., Sampaio, C., Bonifati, V., Wu, R.M., Zabetian, C.P. and Farrer, M.J. (2009) Haplotype analysis of Lrrk2 R1441H carriers with parkinsonism. *Parkinsonism Relat. Disord.* 15: 466-467 (Journal IF: 1.907)
- Sampaio, C. (2009) Biomarkers in Clinical Trials of Alzheimer's Disease (AD): what is expected from regulatory agencies? *J. Nutr. Health Aging* 13: 339-340 (Journal IF: 2.321)
- Sampaio, C. (2009) Can focusing on UPDRS Part II make assessments of Parkinson disease progression more efficient? *Nat. Clin. Pract. Neurol.* 5: 130-131 (Journal IF: 6.979)
- Santos, M.J., Vinagre, F., Nero, P., Barcelos, F., Barcelos, A., Rodrigues, A.M., de Matos, A.A., Silva, C., Miranda, L., Capela, S., Marques, A., Branco, J. and da Silva, J.C. (2009) Predictors of damage progression in Portuguese patients with systemic lupus erythematosus. *Ann. NY Acad Sci.* 1173: 822-888 (Journal IF: 2.303)
- Savina, A., Peres, A., Cebrian, I., Carmo, N., Moita, C., Hacohen, N., Moita, L.F. and Amigorena, S. (2009) Rac2 controls phagosomal functions and crosspresentation selectively in CD8+ dendritic cells. *Immunity* 30: 544-555 (Journal IF: 20.579)
- Schulz, H., Kolde, R., Adler, P., Anastassiadis, K., Bader, M., Billon, N., Boeuf, H., Bourillot, P.Y., Buchholz, F., Dani, C., Doss, X., Forrester, L., Friel, R., Gitton, M., Henrique, D., Hescheler, J., Himmelbauer, H., Hübner, N., Karantzali, E., Kretsovali, A., Lubitz, S., Pradier, L., Reimand J., Rolletschek, A., Sachinidis, A., Savatier, P., Stewart, F., Storm, M.P., Trouillas, M., Vilo, J., Welham, M.J., Winkler, J., Wobus, A. and Hatzopoulos, A.K. (2009) The FunGenES database: a genomics resource for mouse embryonic stem cell differentiation. *PlosOne* 4:e 6804 (Journal IF: NA)
- Sebastião, A.M. and Ribeiro, J.A. (2009) Triggering neurotrophic factor actions through adenosine A2A receptor activation: implications for neuroprotection. *Br. J. Pharmacol.* 158: 15-22 (Journal IF: 4.902)
- Sebastião, A.M. and Ribeiro, J.A. (2009) Tuning and Fine-tuning synapses with adenosine, *Curr. Neuropharmacol.* 7: 180-194 (Journal IF: 1.151)
- Sequeira, A., Artoli, A.M., Silva-Herdade, A.S. and Saldanha, C. (2009) Leukocytes dynamics in microcirculation under shear-thinning blood flow. *Comput. Math. Appl.* 58: 1035-1044 (Journal IF: 0.997)
- Serpa, A., Ribeiro, J.A. and Sebastião, A.M. (2009) Cannabinoid CB1 and Adenosine A1 receptors independently inhibit hippocampal synaptic transmission. *Eur. J. Pharmacol.* 623: 41-46 (Journal IF: 2.787)
- Sillat, T., Pollanen, R., Caetano-Lopes, J., Porola, P., Ma, G., Korhonen, M. and Kontinen, Y.T. (2009) Intracrine androgenic apparatus in human bone marrow stromal cells. *J. Cell. Mol. Med.* 13: 3296-3302 (Journal IF: 5.114)
- Silva Nunes, M.V., Castro-Caldas, A., Del Rio, D., Maestú, F. and Ortiz, T. (2009) The ex-illiterate brain: the critical period, the cognitive reserve and the HAROLD model. *Dement.*

Neuropsychol. 3: 222-227 (Journal IF: NA)

Silva, A.\*, Jotta, P.Y.\*, Silveira, A.B., Ribeiro, D., Brandalise, S.R., Yunes, J.A. and Barata, J.T. (2009) Regulation of PTEN by CK2 and Notch1 in primary T-cell acute lymphoblastic leukemia: rationale for combined use of CK2- and gamma-secretase inhibitors. *Haematologica (Epub December 16, 2009)* \* Joint first authors (Journal IF: 5.978)

Silva, D., Santana, I., Simões do Couto, F., Maroco, J., Guerreiro, M. and de Mendonça, A. (2009) Cognitive deficits in middle-aged and older adults with bipolar disorder and cognitive complaints: Comparison with mild cognitive impairment. *Int. J. Geriatr. Psychiatry* 24: 614-631 (Journal IF: 2.128)

Simões, M.F., Valente, E., Rodriguez-Gomez, M.J., Anes, E\* and Constantino, L.\* (2009) Lipophilic POA amide and ester prodrugs. Stability, activation and activity against *M. tuberculosis*. *Eur. J. Pharm. Sci.* 37: 257-263 \* joint corresponding authors. (Journal IF 3.1)

Sousa, E. and Isenberg, D. (2009) Treating lupus: from serendipity to sense, the rise of the new biologicals and other emerging therapies. *Best Pract. Res. Clin. Rheumatol.* 23: 563-574 (Journal IF: 2.088)

Sousa, E., Caetano-Lopes, J., Pinto, P., Pimentel, F., Teles, J., Canhão, H., Rodrigues, A., Resende, C., Mourão, A.F., Ribeiro, C., Pinto, T.L., Rosa, C.M., da Silva, J.A., Branco, J., Ventura, F., Queiroz, M.V. and Fonseca, J.E. (2009) Ankylosing spondylitis susceptibility and severity - contribution of TNF gene promoter polymorphisms at positions -238 and -308. *Ann. NY Acad. Sci.* 1173: 581-588 (Journal IF: 2.303)

Stevenson, P.G., Simas, J.P. and Efstathiou, S. (2009) Immune control of mammalian gamma-herpesviruses: lessons from murine herpesvirus-4. *J. Gen. Virol.* 90: 2317-2330 (Journal IF: 3.092)

Suarez-Gestal, M., Calaza, M., Endreffy, E., Pullmann, R., Ordi-Ros, J., Domenico Sebastiani, G., Ruzickova, S., Santos, M.J., Papasteriades, C., Marchini, M., Skopouli, F.N., Suarez, A., Blanco, F.J., D'Alfonso, S., Bijl, M., Carreira, P., Witte, T., Migliaresi, S., Gomez-Reino, J.J., Gonzalez, A.; European Consortium Of SLE DNA Collections (2009) Replication of recently identified systemic lupus erythematosus genetic associations: a case control study. *Arthritis Res. Ther.* 11: R69 (Journal IF: 4.49)

Swash, M. (2009) Clinical questions need perceptive answers. *Pract. Neurol.* 9: 117 (Journal IF: NA)

Swash, M. (2009) Peer review and “openness”. *J. R. Soc. Med.* 102: 506-507 (Journal IF: 1.020)

Swash, M. (2010) Apraxia contributes to the motor deficit in Parkinson's Disease and Multiple System Atrophy. *Eur J Neurol. (Epub December 30, 2009)*(Journal IF: 2.732)

Tavanez, J.P., Bengoechea, R., Berciano, M.T., Lafarga, M., Carmo-Fonseca, M. and Enguita, F.J. (2009) Hsp70 Chaperones and type I PRMTs are sequestered at intranuclear inclusions caused by polyalanine expansions in PABPN1. *PLoS One* 4: e6418. (Journal IF: NA)

Telles-Correia, D., Cortez-Pinto, H., Barbosa, A., Mega, I., Monteiro, E. (2009) Quality of life following liver transplantation: a comparative study between Familial Amyloid Neuropathy and liver disease patients. *BMC Gastroenterol.* 9: 54 (Journal IF: 2.136)

Tenreiro, S., Sin, O., Miranda, H.V. and Outeiro, T.F. (2009) Yeast as a model to investigate the role and regulation of alpha-synuclein phosphorylation in Parkinson's disease. 24th International Conference on Yeast Genetics and Molecular Biology, Manchester, UK, July 19-24. *Yeast* 26: 189 (Journal IF: 2.622)

Teodorczuk, A., Firbank, M.J., Pantoni, L., Poggesi, A., Erkinjuntti, T., Wallin, A., Wahlund, L.O., Scheltens, P., Waldemar, G., Schrotter, G., Ferro, J.M., Chabriat, H., Bazner, H., Visser, M., Inzitari, D. and O'Brien, J.T. (2009) Relationship between baseline white-matter changes and development of late-life depressive symptoms: 3-year results from the LADIS study. *Psychol. Med.* 12: 1-8 (Journal IF: NA)

Townes, B.D., Martins, I.P., Castro-Caldas, A., Rosenbaum, G. and DeRouen, T. (2009). The

serial use of child neurocognitive tests: development versus practice effects. *Psychological Assessment*, 29 (4), 361-369 (Journal IF: NA)

Valadas, A., Bentes, C. and Ferro J. (2009) Generalized convulsive status epilepticus treatment: agreement of a standard is not sufficient. *Epilepsia* 50(Supp.10): 53-54 (Journal IF: 3.733)

Vale, N., Prudêncio, M., Marques, C.A., Collins, M.S., Gut, J., Nogueira, F., Matos, J., Rosenthal, P.J., Cushion, M.T., do Rosário, V.E., Mota, M.M., Moreira, R. and Gomes, P. (2009) Imidazoquinones as antimalarial and antipneumocystis agents. *J. Med. Chem.* 52: 7800-7807 (Journal IF: 4.898)

Vaz, C.C., Couto, M., Medeiros, D., Miranda, L., Costa, J., Nero, P., Barros, R., Santos, M.J., Sousa, E., Barcelos, A. and Inês, L. (2009) Undifferentiated connective tissue disease: a seven-center cross-sectional study of 184 patients. *Clin. Rheumatol.* 28: 915-921 (Journal IF: 1.559)

Veiga, A.S., Pattenden, L.K., Fletcher, J.M., Castanho, M.A.R.B. and Aguilar, M.I. (2009) Interactions of HIV-1 antibodies 2F5 and 4E10 with a gp41 epitope prebound to host and viral membrane model systems. *ChemBioChem.* 10: 1032-1044 (Journal IF: 3.322)

Viana-Pereira, M., Almeida, I., Sousa, S., Mahler-Araújo, B., Seruca, R., Pimentel, J. and Reis, R.M. (2009) Analysis of microsatellite instability in medulloblastoma. *Neuro. Oncol.* 11: 458-467 (Journal IF: NA)

Vinagre, F., Santos, M.J., Prata, A., Canas Silva, J. and Santos, A.I. (2009) Assessment of salivary gland function in Sjögren's Syndrome: the role of Salivary Gland Scintigraphy. *Autoimmun. Rev.* 8: 672-676 (Journal IF: 5.371)

Visser, K., Katchamart, W., Loza, E., Martinez-Lopez, J.A., Salliot, C., Trudeau, J., Bombardier, C., Carmona, L., van der Heijde, D., Bijlsma, J.W., Boumpas, D.T., Canhão, H., Edwards, C.J., Hamuryudan, V., Kvien, T.K., Leeb, B.F., Martín-Mola, E.M., Mielants, H., Müller-Ladner, U., Murphy, G., Østergaard, M., Pereira, I.A., Ramos-Remus, C., Valentini, G., Zochling, J. and Dougados, M. (2009) Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. *Ann. Rheum. Dis.* 68: 1086-1093 (Journal IF: 7.188)

Vitor, R.F., Esteves, T., Marques, F., Raposinho, P., Paulo, A., Rodrigues, S., Rueff, J., Casimiro, S., Costa L. and Santos, I. (2009) (99m)Tc-tricarbonyl complexes functionalized with anthracenyl fragments: synthesis, characterization, and evaluation of their radiotoxic effects in murine melanoma cells. *Cancer Biother. Radiopharm.* 24: 1-13 (Journal IF: 1.318)

Yuda, M., Iwanaga, S., Shigenobu, S., Mair, G.R., Janse, C.J., Waters, A.P., Kato, T. and Kaneko, I. (2009) Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. *Mol. Microbiol.* 71: 1402-1414 (Journal IF 5.462)

Zerr, I., Kallenberg, K., Summers, D.M., Romero, C., Taratuto, A., Heinemann, U., Breithaupt, M., Varges, D., Meissner, B., Ladogana, A., Schuur, M., Haik, S., Collins, S.J., Jansen, G.H., Stokin, G.B., Pimentel, J., Hoyer, E., Collie, D., Smith, P., Roberts, H., Brandel, J.P., van Duijn, C., Pocchiari, M., Begue, C., Cras, P., Will, R.G. and Sanchez-Juan, P. (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt Jakob disease. *Brain* 132:2659-2668 (Journal IF: 9.603)

## Seminars and Workshops

### Seminars

14-01-2009

**Sandra Diebold**, Kings College London, UK

*Using synthetic viral nucleic acids as adjuvants for tumour immunotherapy*

15-01-2009

**Maria Leite de Morais**, CNRS UMR 8147, Paris, France

*Invariant Natural Killer T cells can regulate allergic asthma severity*

20-01-2009

**Valerie Verhasselt**, INSERM - Université de Nice-Sophia Antipolis

*Neonatal tolerance under breastfeeding influence*

21-01-2009

**Jeffrey L. Platt**, MD Professor of Surgery and Microbiology and Immunology

University of Michigan, Ann Arbor, MI, USA

*Endogenous activation and control of toll like receptors*

21-01-2009

**Allan Lipton**, PennState University, USA

*Prediction of hormone resistance in breast cancer*

21-01-2009

**Ros Brett**, Strathclyde Institute for Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow

*Cannabis and cannabinoids - use and abuse*

23-01-2009

**Crystal Mackall**, Pediatric Oncology Branch of the National Cancer Institute

*Interleukin-7: Master Regulator of T Cell Homeostasis*

30-01-2009

**Jan Schmoranz**, Columbia University, USA

*Visualizing Polarity in Migrating Cells - Membrane Traffic and Centrosome Positioning*

18-02-2009

**Tecumseh Fitch**, University of St. Andrews, Scotland

*Bridges in Biology: Bio-Linguistics & the Broad Comparative Approach*

7-03-2009

**Patrick Blader**, Centre de Biologie du Développement Université Paul Sabatier, Toulouse, France

*Resolving mixed neural identities in the zebrafish epiphysis: a novel role for Notch*

7-03-2009

**Catarina Almeida**, Instituto de Biologia Molecular e Celular, Porto

*Regulation of the NK cell response*

12-03-2009

**Pedro Geraldes**, Mayo Clinic, Rochester MN

*Ig heavy chain promotes mature B cell survival in the absence of light chain*

13-03-2009

**Ivo Boneca**, Head of Group Biology and Genetics of the Bacterial Cell Wall, Institut Pasteur, Paris, France

*The multiples "personalities" of peptidoglycan: from bacterial shape to pathogenesis*

14-03-2009

**Margitta Dathe**, Leibniz Institute of Molecular Pharmacology, Berlin, Alemanha

*Peptide-modified micellar and liposomal carriers for therapeutic and diagnostic applications*

24-03-2009

**Susan Lindquist**, Whitehead Institute for Biomedical Research, MIT Howard Hughes Medical Institute

*Simple models of protein homeostasis diseases*

24-03-2009

**Jeff Kelly**, Scripps Research Institute, San Diego, USA

*Restoring Protein Homeostasis to Ameliorate a Wide Spectrum of Diseases*

25-03-2009

**Luis Barreiros**, Institut Pasteur, Paris, France

*Detecting Natural Selection on the Human Genome: The Case of Innate Immunity*

3-04-2009

**Arne Akbar**, Department of Immunology, Division of Infection and Immunity, University College London, UK

*The control of end-stage differentiation in human T cells*

4-04-2009

**Rafael Franco**, Membrane Receptors and Intercellular Communication, Dept. Bioquímica i Biologia Molecular Universitat de Barcelona

*Release of glutamate by dendritic cells and its effect on T-cell activation events: the neuroimmune synapse concept*

8-04-2009

**Primal de Lanerolle**, University of Illinois at Chicago

*How myosin phosphorylation regulates cytoskeletal dynamics and cell fate in cancer and hypertension*

14-04-2009

**Brigitte Gicquel**, Mycobacterial Genetics Unit, Institut Pasteur Paris

*Host pathogen interactions in tuberculosis*

17-04-2009

**Elena Cattaneo**, Università degli Studi di Milano, Itàlia

*Developing tools for drug discovery and studies of neurodegenerative diseases, a focus on huntingtin and the NS (Neural Stem) cells*

17-04-2009

**Michael Swash**, London University

*Priorities and Ideas in Neuroscience*

28-04-2009

**Cristina Munõz-Pinedo**, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain

*Crosstalk between energy metabolism and apoptosis*

29-04-2009

**Susanne Lens**, University Medical Center (UMC), Utrecht, Netherlands

*The chromosomal passenger complex and the control of chromosomal stability*

05-05-2009

**Joel Schneider**, University of Delaware, USA

*Material design for Regenerative Medicine*

12-05-2009

**Graham Anderson**, Institute for Biomedical Research Medical School, University of Birmingham

*Development of thymic epithelial cells and the establishment of immunological tolerance*

16-05-2009

### 30 | 2009 IMM Activity Report

**Jorge Pacheco**, Departamento de Física, Faculdade de Ciências, Universidade de Lisboa  
*Dynamics of hematopoiesis*

19-05-2009

**Jorge Pinto**, Iron Genes and Immune System , IBMC, Porto  
*New kids in town: Calreticulin and Erythropoietin in iron homeostasis*

26-05-2009

**Michael Boutros**, German Cancer Research Center, Heidelberg - Germany,  
*Not available*

29-05-2009

**Richard Colvin**, Harvard Medical School, Boston - EUA  
*RNAi screen reveals that lysosomal exocytosis is required for leukocyte chemotaxis*

29-05-2009

**Kira O'Reilly**, Liverpool, UK  
*Performing the body: practices of wounds, cuts and other things, interventions into the body as art practice*

2-06-2009

**Isabel Gordo** , IGC  
*Rate and effects of mutations in Escherichia coli*

04-06-2009

**Leah Mwai**, KEMRI-Wellcome Trust in Kilifi, Kenya  
*Understanding the mechanisms of resistance to piperazine, lumefantrine and amodiaquine in P.falciparum*

04-06-2009

**Inês Antunes**, National Institute for Medical Research, London UK  
*Naturally-occurring regulatory T cells in retroviral infection*

05-06-2009

**Hal Drakesmith**, Weatherall Institute of Molecular Medicine John Radcliffe Hospital, Oxford University  
*Regulation of iron metabolism during infections*

12-06-2009

**Michel Bornens**, Institut Curie, Paris, France  
*Adhesive control of cell shape, cell polarity and division axis*

16-06-2009

**José Silva**, Wellcome Trust Centre Stem Cell Research, Cambridge  
*From neural stem cells to ground state of pluripotency*

19-06-2009

**Carolina Maruta**, UNIC, IMM  
*The Use of Neuropsychological Tests across Europe: The Need for a Consensus in the Use of Assessment Tools for Dementia*

23-06-2009

**Catherine Hawrylowicz**, Kings College, London  
*Regulatory T cells in allergy and asthma: the role of the Vitamin D pathway*

26-06-2009

**Rolf Borlinghaus**, Leica Microsystems CMS  
*Confocal Fluorescence without limits by white laser excitation and Superrresolution by Stimulated Emission Depletion*

26-06-2009

**João Lobo Antunes**  
*Egas Moniz nos 60 anos do prémio Nobel*

30-06-2009

**César Evaristo**, Necker Institute, Paris, France  
*Characterization of the CD8 T cell immune responses to infection*

17-07-2009

**Joel Sussman**, Department of Structural Biology, Israel Structural Proteomics Center (ISPC) & Neurobiology, Weizmann Institute of Science, Rehovot, Israel  
*Intrinsically disordered proteins (IDPs): possible role in nervous system development*

24-07-2009

**Luis Almeida**, CNRS, University, of Nice, France  
*A Mechanical Model for Dorsal Closure and Embryonic Wound Healing*

11-09-2009

**Stefan J. Erkeland**, Hematology department, Erasmus University Medical Center Rotterdam, The Netherlands  
*Identification and functional investigation of oncogenic miRNAs in acute myeloid leukemia*

18-09-2009

**Otilia Vieira**, Universidade de Coimbra  
*Identifying of the molecular machinery involved in phagosomal maturation*

22-09-2009

**Maddy Parsons**, King's College London, UK  
*Integrin regulation of cell motility*

24-09-2009

**Hazel Pinheiro**, PhD Technical Sales Specialist |Exiqon A/S  
*Discovery of microRNA biomarkers for cancer using LNA based technologies*

06-10-2009

**Lucia B. Rothman-Denes**, Department of Molecular Genetics and Cell Biology, University of Chicago  
*A new paradigm for RNA polymerase activation in a virion-RNA Polymerase*

08-10-2009

**Jorge Ramos de Almeida**, Vaccine Research Center, NIH  
*Control of HIV-1 replication by CD8 T cells is reflected by their antigen sensitivity, polyfunctionality and clonal turnover*

14-10-2009

**Scott K. Durum**, Laboratory of Molecular Immunoregulation, National Cancer Institute, USA  
*Interleukin 7 in the life and death of lymphocytes*

16-10-2009

**Ron Hay**, University of Dundee  
*Role of SUMO modification in gene expression*

22-10-2009

**Antonio Giraldez**, Yale Department of Genetics  
*The role of microRNAs in vertebrate development*

03-11-2009

**Dyche Mullins**, Department of Cellular and Molecular Pharmacology University of California, San Francisco, USA  
*A Little Larger than the Entire Cytoskeleton: the Role of Actin in Prokaryotes*

03-12-2009

**Achim Gossler**, Medizinische Hochschule Hannover, Germany  
*Function and regulation of the homeobox gene Noto*

## 32 | 2009 IMM Activity Report

16-12-2009

**Mario Recker**, Department of Zoology, University of Oxford, Oxford, UK

*The role of non-random switching hierarchies among virulence genes in Plasmodium falciparum infections*

Not available

**Leonor Cancela**, Universidade do Algarve

*The growing role of fish as a model organism to unveil molecular determinants of skeletogenesis*

### IMM Seminar Series

19-01-2009

**Herman Waldmann**, Sir William Dunn School of Pathology, Oxford University, UK

*Regulation and Privilege in Therapeutic Tolerance*

26-01-2009

**Helena Cortez-Pinto**, Assistente Graduada de Gastreenterologia no HSM, Professora Auxiliar com Agregação da FML

*The role of ectopic fat in insulin resistance and non-alcoholic fatty liver disease*

02-02-2009

**António Jacinto**, IMM

*Epithelial Repair in Drosophila*

09-02-2009

**Miguel Castanho**, IMM

*Beating the blood-brain-barrier.(S)low (but effective) throughput screening in the quest for a new analgesic drug*

02-03-2009

**Luísa Vasconcelos**, IGC

*The sexually dimorphic brain*

09-03-2009

**Susana Constantino**, IMM

*Undisclosed Effects of Ionizing Radiation in Angiogenesis*

16-03-2009

**Klaus Pantel**, Institut für Tumorbologie, Universitätsklinikum Hamburg-Eppendorf, Germany

*Detection, molecular characterization and clinical relevance of disseminating cancer cells*

06-04-2009

**Luís Graça**, IMM

*Persuading conventional natural killer T cells to adopt unconventional lifestyles*

13-04-2009

**Cláudia Barros**, The Scripps Research Institute, Department of Cell Biology, La Jolla, USA

*Neuregulin-1 signaling and Schizophrenia*

20-04-2009

**Adriano Aguzzi**, University Hospital of Zürich, Zürich, Switzerland

*Molecular Biology of Prions*

27-04-2009

**Thomas Schilling**, Department of Developmental and Cell Biology, University of California, Irvine

*Regulating adhesion during cell migration in zebrafish*

25-05-2009

**Sebastian Amigorena**, Institute Curie, Paris, France

*Regulatory T cells control antitumor immune responses: dynamic imaging in vivo*



01-06-2009

**Mamede Carvalho, IMM**  
*A Moment of Inspiration*

08-06-2009

**Sara Ricardo, Skirball Institute/ NYU Medical Center, USA**  
*An ABC transporter controls the export of a Drosophila germ cell attractant*

15-06-2009

**Ana Sebastião, IMM**  
*Modulation of nicotinic responses in the hippocampus*

22-06-2009

**Mara Almeida, MRC Unit, Oxford Centre for Gene function, University of Oxford, UK**  
*Characterisation of a novel binding partner for the Survival Motor Neuron*

06-07-2009

**Luís Costa, IMM / Hospital de Santa Maria**  
*Cancer metastases: is bone an useful paradigm?*

13-07-2009

**Scott Holley, Yale University**  
*Segmental Patterning and Morphogenesis during Zebrafish Somitogenesis*

20-07-2009

**Marta Agostinho / Margarida Trindade, IMM**  
*An insight into Communication and Funding Support at IMM*

07-09-2009

**Nuno Santos, IMM**  
*Protein-biomembrane interactions as therapeutic targets*

21-09-2009

**Aleksey Kazantsev, MGH, Harvard Medical School**  
*Therapeutic strategies for the treatment of neurodegenerative diseases*

19-10-2009

**Gunnar Mair, IMM**  
*P-bodies and gene expression control in Plasmodium*

26-10-2009

**Mario Ramirez, IMM**  
*What happens when phages want out*

02-11-2009

**Tiago Outeiro, IMM**  
*Mad Cows, neurotic yeast, and back to the future: the world of protein misfolding*

16-11-2009

**Henrique Veiga-Fernandes, IMM**  
*Lymphoid organogenesis and beyond*

23-11-2009

**Domingos Henrique, IMM**  
*Welcome to Notchland*

14-12-2009

**Miguel Soares, IGC**  
*Towards a Molecular Basis For Host Tolerance To Infection: Basic concepts and possible therapeutic implications*

21-12-2009

**Rui Monteiro, MRC Molecular Haematology Unit Weatherall Institute of Molecular Medicine University of Oxford**  
*How to make a blood cell: transcriptional regulation of haematopoiesis in the zebrafish*

Neurosciences Seminar Series

07-09-2009

**Vânia Batalha, IMM**

*Adenosine A2A receptors in the Rat Hippocampus: stress related changes*

21-09-2009

**Paula Pousinha, IMM**

*Cross talk between neurotrophic factor receptors (TrkB) and adenosine A2A receptors: Implications for Amyotrophic Lateral Sclerosis (ALS)*

28-09-2009 11:00 - 12:00

**Vasco Sousa, IMM**

*Modulation of cannabinoid signaling by adenosine A1 receptors and chronic caffeine administration: a behavioural and neurochemical approach*

19-10-2009

**Ana Filipa Ribeiro, IMM**

*Modulation of Neuronal Maturation: Influence of Neurotrophic Factors and Neuromodulators*

26-10-2009

**Leonor Miller Fleming, IMM**

*GOSPEL: a neuroprotective protein that binds to GAPDH upon S-nitrosylation*

02-11-2009

**Armando Cruz, IMM**

*Project presentation Influence of epilepsy on synaptic plasticity in the hippocampus: neuroprotector role of VIP and VIP receptors*

09-11-2009

**Jorge Valadas, IMM**

*Project presentation: Role of A2A Receptors in the neuroprotective effect of corticotropin releasing hormone*

16-11-2009

**Daniela Calçada, IMM**

*Project presentation: Possible roles of beta-amyloid peptide in autophagy: Modulation by BDNF and adenosine A2A receptors*

23-11-2009

**Rosalina Fonseca, Postdoc IGC**

*Shaping the system: Rules of heterosynaptic plasticity*

30-11-2009

**Sofia Cristóvão-Ferreira, PhD student, IFN**

*Role of GABA transporter 3 in GABAergic synaptic transmission at striatal output neurons*

07-12-2009

**Hugo Vicente Miranda, PhD student, INCM**

*Protein glycation and Methylglyoxal Metabolism in Parkinson's Disease*

14-12-2009

**Diana Cunha Reis, IMM**

*Endogenous control of LTD and depotentiation in the hippocampus by VIP*

21-12-2009

**Catarina Gomes, IMM**

*Neurotrophic factors modulation by adenosine receptors and dopamine release in the rat striatum*

29-06-2009

**Diogo Rombo, IMM / Institute of Pharmacology and Neurosciences, FML**

*Adenosine A1 receptors negatively modulate GABAA receptor mediated postsynaptic currents in rat hippocampal CA1 pyramidal cells*

06-07-2009

**Ana Raquel Esteves Tomé, IMM**

*Adenosine Levels in transgenic mice that express altered levels of Adenosine Kinase*

13-07-2009

**Ricardo Reis**, Instituto de Biofísica, Universidade Federal do Rio de Janeiro

*Diferenciação Funcional de neuroesferas de retina de ratinhos*

20-07-2009

**Rita Aroeira, IMM**

*Spatial-temporal characterization of the glycine receptor in rat hippocampus*

27-07-2009

**André Santos, IMM**

*Influence of adenosine A2A receptors on neuroprotective effect of BDNF upon neuronal death induced by amyloid-beta peptide*

### e-PI Seminars

14-10-2009

**Rui Costa, IGC/FC**

*Neural mechanisms of action learning: from intent to habit*

04-11-2009

**Sérgio Dias, IPO**

*Cell interactions in the bone marrow and beyond*

18-11-2009

**Cecília Rodrigues, iMED/FFUL**

*Controlling cell fate: from molecules, to genes, to disease models*

02-12-2009

**Cláudia Lobato da Silva, IBB- Instituto Superior Técnico**

*Ex-vivo Expansion of Human Adult Stem Cells for Cellular Therapies*

### Onboard Talks

14-01-2010

**Anna Bershtey**

*Synthetic Pathogen for vaccines - Biodegradable nanoparticles enveloped by lipid shells, onions, and flowers*

28-01-2010

**Daniel Correia**

*Presentation and discussion of an interesting paper*

11-02-2010

**Sílvia Portugal**

*Interesting but tricky results on malaria infection process*

25-02-2010

**Ana Rita Grosso**

*Tools for genome-wide approaches*

11-03-2010

**Daniel Silva**

*Bioinformatics - more than spreadsheets. Brainstorming the future*

### 36 | 2009 IMM Activity Report

25-03-2010

**Henri Franquelim**

*Atomic Force Microscopy - a powerful tool to unravel membrane events*

08-04-2010

**PhD Students**

*Chill-out evening. Social event for the entire IMM community*

22-04-2010

**Ivo Martins**

*Amyloid biophysics: breaking fibers into pieces*

06-05-2010

**Sara Sousa**

*Regeneration in adult zebrafish*

27-05-2010

**PhD Students**

*Photo session from the II PhD Stds Retreat*

17-06-2009 18:00 - 19:00

**Elsa Abranches, IMM**

*To grow or not to grow: that is the question... in mammalian cell culture!*

01-07-2009

**Bruno Cardoso, IMM**

*T-ALL, Tal1 and HDAC inhibitors...*

15-07-2009

**Pedro Alves, IMM**

*Systematic identification of Splicing factors with a role in IL-1 $\beta$  secretion*

29-07-2009

**Sandra Aguiar, IMM**

*Bacteriocins of Streptococcus pneumoniae*

09-09-2009

**Raquel Dias, IMM**

*Excitotoxicity: the danger of getting neurons too excited...*

23-09-2009

**Vanda Vitorino de Almeida, IMM**

*Flow Chamber in shear-stress experiments – How to use it?*

21-10-2009

**Mariana Campos, IMM**

*Ret in haematopoiesis: a MSc thesis*

18-11-2009

**Inês Domingues, IMM**

*VEGFR-2 - A new role in cell nucleus*

### Immunology Club

12-02-2009

**Rita Cavaleiro (UIC), IMM**

*Dendritic cells in HIV-1 and HIV-2 infection*

12 -03-2009

**Daniel Correia (UNIMOL), IMM**

*Activation of anti-tumor human  $\alpha\alpha$  T cells*

26-03-2009

**Nina Matova (UBCSI), IMM**

*Rel/NF-kappaB double mutants reveal that cellular immunity is central to Drosophila host defense*

2-4-2009

**Henrique Veiga-Fernandes (UIB), IMM**

*Lymphoid organogenesis*

23-04-2009

**Joana Lopes (UIR), IMM**

*Inflammation and bone homeostasis*

30-04-2009

**Telma Lança (UNIMOL), IMM**

*Non-classical MHC proteins in tumor cell recognition by human  $\alpha\alpha$  T cells*

7-05-2009

**Ivonne Wollenberg (UNICEL), IMM**

*New perspectives in the termination of the GC reaction: visualization of GC regulatory T cells*

28-05-2009

**Hélia Neves (UBH), FMUL**

*Early-stages of chick thymus development: The role of BMP signalling*

17-06-2009

**Francisco d'Orey (UNIMOL), IMM**

*Role of CD27 costimulation in the activation of peripheral gd T cells of mice and humans*

9-07-2009

**Pedro Alves (UBCSI), IMM**

*Systematic identification of splicing factors with a role in IL-1 $\alpha$  secretion*

24-09-2009

**Catarina Almeida and Joana Duarte (UNICEL), IMM**

*ECI Berlin 2009 - an inside view*

29-10-2009

**Rita Barbosa (UIC), IMM**

*A link between human memory B cell differentiation and IL-17 producing CD4 T cells?*

26-11-2009

**Ana Pena (UMA), IMM**

*Carbon monoxide and brain sequestration of Plasmodium berghei ANKA in Experimental Cerebral Malaria*

17-12-2009

**Inês Perpétuo (UIR), IMM**

*Differential influence of rheumatoid arthritis therapies on osteoclasts*

### Lisbon Area Neuroscience Meetings

25-11-2009

**Eduardo Dias-Ferreira, IGC**

*Stressing out Decisions*

**Rita Machado de Oliveira, IMM**

*Spotlight on ACE: from bedside to the bench*

16-12-2009

**Sandra Vaz, IMM**

*Regulation of GABA transporters: Brain-derived neurotrophic factor effect on GABA uptake*

**Masayoshi Murakami, IGC**

*Neural mechanisms of self-control in the frontal cortex*

### Tissue Regeneration and Stem Cells Forum

09-07-2009

**Rita Aires**, Organogenesis Lab, IGC

*Electrical control of cell behavior in Regeneration*

23-07-2009

**Catarina Ramos**, IMM

*The role of Stromal Stem Cells in Tissue Regeneration and Wound Repair*

19-11-2009

**Rita Mateus**, IMM

*Growth control in Development and Regeneration*

17-12-2009

**Joana Monteiro**, IGC

*The influence of the microenvironment on regeneration*

### Gene Expression Lab Meetings

05-06-2009

**Sandra Martins** and **Houda Hallay**, IMM

*Lab Meeting: Report of the 14th Annual RNA Meeting, Wisconsin, USA*

03-07-2009

**Célia Carvalho**, IMM

*Live cell imaging reveals cross-talk between spliceosome assembly and RNA Pol II dynamics – an update*

10-07-2009

**Joana Desterro**, IMM

*Auto-Regulation of the heterodimeric splicing factor-U2AF*

### Oncology Series

16-03-2009

**Klaus Pantel**, Director do Instituto de Biologia Tumoral, Universidade de Hamburgo

*Detection, molecular characterization and clinical relevance of disseminating cancer cells*

29-06-2009

**Rakesh Kumar**, George Washington University, Washington DC – USA

*The Biology of Breast Cancer*

31-10-2009

**Josep Tabernero**, Hospital Vall d'Hebron, Barcelona

*Target therapy in Colon Cancer: How to choose*

### Workshops

24-06-2009 a 26-06-2009

**BioImaging**

*TissueFAXS demo*

05-10-2009 a 06-10-2009

**EURASNET**

*Workshop Splicing in Cell Biology*

20-10-2009

**Applied Biosystems**

*Innovative Molecular Biology Solutions in the Clinical Environment*

23-10-2009

**Luis Ferreira Moita**

*IMM Workshop: Pathways of Drug Discovery*

04-11-2009

*iBox Scientia – small animal in vivo fluorescence imaging system*

23-11-2009 a 26-11-2009

**Flow Cytometry Unit**

*Flow Cytometry Workshop*

## IMM International PhD Programme

The IMM is strongly committed to Advanced Training in Biomedicine, in particular through the IMM PhD Programme.

### Programme Team

<i>Director</i>	<i>António Jacinto, PhD (Until June 2009)</i> <i>Miguel Carneiro de Moura, MD, PhD (Since July 2009)</i>
<i>Executive Coordinator:</i>	<i>Inês Crisóstomo, PhD (Since July 2009)</i>
<i>Programme Manager:</i>	<i>Marta Agostinho, PhD (Until June 2009)</i> <i>Joana Costa, PhD (Until October 2009)</i>
<i>Scientific Advisory Board:</i>	<i>António Jacinto, PhD (Since July 2009)</i> <i>Ana Espada de Sousa, MD, PhD</i> <i>Isabel Pavão Martins, MD, PhD</i>

The IMM International PhD Programme in Biomedical Sciences recruits every year highly motivated students to join one of the three IMM research programmes: Cell & Developmental Biology, Immunology & Infectious Diseases and Neurosciences.

IMM's mission is to enable these students to be able to carry out independent, original and scientifically significant research, and become critical thinkers. The core of the IMM PhD Programme is the research project, which is complemented by a "student-oriented" training component. IMM PhD students are accompanied by a supervisor and by a Thesis Committee that includes the student's Tutor. Moreover, students are challenged to organize one or more activities.

The PhD training component has a flexible modular structure that includes:

- Nanocourses, IMM provides a wide selection of courses, aimed to provide:
  - fundamental scientific knowledge;
  - specific topics among the IMM research programmes;
  - transferable skills training;
- Workshops (organized by IMM or by other national or international research institutions);
- Seminars and Scientific meetings.

In 2009 the PhD Team organized the programme - always aiming at improvement, hence the PhD Team set-up a booklet of rules and regulations and increase and diversify the IMM nanocourses. The students commission students initiated a seminar series.

### PhD Programme in numbers

- 94** Total n° of Students (December 31, 2009)
- 85** Total n° of Students with fellowships from Fundação para a Ciência e Tecnologia
- 30** Thesis committee meetings

Other PhD fellowships: 1 Axa Research Fund, 1 Marie Curie Initial Training Network, 2 MD Resident (PFMA-FCT), 1 IMM.



### Students enrolling in 2009 (17)

Ana Água-Doce, Ana Rita Barbosa, Angelica Vittori, Carolina Moreira, Cristina Silva , Inês Cristo, Joana Tato Costa, Marta Amaral, Maurice Itoe, Nádia Correia, Oldriska Marques, Pedro Martins Pereira, Rita Mateus, Rosangela Frita, Susana Gonçalves, Telma Lança and Vera Geraldes.

### IMM Nanocourses (7)

In 2009 IMM organized specifically for its student's seven nanocourses:

#### ***Scientists and the Media***

Organizer: Marta Agostinho

Dates: February 12-13

This course aimed to bring scientists and journalists together and discuss how and why one should communicate their science. Also the students were given insights on the way the media works. The course had a practical component of writing and interviews.

#### ***Science outside academia***

Organizer: Miguel Castanho

Date: March 9

This nanocourse aimed to provide information and showing examples of people that found different ways of life outside academia. Some of these people are enablers of new opportunities, some are just good role models but they all have something to say that might help the students.

#### ***Protein Expression and Purification***

Organizer: Francisco Enguita

Dates: April 15-17

This course aimed at training students in the theoretical and practical topics related with heterologous protein expression in several hosts

#### ***Flow Cytometry***

Organizer: Flow Cytometry Unit

Dates: April 20-23

This workshop was designed for the beginner to intermediate flow cytometry users. It had both theoretical and practical modules. The latter allowed for students to have direct contact (“hands-on”) with essential aspects of acquisition and analysis of biological samples by flow cytometry.

#### ***Biology of Cancer***

Organizer: João Barata

Dates: April 27-30

The course covered different aspects of cancer research and therapeutics, from the basics, to the bench, to the bedside.

#### ***Nanomedicine***

Organizer: Nuno Santos

Dates: May 4-6

The lectures focused on the following: i) nanotechnology-based diagnostics; ii) nanotechnology-based imaging; iii) targeted drug delivery and release; and, iv) regenerative medicine. These were complemented with practical sessions on atomic force microscopy (AFM) and “force spectroscopy”.

***BioImaging Nanocourse on Microscopy***

Organizer: José Rino, IMM

Date: 27-28 October (Afternoons 15h-18h30m)

This nanocourse aimed at beginning or intermediate microscope users, gave students the opportunity to become familiar with both basic microscopy techniques and more advanced 3D microscopy and live imaging during two intensive afternoon sessions.

**Courses under PFMA – “Programa de Formação Médica Avançada”**

Besides IMM nanocourses and under the IMM partnership with the Doctoral Program for Physicians (PFMA) IMM students attended the following courses:

Immune Disorders; Ageing; Neurodegenerative Disorders; Regenerative Medicine; Scientific Integrity; Preparing for Research; Cell Cycle and Disease and Computational Biology.

**PhD Students Activities**

PhD students are challenged to actively suggest activities to foster both scientific and social networking between the IMM research community. Every year a group of students volunteers to form the students’ commission, who is responsible for the organization of the different initiatives: “Students’ Annual Retreat”, the “Annual IMM PhD Students Meeting”. In 2009 the students’ commission launched a new activity: a seminar series, the “Onboard Talks”.

***2009 Students committee:***

Daniel Silva, Henri Franquelim, Joana Tato, Manuel Melo, Marco Domingues, Marta Ribeiro, Pedro Matos, Raquel Mendes, Sara Sousa, Susana Gonçalves

***II IMM PhD Students Retreat***

**Date:** 15 – 17 May 2009

**Place:** Quinta dos Amarelos, Vaiamonte, Alentejo

**Participants:** 49, PhD students, post-docs, master students and technicians

**Programme:**

- Presentation of the group using the game "Indiscrete friend"
- Round table under the theme "Working at the IMM"
- Workshop about "Multidisciplinary in scientific projects"
- Leisure activity in groups : "Treasure Hunt"
- Visit to Herdade do Esporão

***III IMM PhD Students Annual Meeting***

**Date:** October 12-13, 2009

**Place:** IMM

**Participants:** 54 PhD students, 12 Guests, 9 Moderators, 3 Members of Jury, 4 PhD Team (Total: 82), plus audience

**Programme:**

October 12, 2009

***Opening Session***

Pedro Alves, Inês Crisóstomo, and Miguel Carneiro de Moura

***Cell and Developmental Biology Programme***

Keynote Speaker: Leonor Saúde, IMM, Portugal

(Moderators: Rita Fior and Manuel Melo)

Talks: Leila Martins, Vanda Almeida, Leila Martins, Vanda Almeida, Ana Inês Domingues, Inês

Vala, Jorge Andrade, Ana Inês Domingues, Inês Vala, Jorge Andrade, Pedro Alves and Filomena Carvalho.

(Moderators: Soren Prag and Catarina Moita)

Talks: Marco Domingues, Henri Franquelim, Sara Sousa, Mariana Simões, Aida Costa, and Filipe Vilas-Boas

**Neurosciences Programme**

Keynote Speaker: Tiago Outeiro, IMM, Portugal

(Moderators: Luísa Lopes and Zrinka Marijanovic)

Talks: Susana Pinto; Hugo Miranda; Raquel Dias

**Poster Session**

**Round-table discussion**

“What to do with a discovery?”

Moderator: Miguel Castanho, IMM, Portugal

Invited Speakers: João Marcelino (INPI), Pedro Vilarinho (COHiTEC), Paulo Santos (Crioestaminal) and Nuno Afonso (IMM)

October 13, 2009

**Immunology and Infectious Diseases Programme**

Keynote Speaker: Pedro Simas, IMM, Portugal

(Moderators: João Carriço and Afonso Almeida)

Talks: Joana Lopes, Daniel Correia, Ana Barros, Marta Caridade, Ivonne Wollenberg and Rita Tendeiro

(Moderators: Lénia Rodrigues and Ana Água-Doce)

Talks: Adriana Albuquerque, Helena Cabaço, Patrícia Inácio, Sílvia Portugal and Carina dos Santos, Margarida Carrolo, Elisabete Martins and Sandra Aguiar

**Poster Session**

**Closing Session**

“Principles in Biology? Towards a Physics of Living Matter”

Keynote Speaker: Alfonso Martinez-Arias

University of Cambridge, UK

“Getting in and especially out of Mitosis”

Keynote Speaker: Tim Hunt, Nobel Prize in Physiology or Medicine,

ICRF Clare Hall Laboratories, UK

**Closing Ceremonies and Awards**

Maria do Carmo Fonseca, IMM Executive Director

Manuel Heitor, Secretário de Estado da Ciência e Tecnologia

**Dinner**

Entertainment by "Meninos do Coro Aiéméme" (Group of IMM PhD Students)

**Onboard Talks**

**Date:** January - November, 2009

**Place:** IMM

**Participants:** 18 speakers plus audience (More on Seminars and Workshops)

**PhD Thesis in 2009 (11)**

**Maria Vânia Nunes** (2009) Estudo das alterações do sistema mnésico com a idade. Uma abordagem integrada no âmbito das neurociências do envelhecimento. Supervisor: A. Castro Caldas. *Faculdade de Medicina da Universidade de Lisboa, February 18-19.*

**Maria José Diógenes** (2009) Cross talk between adenoise A24 receptors and the TrK receptors for brain-derived neurotrophic factor (BDNF). Supervisor: J.A. Ribeiro. *Faculdade de Medicina*

*da Universidade de Lisboa, March 4-5.*

**Inês Mollet** (2009) Genome wide mining of alternative splicing in metazoan model organisms. Supervisor: M. Carmo-Fonseca. *Faculdade de Medicina da Universidade de Lisboa, April 13-14.*

**João Parracho Costa** (2009) Evaluation of human subcortical circuits and pathways through stimulation and direct recording from electrodes implanted for chronic deep brain stimulation. Supervisor: M de Carvalho. *Faculdade de Medicina da Universidade de Lisboa, May 12-13.*

**Joaquim Ferreira** (2009) Anti-Parkinsonian drugs and sleep disorders in Parkinson's disease. Supervisors: C. Sampaio and O. Rascol. *Faculdade de Medicina da Universidade de Lisboa, Julho 7-8.*

**Henrique do Rosário** (2009) Matrix metalloproteinase-9 and pancreatic trypsin in the intestinal wall – A contribution to the understanding of intestinal ischemia-reperfusion. Supervisor: J. Martins e Silva and Co-supervisor: G. Schmid-Schönbein. *Faculdade de Medicina da Universidade de Lisboa, July 16-17.*

**Rita Cavaleiro** (2009) Modulação de Células Dendríticas e Monócitos pelo Vírus da Imunodeficiência Humana Tipo 2. Supervisor: A. Espada de Sousa and Co-supervisor: R.M.M. Victorino. *Faculdade de Medicina da Universidade de Lisboa, December 3, 2009.*

**Susana F. Rocha** (2009) The role of multiple DSL ligands - Dll1, Dll4 and Jag1 – in vertebrate neurogenesis. Supervisor: D. Henrique. *Faculdade de Medicina da Universidade de Lisboa, December 4.*

**Russell Foxall** (2009) HIV-specific responses, T-cell activation and regulatory T-cells in AIDS pathogenesis: lessons from the HIV-2 model. Supervisor: A. Espada de Sousa; Co-supervisor: R.M.M. Victorino. *Faculdade de Medicina da Universidade de Lisboa, December 10.*

**Patrícia Canhão** (2009) Doença trombótica das veias e seios venosos cerebrais. Supervisor: J.M. Ferro. *Faculdade de Medicina da Universidade de Lisboa, December 16.*

**Catarina S. Cortesão** (2009) Studies on a human model and a murine model of immunodeficiency: HIV-2 infection and TACI deficiency. Co-supervisors: A. Espada de Sousa and M. Cascalho. *Faculdade de Medicina da Universidade de Lisboa, December 21.*

## Partnerships

**Centro Académico de Medicina de Lisboa:** IMM is associated with the Faculdade de Medicina da Universidade de Lisboa and with the Santa Maria teaching hospital through the Medical Academic Centre of Lisbon (CAML). CAML is a newly formed consortium that represents an infrastructure aiming to promote the academic dimension in clinical practice, renewing the teaching hospital concept.

IMM is also a partner of the **Harvard Medical School – Portugal programme**, sponsored by Fundação para a Ciência e Tecnologia. This programme, directed by M. Carmo-Fonseca (IMM/FML), results from a Memorandum of Understanding between Portuguese Ministry of Science, Technology and Higher Education and Harvard Medical School to encourage internationalization and cooperation between Portuguese schools of medicine and major national research centers working in biomedical and health sciences.

Furthermore, IMM is associated with the **Doctoral Programme for Physicians, PFMA**, supported by the Gulbenkian and Champalimaud Foundations, the Ministry of Health and the Foundation for Science and Technology.

Besides education IMM also fosters scientific ideas to turn into products and technologies that make difference in health care. To achieve this goal IMM develops ties and strategic plans with companies, namely companies incubated at IMM: **Genomed** and **Technophage** (See Companies). In addition, IMM is one of the leading founders of the **Health Cluster Portugal**. This consortium promotes initiatives and research projects that increase the national competitiveness, innovation and technology and also encourages cooperation between companies, organizations, universities and public entities, seeking to expand economic areas related to health and to the improvement of health care.



## **RESEARCH**

In 2009, IMM hosted 31 research units.

Of these:

- 28 are located at Edifício Egas Moniz (IMM),
- 1 at Hospital Santa Maria (Neurological Clinical Research Unit)
- 2 at the Faculdade de Farmácia da Universidade de Lisboa (Mycobacteria Host Interactions Unit and Retrovirus Research Unit)

One unit, the Parasite Molecular Genetics, started its activity in 2009 as a result of the Group Leader recruitments that were held October 2008:





## Cell and Developmental Biology programme

**Programme Coordinators:** António Jacinto and Luís Moita

### **Heads of Unit:**

M. Carmo Fonseca, MD, PhD

João Ferreira, MD, PhD

Domingos Henrique, PhD

António Jacinto, PhD

Carlota Sadanha, PhD

Susana Constantino Santos, PhD

Nuno C. Santos, PhD

Miguel Castanho, PhD

Luís Ferreira Moita, MD, PhD

João Taborda Barata, PhD

Luis Costa, MD, PhD

Leonor Saúde, PhD

Musa Mhlanga, PhD

### **Principal Investigators:**

M. Carmo-Fonseca, Francisco Enguita

João Ferreira

Domingos Henrique

António Jacinto, Soren Prag

Carlota Sadanha

Susana Constantino Santos

Nuno C. Santos

Miguel Castanho

Luís Ferreira Moita, Nina Matova

João Taborda Barata

Luis Costa

Leonor Saúde

Musa Mhlanga



## Cell Biology Unit

### Description and Objectives

Understanding the genomic programme of the human organism requires knowledge of how the information encoded in DNA is processed to generate messenger RNAs that can be translated into proteins, the building blocks of the different tissues in the body. In the cell, extensive modification and alternative processing of the initial products of gene transcription can profoundly affect the diversity and function of the proteins that are generated from a single gene. Our lab is interested in identifying mechanisms of mRNA biogenesis to better understand diseases caused by errors that affect these processes. Making use of a multidisciplinary approach that combines live-cell microscopy, computational modeling, molecular biology, biochemistry and bioinformatics, our group developed expertise to study how the dynamic properties of RNA-protein complexes contribute to post-transcriptional gene regulation.

### Head of Unit

Maria Carmo-Fonseca, MD, PhD – Full Professor, FMUL

### Principal Investigators

Francisco Enguita, PhD - Auxiliary Professor, FMUL

### Research Team

Teresa Carvalho, MD, PhD	- Auxiliary Professor, FMUL
Joana Desterro, PhD	- Auxiliary Professor, FMUL
Célia Carvalho, PhD	- Auxiliary Professor, FMUL
Noélia Custódio, PhD	- Auxiliary Professor, FMUL
Sandra Martins, PhD	- Auxiliary Professor, FMUL
José Rino, PhD	- Researcher, IMM
Natalia Kozlova, PhD	- Post-Doc fellowship, FCT
Marco Campinho, PhD	- Post-Doc fellowship, FCT
Houda Hallay, PhD	- Post-Doc fellowship, MCTN
Sérgio Almeida, PhD	- Post-Doc fellowship, FCT
Inês Mollet	- PhD fellowship, EURASNET
Ana Rita Grosso	- PhD fellowship, FCT
Jorge Andrade	- PhD fellowship, FCT
Marisa Cabrita	- Technician, FMUL
Sérgio Marinho	- Technician (trainee)

### Research Areas

Cell and Molecular Biology  
Biogenesis of messenger RNA  
RNA splicing  
Diseases of RNA metabolism

### Major Scientific Achievements

Using fluorescence in situ hybridization (FISH) and confocal microscopy, we have showed in the past that mRNA processing defects induce an accumulation of  $\beta$ -globin transcripts in discrete nuclear foci localized in close proximity to the site of transcription. Our group has since been

interested in further dissecting the molecular mechanisms involved in this RNA surveillance process. To investigate the molecular mechanisms involved in quality control of deficiently processed pre-mRNA in mammalian cells, we reduced the levels of Rrp6 by RNA interference. We observe that Rrp6 is required for retention of unprocessed globin RNA at the site of transcription, but loss of Rrp6 is not sufficient to trigger export of unspliced RNA to the cytoplasm. ChIP analysis further reveals that unprocessed transcripts are not efficiently terminated and remain stalled in association with Pol II with at the 3'-end of the mutant gene. In contrast, in the absence of Rrp6, Pol II and unprocessed transcripts are released from the DNA template. Taken together these results suggest a novel link between nuclear RNA surveillance, the exosome and Pol II transcriptional termination.

In parallel, we have developed methodologies to study how splicing factors are recruited to nascent transcripts in the living cell nucleus, and we are currently developing live-cell microscopy tools to visualize the dynamics of transcription, spliceosome assembly and splicing. In particular, we have been developing tools to visualize in real-time the dynamics of co-transcriptional splicing and spliceosome assembly. In order to simultaneously study the dynamics of transcription by RNA polymerase II (Pol II) and pre-mRNA splicing *in vivo*, we used the well characterized human  $\beta$ -globin (*HBB*) gene as a model system. We engineered human osteosarcoma-derived cell lines (U2-OS) to stably express a series of tetracycline-inducible  $\beta$ -globin transgenes integrated in tandem in the genome. Multiple copies of the MS2 coat protein binding site were inserted in exon 3 and intron 2 of the human transgene. Expression of the MS2 coat protein fused to the red fluorescent protein Cherry (MS2-Cherry) allows the transcribed  $\beta$ -globin RNA to be visualized. Real-time transcription is monitored in the same cell lines by transient expression of green fluorescent protein (GFP) fused to an  $\alpha$ -amanitin – resistant mutant of Pol II large subunit. Kinetic analysis is performed using photobleaching and photoactivation microscopy techniques.

We have also established and further developed computational approaches to generate microarray-based predictions for understanding the alternative splicing code. We performed a large-scale computational analysis of expression profiles in cancer for 262 genes encoding proteins shown to be involved in the splicing process and we identified 192 splicing-related genes that were misregulated in cancer. The genes with expression variations encoded for the major splicing protein families snRNPs, hnRNPs, SRs, SR-kinases, RNA-helicases-like and other splicing regulators. The majority of misregulated splicing factors were up-regulated in cancer, and some splicing-related genes (including hnRNPs and SR proteins) were commonly misregulated in several cancer types. Next, we searched for misregulated splicing events using splicing-sensitive microarray data. To reduce false positive predictions of alternative splicing events, filtering steps were applied to the data prior statistical analysis. To evaluate the quality of the alternative splicing events selected we compared our results with previous exon-microarray studies where validations were performed by RT-PCR. The next goal is to further develop computational approaches to mine microarray datasets in order to identify cancer-specific alternative splicing events and correlate them with mis-regulated expression of splicing factors.

### Recent Most Relevant Publications

Braga, J., Desterro, J.M. and Carmo-Fonseca, M. (2004) Intracellular macromolecular mobility measured by fluorescence recovery after photobleaching with confocal laser scanning microscopes. *Mol. Biol. Cell* 15: 4749-4760 (Times cited: 71) (Journal IF: 5.942)

Calapez, A., Pereira, H.M., Calado, A., Braga, J., Rino, J., Carvalho, C., Tavanez, J.P., Wahle, E., Rosa, A.C. and Carmo-Fonseca, M. (2002) The intranuclear mobility of messenger RNA binding proteins is ATP-dependent and temperature-sensitive. *J. Cell Biol.* 159: 795-805 (Times cited: 76)(Journal IF: 9.120)

Carmo-Fonseca, M. (2002) The contribution of nuclear compartmentalization to gene regulation. *Cell* 108:1-20 (Times cited: 118)(Journal IF: 31.253)

Calado, A., Tomé, F.M.S., Brais, B., Rouleau, G.A., Kühn, U., Wahle, E. and Carmo-Fonseca, M. (2000) Nuclear inclusions in oculopharyngeal muscular dystrophy consist of poly(A) binding

protein 2 aggregates which sequester poly(A) RNA. *Hum. Mol. Gen.* 9: 2321-2328 (Times cited: 124)(Journal IF: 7.249)

Carmo-Fonseca, M., Mendes-Soares, L. and Campos, I. (2000) To be or not to be in the nucleolus. *Nat. Cell Biol.* 2: E107-E112 (Times cited: 191)(Journal IF: 17.774)

### Ongoing Research Projects

2006/2010 EURASNET: European Alternative Splicing Network of Excellence, European Commission (FP6-LSHG-CT-2005-518238)

2006/2010 Chromatin Plasticity, European Commission (FP6-Marie Curie Training Network)

2007/2009 Mechanisms of splicing regulation by U2AF proteins, FCT. Coordinator: M. Carmo-Fonseca (PTDC/SAU-GMG/69739/2006)

### Publications in Peer Review International Journals (2009)

Rino, J., Braga, J., Henriques, R. and Carmo-Fonseca, M. (2009) Frontiers in Fluorescence microscopy. *Int. J. Dev. Biol.* 53: 1569-1579 (Journal IF: 2.359)

Tavanez, J.P., Bengoechea, R., Berciano, M.T., Lafarga, M., Carmo-Fonseca, M. and Enguita, F.J. (2009) Hsp70 Chaperones and type I PRMTs are sequestered at intranuclear inclusions caused by polyalanine expansions in PABPN1. *PLoS One* 4: e6418. (Journal IF: NA)

Rino, J. and Carmo-Fonseca, M. (2009) The spliceosome: a self-organized macromolecular machine in the nucleus? *Trends Cell Biol.* 19: 357-384 (Journal IF: 13.385)

### Communications in International Conferences

Carvalho, T. et al. Splicing affects in vivo dynamics of pre mRNA transcription. EMBO Conference Series on Nuclear Structure and Dynamics. Avignon, September 30 - October 4, 2009 (Poster presentation)

Desterro, J.M. et al. Auto-regulation of the heterodimeric splicing factor U2AF. The EMBO Conference Series on Protein Synthesis and Translation Control, EMBL Heidelberg, September 9-13, 2009 (Poster presentation)

Hallay, H. et al. Splicing in Human Aging. The International Workshop on High Throughput Technologies for Alternative Splicing. Valencia, February 5-7, 2009 (Poster presentation)

Hallay, H. et al. Global Analysis of Splicing in a Cellular Model of Human Aging. RNA 2009 (Annual Meeting of the RNA Society). Madison, Wisconsin, May 26-30, 2009 (Platform presentation)

Grosso, A.R. et al. Genome-wide bioinformatics search for cancer-associated splicing misregulation. The International Workshop on High Throughput Technologies for Alternative Splicing. Valencia, February 5-7, 2009 (Poster presentation)

Martins, S. et al. Live cell imaging reveals cross-talk between splicing and RNA Pol II dynamics. RNA 2009 (Annual Meeting of the RNA Society). Madison, Wisconsin, May 26-30, 2009 (Platform presentation)

Martins, S. and Carmo-Fonseca, M. RNAi-mediated knockdown of the Ski-interacting protein (SKIP) affects splicing of cell cycle genes. EMBO Conference Series on Nuclear Structure and Dynamics. Avignon, September 30 - October 4, 2009 (Poster presentation)

### Communications in National Conferences

Andrade, J. Functional analysis of the U2AF35 family of splicing factors. 3<sup>rd</sup> IMM PhD Students

Meeting, IMM, Lisboa. October 12-13, 2009

De Almeida, S. et al. The human exosome surveys Pol II termination. Platform presentation at RNA 2009. Cascais, November 5-6, 2009.

### Invited Lectures and Seminars

Carmo-Fonseca, M. A stochastic View of Spliceosome Assembly in the Nucleus. University of Massachusetts Medical School, Department of Biochemistry and Molecular Pharmacology Worcester, 5 January 2009.

Carmo-Fonseca, M. O genoma Humano e a medicina personalizada. Instituto de Medicina Legal, Lisboa, 9 Janeiro 2009.

Carmo-Fonseca, M. Splicing Alive. Wellcome Trust Conference “The future of light microscopy: breaking the barriers”. Hinxton, Cambridge, 14-16 January 2009.

Carmo-Fonseca, M. Desafios da Investigação Médica para o século XXI. VI Jornadas do Jovem Médico, Coimbra, 7 Março 2009.

Carmo-Fonseca, M. Real-time dynamics of pre-mRNA transcription and splicing. “Experimental Biology 2009”. New Orleans, LA, 18-22 April 2009.

Carmo-Fonseca, M. Co-transcriptional RNA checkpoints. EMBO Conference on Chromatin and Epigenetics, EMBL, Heidelberg, 13-17 May 2009.

Carmo-Fonseca, M. Live-cell imaging reveals coupling between splicing and transcription. EMBO Conference on Nuclear Structure and Dynamics. Avignon, September 30-October 4, 2009.

Carmo-Fonseca, M. In vivo tools to visualize co-transcriptional RNA processing. Vienna Biocenter Seminar Series, 4 June, 2009.

Carmo-Fonseca, M. Insights into RNA processing in the nucleus. CNR Seminar Series, Pisa, 17 November, 2009.

Carmo-Fonseca, M. Live-cell imaging of pre-mRNA splicing. RNA 2009, Carcavelos, 5-6 November, 2009.

De Almeida, S. et al. The nuclear exosome regulates transcription termination by RNA Pol II. Platform presentation at EURASNET Workshop on Splicing in Cell Biology. Lisboa, October 5-6, 2009.

Rino, J. Quantitative FRAP – measuring dynamics in living cells. “EMBO course on Light Microscopy in Living Cells”, Oeiras, 4 June 4, 2009.

Rino, J. Real-time imaging of gene expression in living cells. First SPBf Seminar organized by the Sociedade Portuguesa de Biofísica, Coimbra, 25 March, 2009.

### Organisation of National and International Conferences

M. Carmo-Fonseca. Organizer of EURASNET Workshop on Splicing in Cell Biology. Lisboa, October 5-6, 2009.

### Thesis Completed

#### PhD Thesis

Inês Maria de Stoop Camões Teixeira Guerra Mollet (2009) “Genome wide mining of alternative splicing in metazoan model organisms” Faculdade de Medicina da Universidade de Lisboa. Supervisor: M. Carmo-Fonseca.

## Chromatin Biology Unit

### Description and Objectives

Topoisomerase 2 is a vital enzyme that controls DNA topology by passing an intact double helix through a transient, potentially cytotoxic, double-stranded DNA break (DSB) generated by the enzyme during its catalytic cycle. Under certain conditions DSBs generated by topoisomerase 2 activity may become stabilized and induce severe DNA damage and subsequent cell death, with the enzyme acting as “the enemy within”. Since the discovery of drugs that stabilize topoisomerase 2-induced DNA DSBs (eg, etoposide, anthracyclines) topoisomerase 2 has become a main target in cancer chemotherapy.

Chromatin structure is increasingly recognized as a major participant in the cellular response to DNA damage, and the role of regulators of chromatin structure in this response is an emerging field of research. Importantly, histone deacetylases (HDACs) were previously shown to be required for the proper repair of topoisomerase 2-mediated DNA damage, and inhibitors of HDACs synergize with drugs targeting topoisomerase 2.

The main objective of our research unit is to understand how human cells handle DNA damage introduced by cellular topoisomerases. We are currently focusing our research on identifying chromatin structure regulators (mostly non-HDACs) that may control cellular responses to topoisomerase 2-mediated DNA damage and influence cell fate decisions (apoptosis, accelerated senescence) in cancer cells. To this end we are utilizing a combination of approaches both in the small and the large-scale (expression microarray analysis, siRNA screens). Results originating from this research shall unravel additional (non-HDAC) chromatin structure regulators that may become novel targets for the chemotherapy of cancer.

### Head of Unit

João Ferreira, MD, PhD – Associate Professor, FMUL

### Research Team

Joana Cardoso, PhD	- Post-Doc fellowship, FCT
Pedro Pereira	- PhD fellowship, FCT
Inês Alves	- Master student (left in July 2009)
António Caetano	- Medical student (left in November 2009)

### Research Areas

Topoisomerases  
Chromatin  
DNA damage  
Cellular senescence

### Major Scientific Achievements

In the past we have contributed to the study of the organization of chromosomes in the cell nucleus during interphase (Ferreira et al, **J Cell Biol** 139:1597-1610, 1997). The data obtained in this research led to the set-up of a model that still stands at the basis of our current understanding of the large-scale, functional organization of chromatin within the mammalian cell nucleus (cf Review by Takizawa, Meaburn and Misteli, **Cell** 135:9-13, 2008, and references therein).

Afterwards, we have described a novel approach to detect topoisomerase activities in situ, in the context of the cell nucleus. This was the first time that evidence for the activity of a non-polymerase enzyme could be obtained with subcellular resolution (Agostinho et al., **Mol. Biol.**

Cell 15:2388-2400, 2004), and was soon followed by the identification of sites of DNA methyltransferase activity by others. This novel technique allowed us to identify factors that influence the targeting of human topoisomerase 2 to sites of catalytic activity, namely histone deacetylases.

In a collaborative research with the laboratory of Dr. Patrick Varga-Weisz (Babraham Institute, Cambridge, UK), we have contributed to the original finding that a chromatin remodeling factor (WSTF/Williams Syndrome Transcription Factor), classically connoted with transcriptional activity, also acted during DNA replication to maintain epigenetic states via chromatin remodeling (Poot et al, **Nature Cell Biol** 6:1236-1244, 2004). This also provided the first evidence that chromatin remodeling factors may work at the replication fork.

More recently, we have shown that topoisomerase 2- $\alpha$  is extensively modified post-translationally by SUMO-proteins, and that this modification exerts profound alterations in the physiology of the enzyme, namely in genome propagation. In this work we have also assigned a function to a “mythical” structure in the field of chromosome biology, the mitotic chromosome axis. We have provided compelling evidence that the chromosomal axis functions as a sumoylation centre for topoisomerase 2- $\alpha$  (Agostinho et al., **Cancer Research**, 68:2409-18, 2008), and that this is key to its participation in chromatid segregation during mitosis.

### Recent Most Relevant Publications

Agostinho, M., Santos, V., Ferreira, F., Costa, R., Cardoso, J., Pinheiro, I., Rino, J., Jaffray, E., Hay, R.T. and Ferreira, J. (2008) Conjugation of human topoisomerase 2 with SUMO-2/-3 in response to topoisomerase inhibitors: cell cycle stage and chromosome domain specificity. *Cancer Res.* 68: 2409-2418 (Times cited: 5)(Journal IF: 7.514)

Agostinho, M., Rino, J., Braga, J., Ferreira, F., Steffensen, S. and Ferreira, J. (2004) Human topoisomerase II $\alpha$ : targeting to subchromosomal sites of activity during interphase and mitosis. *Mol. Biol. Cell* 15: 2388-2400 (Times cited 14)(Journal IF: 5.558)

Poot, R., Bozhenok, L., van den Berg, D.L.C., Steffensen, S., Ferreira, F., Grimaldi, M., Ferreira, J. and Varga-Weisz, P. (2004) The Williams Syndrome transcription factor interacts with PCNA to target chromatin remodeling by ISWI to replication foci. *Nat. Cell Biol.* 6: 1236- 1244 (Times cited 45)(Journal IF: 17.774)

Carvalho, C., Pereira, H.M., Ferreira, J., Pina, C., Mendonça, D., Rosa, C. and Carmo-Fonseca, M. (2001) Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in the nucleus. *Mol. Biol. Cell* 12: 3563-3572 (Times cited: 27)(Journal IF: 5.558)

Ferreira, J., Paoletta, G., Ramos, C. and Lamond, A.I. (1997) Spatial organization of large-scale chromatin domains in the nucleus: a magnified view of single chromosome territories. *J. Cell Biol.* 139: 1597-1610 (Times cited 139)(Journal IF: 9.120)

### Ongoing Research Projects

2009/2012 Novel therapeutic targets for the treatment of Acute Myeloid Leukemia, Fundação Gulbenkian Portugal. Coordinator: J. Ferreira (Project: 96526/2008)

2009/2010 Characterization of two novel anti-cancer drugs, collaborative project with “ELARA Pharmaceuticals GmbH, Heidelberg, Germany (based at the European Molecular Biology Laboratory/EMBL, Heidelberg). Coordinators: J.D. Lewis and J. Ferreira.

### Communications in International Conferences

Cardoso, J. et al. The contribution of alternative splicing to drug-induced accelerated senescence. Poster presentation at EMBO Meeting. Amsterdam, The Netherlands. August/September 2009 (Poster presentation).



Cardoso, J. et al. The contribution of alternative splicing to drug-induced accelerated senescence, International Workshop on High Throughput Technologies for Alternative Splicing, Valencia, Spain. February 2009. (Poster presentation).

Cardoso, J. et al. The contribution of mitosis-related genes to drug-induced accelerated senescence, the Mitosis and Cancer Symposium, Amsterdam, The Netherlands. February 2009. (Poster presentation).

Hallay, H. et al. Splicing in human aging, International Workshop on High Throughput Technologies for Alternative Splicing, Valencia, Spain. February 2009. (Poster presentation).

### **Advanced Teaching**

Ferreira, J. organized and lectured in the Gene Expression module of the “Doctoral Program for Physicians - PFMA” held at the Instituto de Medicina Molecular/IMM, Lisbon, October 1-21, 2009.

### **Public Perception of Science**

Ferreira, J. (2009) “O Gabinete de Apoio à Investigação Científica-GAPIC na formação científica dos futuros médicos”, Semana de Introdução ao Curso de Medicina, Faculdade de Medicina, 2 de Outubro, Lisboa.

Ferreira, J. (2009) “Os médicos na investigação de fronteira – que papel?”, Acta Reumatológica Portuguesa 34, 577-578 (Editorial).

### **Thesis Completed**

#### **Master Thesis**

Inês Teles Alves (2009) Topoisomerase II-mediated DNA damage: role of BRCA1 and other chromatin structure regulators. Mestrado em Biologia Molecular Humana, Faculdade de Ciências da Universidade de Lisboa. Supervisors: João Ferreira and Júlio Duarte.

## Developmental Biology Unit

### Description and Objectives

The main objective of the Developmental Biology Unit is to investigate the molecular mechanisms that regulate the genesis of neurons in vertebrate embryos. Our aim is to characterize the molecular events that control the generation of neural stem cells in the embryo, how these cells are maintained and how they give rise to the multitude of neurons that compose the adult CNS. In vertebrates, neurogenesis starts very early during embryonic life and the majority of neurons is produced before birth. A conserved genetic circuitry, comprising the action of both proneural and neurogenic genes, is employed to regulate the production of neurons during embryonic development. How this circuitry functions to regulate the establishment of neural precursors in vertebrate embryos and their commitment to neurons is the main aim of our work. A better knowledge about these fundamental mechanisms is a pre-requisite for the development of cellular replacement therapies to treat neurodegenerative diseases, with a significant impact on human health.

### Head of Unit

Domingos Henrique, PhD – Investigator, FMUL

### Research Team

Evguenia Bekman, PhD	- Post-Doc fellowship, FCT
Elsa Abranches, PhD	- Post-Doc fellowship, FCT
Catarina Ramos, PhD	- Post-Doc fellowship, FCT
Cláudia Gaspar, PhD	- Post-Doc fellowship, FCT
Filipe Vilas Boas	- PhD Student, FCT
Aida Costa	- PhD Student, FCT
Sara Ferreira	- Technician/Lab Manager, Fellowship FCT
Ana Margarida Cristóvão	- Technician, Fellowship FC

### Research Areas

Developmental Biology  
Cell and Molecular Biology of Notch signalling  
Neural Stem cell biology  
Sensory Hair Cells

### Major Scientific Achievements

Our recent work has focused on two main areas:

1. the function of the Delta/Notch signalling pathway during neurogenesis and
2. the use of Embryonic Stem cells for *ex vivo* production of neurons

Concerning the first area, our work has contributed to elucidate some molecular mechanisms that control cell fate determination in the vertebrate nervous system, and this will continue to be the main research area in our group. We have shown that cell-cell communication mediated by the Notch pathway is a major player in the mechanisms that regulate neurogenesis in the developing spinal cord and neural retina (Rocha et al, 2009), and have also identified several components of the pathway and how they function to control neuronal differentiation (Fior et al, 2005). Our analysis of Dll1 and Jag1 mouse mutants confirmed the function of Notch signaling in maintaining neural progenitors but indicated that neither Dll1 nor Jag1 are directly involved in creating cell fate diversity (Rocha et al, 2009). This correlates with the view that neuronal type

specification in the spinal cord is mainly controlled by the DV patterning system, which delineates distinct domains along the DV axis of the neural tube. From each domain, different neuronal sub-types arise in a precise temporal order and this is determined by the combination of TFs (namely, homeodomain and bHLH family members). In this process, extrinsic signalling mediated by Notch seems to be only used to maintain a pool of progenitors, able to generate all the successive cell types (Rocha et al, 2009).

We have also addressed the role of two *Delta-like* genes, *Dll1* and *Dll4*, during mouse embryonic neurogenesis and show that they are active components of the Notch pathway that regulate neuronal production in the retina and spinal cord's pV2 domain. We showed that the two *Dll* genes are sequentially expressed in differentiating neurons and that *Dll1* starts to be expressed before *Dll4*, as cells commit to differentiation. Analysis of *Dll1* mutants reveals that this gene is necessary and sufficient to maintain a pool of progenitors in the spinal cord and retina, but dispensable for cell fate diversity in these regions. Our results support a model where *Dll1* and *Dll4* are part of a unique genetic circuitry that regulate subsequent steps of neurogenesis in the retina and pV2 domain: while *Dll1* serves to prevent the untimely differentiation of neural progenitors, *Dll4* functions to generate diversity within the population of differentiating neurons.

Concerning the second research topic of the lab, we have characterized the main stages and transitions that occur when ES cells are driven into a neural fate, using an adherent monolayer culture system. We established improved conditions to routinely produce highly homogeneous cultures of neuroepithelial progenitors, which organize into neural tube-like rosettes when they acquire competence for neuronal production. Within rosettes, we have shown that neuroepithelial progenitors display morphological and functional characteristics of their embryonic counterparts, namely, apico-basal polarity, active Notch signalling, and proper timing of production of neurons and glia.

In order to characterize the global gene activity correlated with each particular stage of neural development, the full transcriptome of different cell populations that arise during the *in vitro* differentiation protocol was determined by microarray analysis. By using embryo-oriented criteria to cluster the differentially expressed genes, we have defined five gene expression signatures that correlate with successive stages in the path from ES cells to neurons. These include a gene signature for a primitive ectoderm-like stage that appears after ES cells enter differentiation, and three gene signatures for subsequent stages of neural progenitor development, from an early stage that follows neural induction to a final stage preceding terminal differentiation. Overall, this work confirms and extends the cellular and molecular parallels between monolayer ES cell neural differentiation and embryonic neural development, revealing in addition novel aspects of the genetic network underlying the multistep process that leads from uncommitted cells to differentiated neurons.

### Recent Most Relevant Publications

Rocha, S.F., Lopes, S.S., Gossler, A. and Henrique, D. (2009) *Dll1* and *Dll4* function sequentially in the retina and pV2 domain of the spinal cord to regulate neurogenesis and create cell diversity. *Dev. Biol.* 328: 54-65 (Journal IF: 4.416)

Abranches, E., Silva, M., Pradier, L., Schulz, H., Hummel, O., Henrique, D. and Bekman, E. (2009) Neural differentiation of embryonic stem cells *in vitro*: a road map to neurogenesis in the embryo. *PLoS ONE* 4:e6286 (Journal IF: NA)

Fior, R. and Henrique, D. (2009) "Notch-Off": a perspective on the termination of Notch signalling. *Int. J. Dev. Biol.* 53: 1379-1384 (Journal IF: 2.359)

Diogo, M.M., Henrique, D. and Cabral, J.M.S. (2007) Optimization and integration of expansion and neural commitment of mouse embryonic stem cells. *Biotechnol. Appl. Biochem.* 49: 105-112 (Journal IF: 1.288)

Afonso, C. and Henrique, D. (2006) PAR3 acts as a molecular organizer to define the apical domain of chick neuroepithelial cells. *J. Cell Science* 119: 4293-4304 (Journal IF: 6.247)

Fior, R. and Henrique, D. (2005) A novel *hes5/hes6* circuitry of negative regulation controls

Notch activity during neurogenesis. *Dev. Biol.* 281: 318-333 (Journal IF: 4.416)

### Ongoing Research Projects

2007-2010 Stem cell based therapy for inner ear hair cell regeneration, FCT. Coordinator: D. Henrique (PTDC/SAU-NEU/71310)

2007-2010 Notch signaling and regulatory mechanisms during mammalian neurogenesis. Coordinator: D. Henrique (PTDC/SAU-OB/75106)

2007-2010 NEURAL TALK - Scaffold-driven stem-cell regenerative therapy for the spinal cord injury. Biomimetic neurogenesis in the CNS. Coordinator: A.P. Pêgo (PTDC/SAU-BEB/65328)

### Publications in Peer Review International Journals (2009)

Rocha, S.F., Lopes, S.S., Gossler, A. and Henrique, D. (2009) Dll1 and Dll4 function sequentially in the retina and pV2 domain of the spinal cord to regulate neurogenesis and create cell diversity. *Dev. Biology* 328: 54-65 (Journal IF: 4.416)

Fernandes, A., Falcão, A.S., Abranches, E., Bekman, E., Henrique, D., Lanier, L.M. and Brites, D. (2009) Bilirubin as a determinant for altered neurogenesis, neuritogenesis and synaptogenesis. *Dev. Neurobiol.* 69: 568-562 (Journal IF: 2.333)

Abranches, E., Silva, M., Pradier, L., Schulz, H., Hummel, O., Henrique, D. and Bekman, E. (2009) Neural differentiation of embryonic stem cells “in vitro”: a road map to neurogenesis in the embryo. *PLoS ONE* 4: e6286 (Journal IF: NA)

Fior, R. and Henrique, D., (2009) “Notch-Off”: a perspective on the termination of Notch signalling. *Int. J. Dev. Biol.* 53: 1379-1384 (Journal IF: 2.359)

Schulz, H., Kolde, R., Adler, P., Anastasiadis, K., Bader, M., Billon, N., Boeuf, H., Bourillot, P.Y., Buchholz, F., Dani, C., Doss, X., Forrester, L., Friel, R., Gitton, M., Henrique, D., Hescheler, J., Himmelbauer, H., Hübner, N., Karantzali, E., Kretsovali, A., Lubitz, S., Pradier, L., Reimand J., Rolletschek, A., Sachinidis, A., Savatier, P., Stewart, F., Storm, M.P., Trouillas, M., Vilo, J., Welham, M.J., Winkler, J., Wobus, A. and Hatzopoulos, A.K. (2009) The FunGenES database: a genomics resource for mouse embryonic stem cell differentiation. *PlosOne* 4:e 6804 (Journal IF: NA)

### Books/Book Chapters

Fior, R. and Henrique, D. (2009): “Notch-Matics”, in *Mathematics, Developmental Biology and Tumour Growth*, F.G.A.M.A. Herrero, Editor. American Mathematical Society (co-publication with the Real Sociedad Matemática Española).

### Communications in International Conferences

Vilas-Boas, F. et al. Two *hes6* genes in the chick with different functions during embryonic development. The Notch Meeting, Athens, September 2009 (Poster presentation)

Amaral, I.F. et al. Evaluation of fibrin gel for the culture of embryonic neural stem cells derived in serum free medium. 22nd European Conference on Biomaterials, Lausanne, Switzerland. September 7-11, 2009 (Poster presentation)

Beça, F. et al. Co-transplantation of embryonic stem cell-derived neural stem cells with endothelial cells in a fibrin gel: optimization of cell culture parameters. 4th Annual International Meeting of the Portuguese Society for Stem Cells and Cell Therapies, Lisbon, Portugal, April 29-30, 2009 (Poster presentation)

Bekman, E. et al. Stem cell based therapies for inner ear hair cell regeneration. 4th Annual

International Meeting of the Portuguese Society for Stem Cells and Cell Therapies, Lisbon, Portugal, April 29-30, 2009 (Poster presentation)

Santos, D.M. et al. Surviving death while differentiating – Is tauroursodeoxycholic acid a Neural Stem Cell's best friend? Perspectives in Stem Cell Proteomics Conference, Cambridge, UK, March 21-23, 2009 (Poster presentation)

Abranches, E. and Henrique, D. Notch signaling and neural differentiation in ES cells. ESTOOLS scientific winter-school on hES and iPS cell technologies, Saariselka, Finland, January 26-30, 2009 (Poster presentation)

Henrique, D. et al. Two *hes6* genes in the chick with different functions during embryonic development. EMBO Workshop on bHLH Transcription Factors, London, UK, May 7-8, 2009 (Poster presentation)

### Communications in National Conferences

Abranches, E. and Henrique, D. From ES cells to Neurons: A Road Map to Neurogenesis in the Embryo, Encontro Ciência 2009, Lisbon, Portugal, July 29-30, 2009 (Oral Presentation)

Henrique, D. How to make neurons. Encontro Ciência 2009, Lisbon, Portugal, July 29-30, 2009 (Oral Presentation)

### Invited Lectures and Seminars

Henrique, D. Notch Ligands and Neurogenesis. College of Life Sciences, Dundee University, Scotland, UK, September 11, 2009.

Henrique, D. How to make neurons: from the embryo to ES cells. F. Hoffmann-La Roche Ltd, Dept. PRMBT, Basel, Switzerland. November 16, 2009.

Henrique, D. How to make neurons: from the embryo to ES cells. PRBB, Barcelona, Spain, December 2, 2009.

### Advanced Teaching

Henrique, D. organized and lectured the Regenerative Medicine module of the “Doctoral Program for Physicians - PFMA” held at the Instituto de Medicina Molecular/IMM, Lisbon, February 16-20, 2009.

Henrique, D. “Development of the nervous system” Masters Program on Neuroscience, Faculdade Medicina da Universidade de Lisboa.

Henrique, D. “Neurons and stem cells” MIT/Portugal PhD programme on Bioengineering.

Henrique, D. “From Neurons to Stem Cells”, PhD course about "Neural Stem Cell Environment" at the CNC-Coimbra.

Abranches, E. and Bekman, E. Course on Stem Cell Biology, Mestrado em Ciências Biológicas, Universidade do Algarve, Faro, October 28-29, 2009.

### Thesis Completed

#### PhD Thesis

Susana F. Rocha (2009) The role of multiple DSL ligands - Dll1, Dll4 and Jag1 – in vertebrate neurogenesis. Faculdade de Medicina da Universidade de Lisboa. Supervisor: Domingos Henrique

#### Master Thesis

**62 | 2009 IMM Activity Report**

Ruben Duarte Magalhães Alves Pereira (2009) Establishment of mouse ES cell lines with a reporter of Dll1 activity. Instituto Superior Técnico. Supervisor: D. Henrique, Co-supervisor: E. Abranches

## Tissue Morphogenesis and Repair Unit

### Description and Objectives

Epithelia have the essential role of acting as a barrier that protects living organisms and its organs from the surrounding environment. It is crucial for epithelial tissues to have robust ways of maintaining their integrity despite the frequent damage caused by injury, inflammation and normal cell turnover. Our objective is to understand molecular and cellular processes that regulate epithelial resealing, a conserved fundamental repair mechanism that acts in several types of simple epithelia, both in embryos and in adults, and across species. The organism of choice has been the fruit fly (*Drosophila melanogaster*) due to the amenability of this model system to genetic manipulation and live imaging analysis. We are also using flies to investigate the mechanisms that control the recruitment of macrophage-like blood cells to wound sites to clear damaged cells and to study a natural occurring morphogenetic movement, dorsal closure, that has striking similarities to wound repair. Recently we have started using Zebrafish to translate our *Drosophila* findings to a vertebrate model and to explore more complex tissue repair mechanisms, such as epimorphic regeneration.

### Head of Unit

António Jacinto, PhD - Assistant Professor, FMUL

### Principal Investigators

Soren Prag, PhD - Staff Scientist

### Research Team

Isabel Campos, PhD	- Post-Doc fellowship, FCT
Dulce Azevedo, PhD	- Post-Doc fellowship, FCT
Nuno Afonso, PhD	- Post-Doc fellowship, FCT
Patricia Abrantes, PhD	- Post-Doc fellowship, FCT
Anna Zaidman, PhD	- Post-Doc fellowship, FCT
	(Association pour la Recherche sur le Cancer until March)
Ana Cristina Silva, PhD	- Post-Doc fellowship, FCT
Jennifer Regan, PhD	- Post-Doc fellowship, EMBO (since July)
	(CELLIO until February, ERC from March to June)
Lara Jesus Carvalho, PhD	- Post-Doc fellowship, FCT grant
João Cordeiro, PhD	- Post-Doc fellowship, ERC (since March)
Marta Carapuço, PhD	- Post-Doc fellowship, ERC
Sara Maurício de Sousa	- PhD fellowship, FCT
Mariana Simões	- PhD fellowship, FCT
Jennifer Geiger	- PhD fellowship, FCT
Marco Antunes	- PhD fellowship, FCT
Rita Mateus	- PhD fellowship, FCT (since October)
Carolina Moreira	- PhD fellowship (since November)
	(April 16- October 15 Research fellowship, FCT Grant)
Telmo Pereira	- Research fellowship, FCT Grant (since November)
Inês Tenente	- Masters student (since September)
Pedro Saavedra	- Training student (until July 2009)
Ding Zhang	- Medical student, GAPIC (left in December)
Inês Matos	- Technician, ERC
Ana Cunha	- Technician, NoE “Cells into Organs”(left in February)

## Research Areas

Cell and Developmental Biology  
Drosophila Genetics  
Cell Migration and Adhesion  
Tissue Repair and Regeneration

## Major Scientific Achievements

We have completed a genetic screen designed to identify novel genes involved in epithelial resealing. We have tested 655 piggy-Bac and P-element insertion mutations and we were able to identify 30 genes that consistently showed over 30% of unclosed wounds in our wounding assay. Currently we are investigating the most interesting mutants that may help us to uncover the signaling networks that regulate epithelial repair. We have developed a new wound healing assay using the *Drosophila* pupa that allows us to analyze the process with unprecedented spatial and temporal resolution and to manipulate the system with the full range of fly genetic tools.

We have characterized the chemotaxis of hemocytes towards epithelial wounds and we have established assays that will be used to identify the molecular basis of this process.

Our zebrafish fin regeneration projects are giving the first promising results. We have characterized molecularly the process of bone regeneration during caudal fin regeneration, and we have demonstrated that, similarly to amphibians, re-innervation is essential for fin regeneration in zebrafish. In the end of 2009 we have initiated a chemical screen designed to identify small molecules that can interfere with the ability of fish to regenerate their caudal fins and to uncover the signaling pathways that control regeneration.

## Recent Most Relevant Publications

Campos, I., Geiger, J.A., Santos, A.C., Carlos, V. and Jacinto, A. (2010) Genetic screen in *Drosophila melanogaster* uncovers a novel set of genes required for embryonic epithelial repair. *Genetics* 184: 129-140 (Journal IF: 4.002)

Wood, W. and Jacinto, A. (2007) *Drosophila melanogaster* embryonic haemocytes: masters of multitasking. *Nat. Rev. Mol. Cell Biol.* 8: 542-551 (Journal IF: 35.423)

Simões, S., Denholm, B., Azevedo, D., Sotillos, S., Martin, P., Skaer, H., Castelli-Gair Hombria, J. and Jacinto, A. (2006). Compartmentalization of Rho regulators directs cell invagination during tissue morphogenesis. *Development* 133: 4257-4267 (Journal IF: 6.812)

Wood, W., Faria, C. and Jacinto A. (2006) Distinct mechanisms regulate hemocyte chemotaxis during development and wound healing in *Drosophila*. *J. Cell Biol* 174: 405-416.

Wood, W., Jacinto, A., Grose, R., Gale, J., Wilson, C. and Martin, P. (2002) Wound healing recapitulates morphogenesis, co-ordinated regulation of actin cytoskeletal elements by small GTPases during repair of epithelial wounds in *Drosophila* embryos. *Nat. Cell Biol.* 4: 907-912 (Journal IF: 17.774)

## Ongoing Research Projects

2006/2009 Cells into organs: Functional genomics for developmental disease of mesodermal organ systems, European Commission. Coordinator: A. Jacinto (CELLIO)

2007/2009 Molecular mechanisms of bone formation during caudal fin regeneration in zebrafish, FCT. Coordinator: A. Jacinto (PTDC/SAU-OBD/73112/2006)

2007/2010 Integrating the genetics, mechanics, phenomenology of embryonic wound healing, Human Frontier Science Program. Coordinator: A. Jacinto (HFSP-RGP21-07)

2009/2010 The role of re-innervation in zebrafish fin regeneration, GAPIC Coordinator: A. Jacinto (GAPIC - 2009041)



2008/2011 Embryonic wound healing: a genetic approach, FCT. Coordinator: A. Jacinto (PTDC/BIA-BCM/65872/2006)

2008/2014 ERC-Epithelial Resealing, European Commission. Coordinator: A. Jacinto (ERC - AJ208631 EPITHELIAL RESEALING)

### Publications in Peer Review International Journals 2009

Campos, I., Geiger, J.A., Santos, A.C., Carlos, V. and Jacinto, A. (2010) Genetic screen in *Drosophila melanogaster* uncovers a novel set of genes required for embryonic epithelial repair. *Genetics* 184: 129-140 (*Epub November 2, 2009*)(Journal IF: 4.002)

Garcia-Fernandez, B., Campos, I., Geiger, J., Santos, A.C. and Jacinto, A. (2009) Epithelial resealing. *Int. J. Dev. Biol.* 53: 1549-1556 (Journal IF: 2.359)

### Communications in International Conferences

\*Geiger, J.A., \*Campos, I., \*Santos, A. et al. Searching *Drosophila* for New Genes Involved in Wound Healing. International Society Developmental Biology 2009 Conference, Edinburgh, Scotland. September 6-10, 2009 (Poster presentation)

Antunes, M. et al. Establishing a wound healing model in the *Drosophila* pupa. International Society Developmental Biology 2009 Conference, Edinburgh, Scotland. September 6-10, 2009 (Poster presentation)

Azevedo, A. et al. Regeneration in zebrafish caudal fin: the role of telomerase. International Society Developmental Biology 2009 Conference, Edinburgh, Scotland. September 6-10, 2009 (Poster presentation)

Azevedo, D. et al. DRhoGEF2 regulates contractile forces in amnioserosal cells during dorsal closure in *Drosophila*. EMBO Conference Series on Morphogenesis and Dynamics of Multicellular Systems. EMBL, Heidelberg, Germany. October 2-6, 2009 (Poster presentation)

Campos, I. et al. The search for new genes involved in wound healing in *Drosophila*. International Society Developmental Biology 2009 Conference, Edinburgh, Scotland. September 6-10, 2009, (Poster presentation)

Carvalho, L. et al. Studying wound healing mechanisms in *Drosophila* epithelia, 21<sup>st</sup> European *Drosophila* Research Conference, Nice, France. November 18-21, 2009 (Poster presentation)

Prag, S. and Jacinto, A. Regulation of GTPase Activity during *Drosophila* Haemocyte Migration. IRB BioMed Conference Modelling Cancer in *Drosophila*. Barcelona, Spain. September 14-16, 2009 (Poster presentation)

Prag, S. and Jacinto, A. Regulation of GTPase Activity during *Drosophila* Haemocyte Migration. EMBO Conference Series on Morphogenesis and Dynamics of Multicellular Systems. EMBL, Heidelberg, Germany. October 2-6, 2009 (Poster presentation)

Simões, M. and Jacinto, A. Zebrafish as a model to study the role of nerves in regenerating appendages. International Society Developmental Biology 2009 Conference, Edinburgh, Scotland. September 6-10, 2009, (Poster presentation)

Simões, M. and Jacinto, A. Zebrafish as a model to study the role of nerves in regenerating appendages Workshop on Mechanisms of organ Regeneration in model systems. Baeza, Spain. October 5-7, 2009 (Poster presentation)

Sousa, S. et al. Bone dynamics during adult zebrafish caudal fin regeneration. International Society Developmental Biology 2009 Conference, Edinburgh, Scotland. September 6-10, 2009 (Poster presentation)

Zaidman-Rémy, A. et al. *Drosophila* hemocytes: the right place at the right moment. 21<sup>st</sup> European *Drosophila* Research Conference, Nice, France. November 18-21, 2009 (Poster presentation)

### Communications in National Conferences

Afonso, N. et al. Is cartilage required to regenerate dermal bone in the zebrafish caudal fin? I Meeting on Interdisciplinary Approaches in Fish Skeletal Biology, Tavira, Portugal. April 27–29, 2009 (Oral presentation)

Antunes, M. et al. Establishing a Wound Healing model in the *Drosophila* Pupa, 3rd IMM PhD Student Meeting, IMM, Lisbon, Portugal. October 12-13, 2009 (Poster presentation)

Azevedo, D. et al. DRhoGEF2 regulates contractile forces in amnioserosal cells during dorsal closure in *Drosophila*. Biophysical Mechanisms of Development. Instituto Gulbenkian de Ciência, Oeiras, Portugal. May 25-27, 2009 (Poster presentation)

Campos, I. et al. The search for new genes involved in wound healing in *Drosophila*. Biophysical Mechanisms of Development. Instituto Gulbenkian de Ciência, Oeiras, Portugal. May 25-27, 2009 (Poster presentation)

Carvalho, L. et al. Studying wound healing mechanisms in *Drosophila* epithelia. Portuguese Annual *Drosophila* Meeting, Instituto de Biologia Molecular e Celular, Universidade do Porto. December 18, 2009 (Poster presentation)

Prag, S. and Jacinto, A. Regulation of GTPase Activity during *Drosophila* Haemocyte Migration. Biophysical Mechanisms of Development. Instituto Gulbenkian de Ciência, Oeiras, Portugal. May 25-27, 2009 (Poster presentation)

Regan, J. et al. Analysing chemotaxis by tracking hemocytes in the *Drosophila* pupa. Biophysical Mechanisms of Development, Instituto Gulbenkian de Ciência, Oeiras, Portugal. May 25-27, 2009 (Poster presentation)

Simões, M. and Jacinto, A. Zebrafish as a model to study the role of nerves in regenerating appendages, 3rd IMM PhD Student Meeting, IMM, Lisbon, Portugal. October 12-13, 2009 (Oral presentation)

Sousa, S. and Jacinto, A. Bone dynamics during adult zebrafish caudal fin regeneration. 3<sup>rd</sup> IMM PhD Student Meeting, Lisbon. October 12-13, 2009 (Oral presentation)

Sousa, S. and Jacinto, A. Bone dynamics during adult zebrafish caudal fin regeneration. Reunião anual do Programa Doutoral em Biologia Experimental e Biomedicina, Coimbra. December 21-22, 2009 (Oral presentation)

Zaidman-Rémy, A. et al. *Drosophila* hemocytes: the right place at the right moment. Portuguese Annual *Drosophila* Meeting, Instituto de Biologia Molecular e Celular, Universidade do Porto. December 18, 2009 (Poster presentation)

### Invited Lectures and Seminars

Afonso, N. “The zebrafish as a model system in tissue regeneration studies” First SPCAL meeting, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisboa, Portugal. June 18-19, 2009.

Jacinto A. Lecturer at the ERC Starting Grants – StG3, Sessão de Informação 7<sup>o</sup>PQ. Reitoria da Universidade Nova de Lisboa, Lisboa, Portugal, September 16, 2009.

Prag, S. “Staff Scientist - Ciência2007”. Ciência 2007/2008. Reitoria da Universidade de Lisboa. September 23, 2009

### Organisation of National and International Conferences

Jacinto A. organized the Cells into Organs Symposium on Tissue Specification and Organogenesis. February 4-6, 2009, Lisbon, Portugal.

### **Prizes, Honours and Awards**

Ding Zhang , Poster Prize GAPIC

### **Advanced Teaching**

Jacinto, A. IGC organizer of the Between Cells course. PhD Programme, Instituto Gulbenkian de Ciência, Portugal, November 30 - December 4, 2009.

Prag, S. “Regulation of Hemocyte Migration *in vivo*” BioImaging course. Faculdade Ciências da Universidade de Lisboa, Portugal. March 18, 2009.

Prag, S. “Cell Migration” Between Cells course. PhD Programme, Instituto Gulbenkian de Ciência, Portugal, November 30 - December 4, 2009.

### **Public Perception of Science**

Developmental Biology lab rotation of 3 students from FCUL. March 6, 13 and 27, 2009, IMM. Activity coordination: Pedro Saavedra, Mariana Simões, Sara Sousa, Nuno Afonso, Jennifer Regan, António Jacinto.

Visit from 47 students of Escola EB 2,3 /S Prof. Mendes dos Remédios, Nisa. March 4, 2009, IMM. Activity coordination: Marco Antunes, Marta Carapuço.

Visit from 36 students of Escola EB 2,3 Abade Correia da Serpa, Serpa. April 28, 2009, IMM. Activity coordination: Pedro Saavedra, Isabel Campos, Marta Carapuço.

Members of the Morphogenesis Unit participated as volunteers in the Researchers’ Night 2009, September 2009.

## Microvascular Biology and Inflammation Unit

### Description and Objectives

As part of the host immune response, inflammation aims the recognition and elimination of the agent of infection or lesion so as to promote tissue repair and to attain cellular homeostasis. The inflammatory process involves the interplay between inflammatory cells (neutrophils, monocytes/macrophages and lymphocytes), vascular cells and inflammatory mediators. During inflammation, the expression and release of pro-inflammatory mediators, such as vasoactive compounds and several cytokines by diverse cellular types, directs the several events involved. Among other functions, pro-inflammatory cytokines induce the synthesis and release of acute phase proteins, such as the fibrinogen by the liver. Fibrinogen constitutes an important player in the inflammatory process and it is even considered as an important inflammatory marker.

On the other hand, the activation of anti-inflammatory mechanisms is of crucial importance to limit and balance the pro-inflammatory response and finally, trigger the resolution of the inflammatory process. Among these, the recently identified cholinergic anti-inflammatory pathway has been shown to play an important role in the context of inflammatory disorders by limiting the synthesis and release of important pro-inflammatory cytokines.

Taking all this into consideration, our research is focused: (1) on the role of fibrinogen in modulating blood-vessel wall interaction in inflammation, (2) on the role of non-neuronal cholinergic systems in the inflammatory response and (3) on the development of theoretical simulation models at the level of leukocyte-vascular wall interaction in the microvascular network.

### Head of Unit

Carlota Saldanha, PhD – Associated Professor with Habilitation, FMUL

### Research Team

Ângelo Calado, PhD	- Auxiliary Professor, FMUL
Henrique Sobral do Rosário, MD, PhD	- Auxiliary Professor, FMUL
Ana S. Silva-Herdade, Ms	- Assistant, FMUL
Vanda Vitorino de Almeida	- PhD fellowship, FCT
Sofia de Oliveira	- Research fellowship, FCT grant
Paula Oliveira, Ms	- Research fellowship, FCT grant
Teresa Freitas	- Lab technician

### Research Areas

Inflammation / Resolution  
Leukocyte-endothelium interaction / Microcirculation  
Hemorheology  
Non-Neuronal Cholinergic System

### Major Scientific Achievements

#### **1) Fibrinogen-dependent signalling in microvascular blood cell function-implications for inflammation and cardiovascular disease.**

##### a) Interaction of soluble fibrinogen with the erythrocyte

Fibrinogen–erythrocyte membrane interaction and its modulation have been monitored by zeta potential light scattering spectroscopy. Using isolated young and old erythrocytes this technique showed that the interaction between fibrinogen and young erythrocytes is higher than the

interaction between fibrinogen and old ones. Accordingly and by using flow cytometry and confocal microscopy, young erythrocytes showed more affinity to bind fluorescent fibrinogen than old ones.

On the other hand, fibrinogen modulates the erythrocyte nitric oxide (NO) metabolism by decreasing NO efflux levels and enhancing S-nitrosoglutathione, nitrites and nitrates concentration.

b) Interaction of soluble fibrinogen with the neutrophil

For the characterization of the effect of soluble fibrinogen in regulating the activation state of the neutrophil under static conditions, we used flow cytometry and fluorescence microscopy approaches.

Fibrinogen leads to an increase in neutrophil free radical production. However the membrane expression and activation of the  $\beta$ 2-integrin Mac-1 is apparently not modified in the presence of fibrinogen.

We also studied the interaction between neutrophils and endothelial cells, in presence of physiological concentrations of fibrinogen under flow. Using flow chamber techniques, we observed that neutrophil adhesion is diminished in the presence of fibrinogen.

**2) Non-neuronal cholinergic systems in inflammation**

a) Monocyte/macrophage cholinergic systems

Acetylcholine (ACh) plays an anti-inflammatory role by downregulating the expression/release of pro-inflammatory cytokines by monocytes and macrophages. As ACh content is regulated at its synthesis and degradation steps, we hypothesized that the expression of particular cholinergic factors could be modulated during the inflammatory response. Previously, we have shown that in THP-1 cells (human acute leukemic monocytic cell line) the mRNA expression of AChE (enzyme responsible for ACh hydrolysis) is up-regulated by LPS treatment whereas its protein content is not significantly altered under these conditions. These results suggested to us the existence of post-transcriptional mechanisms that silence AChE expression in monocytes during the inflammatory response. During the past year and in order to address the physiologic role of these findings, we have extended our study to human monocytes isolated from peripheral blood and macrophages derived ex vivo from those cells. For both these cellular types, an up-regulation of AChE mRNA expression is similarly observed under those inflammatory conditions. We are currently evaluating its protein expression so as to fully understand how AChE expression is regulated in these cellular models in inflammation. In our studies, we have simultaneously analyzed the cellular expression of other cholinergic factors under these inflammatory conditions. Here, we have unravelled that in clear contrast to monocytes, monocyte-derived macrophages express the choline acetyltransferase (enzyme required for ACh synthesis) at the mRNA level. Importantly, LPS stimulation abrogates this expression. These results suggest a differential ability of monocytes and macrophages to synthesize ACh.

b) Erythrocytic cholinergic systems

ACh functions as a global signaling player with a central role in microvasculature. Circulating ACh can be produced by T lymphocytes and endothelial cells. Red blood cells account for the blood elements with highest expression of the membrane enzyme AChE. We demonstrated that the ACh-AChE complex activity is dependent on protein band 3 phosphorylation status and unravelled a possible conformational linkage between Gi protein and band 3.

**3) Leukocyte-endothelial cell interaction**

a) Animal experimentation

Leukocyte recruitment and adhesion to the vessel wall are essential stages of the inflammatory response and animal models are essential tools for the study of cell-cell and leukocyte-endothelial cell interactions. Taking into account our future goals, we have developed two different animal models of inflammation – the air-pouch and the intraperitoneal inflammation models. The air-pouch model of inflammation is produced by a subcutaneous injection of sterile air into the back of a mouse. The formation of an air pouch provides a localized environment for

study of inflammation and cellular response. The air-pouch model offers a unique window, amongst in vivo inflammation models, through differential cell analysis of the inflammatory exudates. The intraperitoneal inflammation model is achieved by administering an inflammatory agent in the intraperitoneal cavity of the mouse. This model provides an inflammatory environment that can be observed by intravital microscopy of the mesentery in order to study leukocyte-endothelial cell interactions during an inflammatory response.

#### b) Mathematical Simulation

It is thought that dynamic forces play an important role in the leukocyte recruitment and adhesion to the vessel wall. In collaboration with the group of Adélia Sequeira (IST, Portugal) we have been developing theoretical models on leukocyte-vessel wall interactions in order to understand the role of the dynamic forces in leukocyte recruitment. We have already shown that there is a relationship between leukocyte recruitment and wall shear stress. The development of numerical simulations can provide us with tools for better understanding leukocyte-vessel wall interaction in particular inflammatory and cardiovascular diseases.

#### 4) Erythrocyte properties in inflammation

Red blood cells (RBC) are the major rheological factors of blood flow in view of the association between flow retardation with progressive erythrocyte aggregation and of flow acceleration with progressive RBC deformability. As RBC are more deformed, oriented and displaced to the vessel axis the higher blood fluidity is. The RBC membrane elasticity is the dominant factor at the microcirculation vessels.

At physiological conditions, the shear stress at threshold values assures maximal erythrocyte deformability. This is impaired in the inflammatory response inducing erythrocyte rheological abnormalities. Taken this, changes in RBC normal immune and anti-inflammatory functions in vascular disorders are associated with worse disease progression. We have shown that by acting at the signal transduction axis, ACh-AChE-band 3 phosphorylation status, the reduced erythrocyte deformability may be restored at physiological and higher shear stress in dependence of erythrocyte nitric oxide level or thiol redox status.

#### Recent Most Relevant Publications

Artoli, A.M., Sequeira, A., Silva-Herdade, A.S. and Saldanha, C. (2007) Leukocytes rolling and recruitment by endothelial cells: hemorheological experiments and numerical simulations. *J. Biomech.* 40: 3493-3502 (Times cited: 4)(Journal IF: 3.520)

Santos, S.C.R., Vala, I., Miguel, C., Barata, J.P., Garção, P., Agostinho, P., Mendes, M., Coelho, A.V., Calado, A., Oliveira, C.R., Martins e Silva, J. and Saldanha, C. (2007) Expression and subcellular localization of a novel nuclear acetylcholinesterase protein. *J. Biol. Chem.* 282: 25597-25603 (Times cited: 6)(Journal IF: 5.520)

Lund, E., Guttinger, S., Calado, A., Dahlberg, J.E. and Kutay, U. (2004) Nuclear export of microRNA precursors. *Science* 303: 95-98 (Times cited: 477)(Journal IF: 28.103)

Rosário, H.S., Waldo, S.W., Becker, S.A., Schmid-Schönbein, G.W. (2004) Pancreatic trypsin increases matrix metalloproteinase-9 accumulation and activation during acute intestinal ischemia-reperfusion in the rat. *Am. J. Pathol.* 164: 1707-1716 (Times cited: 20)(Journal IF: 5.697)

Waldo, S.W., Rosario, H.S., Penn, A.H. and Schmid-Schönbein, G.W. (2003) Pancreatic digestive enzymes are potent generators of mediators for leukocyte activation and mortality. *Shock* 20: 138-143 (Times cited: 18)(Journal IF: 3.394)

#### Ongoing Research Projects

1998/ Acetylcholinesterase and chronic venous diseases. Coordinator: José Albino, HPV.

2006/ Effects of long-term therapy with acetylcholinesterase and butyrylcholinesterase

inhibitors in Alzheimer disease. Coordinators: A. de Mendonça, F. Simões do Couto, IMM, FMUL.

2006/ Factores preditivos de lesão endotelial e cardiovascular no prognóstico da esclerose sistémica e sua correlação com a terapêutica. Coordinator: C. Resende, HSM.

2007/09 Fibrinogen-dependent signalling in microvascular blood cell function – implications of inflammation and cardiovascular disease, FCT. Coordinator: C. Saldanha (PTDC/SAU-OSM/73449/200)

2007/09 Multiscale Mathematical Modelling in Biomedicine, FCT. Coordinator: A. Sequeira, IST, UTL (PTDC/MAT/68166/2006)

2007/09 Identificação de um marcador de interação eficaz na terapêutica de doença de Alzheimer, Fundação Merck Shape & Dohme. Coordinator: S. Abreu, IMM, FMUL.

2009/2011 Cardiovascular risk and inflammation- contribution to the etiopathogenesis of atherosclerosis and cardiovascular disease, FCT. Coordinator: J.E. Fonseca, IMM, FMUL (PIC/IC/82920/2007)

2009/2011 Development and validation of vaso-occlusion early predictors in a mendelian model of vascular disease, FCT. Coordinator: J. Lavinha, INSA (PIC/IC/83084/2007)

#### **Publications in Peer Review International Journals (2009)**

De Oliveira, S. and Saldanha, C. (2009) An overview about erythrocyte membrane. Clin. Hemorheol Microcirc. doi 10-3233/CH-2009-1253. (Journal IF: 1.814).

de Almeida, J.P.L., Carvalho, F.A., Silva-Herdade, A.S., Santos-Freitas, T. and Saldanha C. (2009) Redox thiol status plays a central role in the mobilization and metabolism of nitric oxide in human red blood cells. Cell Biol. Int. 33: 268-275 (Journal IF: 1.619)

Sequeira, A., Artoli, A.M., Silva-Herdade, A.S. and Saldanha, C. (2009) Leukocytes dynamics in microcirculation under shear-thinning blood flow. Comput. Math. Appl. 58: 1035-1044 (Journal IF: 0.997)

de Almeida, J.P., Freitas-Santos, T. and Saldanha, C. (2009) Fibrinogen-dependent signaling in microvascular erythrocyte function: implications on nitric oxide efflux. J. Membr. Biol. 231: 47-53 (Journal IF: 2.320)

Carvalho, F.A., de Almeida, J.P., Freitas-Santos, T. and Saldanha, C. (2009) Modulation of erythrocyte acetylcholinesterase activity and its association with G protein-band 3 interactions. J. Membr. Biol. 228: 89-97 (Journal IF: 2.320)

#### **Publications in Peer Review National Journals (2009)**

Cascão, R., Rosário, H.S., Fonseca, J.E. (2009) Neutrophils: warriors and commanders in immune mediated inflammatory diseases. Acta Reumatol. Port. 34: 313-326. (Journal IF: NA)

#### **Communications in International Conferences**

de Oliveira, S. et al. Fibrinogen and erythrocyte membrane interaction. 15<sup>th</sup> Conference of the European Society for Clinical Hemorheology and Microcirculation (ESCHM), Pontresina/St.Moritz, Switzerland. June 28 - July 1, 2009 (Oral presentation)

Oliveira, P. et al. Acetylcholinesterase expression in human peripheral blood monocytes under inflammatory conditions. 10<sup>th</sup> International Meeting on Cholinesterases, Sibenik, Croatia. September 20-25, 2009 (Poster presentation)

Vitorino de Almeida, V. et al. Fibrinogen and neutrophil recruitment. Tri-Society Annual Conference 2009 of the Society for Leukocyte Biology, International Cytokine Society & International Society for Interferon and Cytokine Research: Cellular and Cytokine Interactions in

Health and Disease, Lisbon, Portugal. October 18-21 (Poster presentation)

Santos, M.J. et. al. Hemorheological changes associated with systemic lupus erythematosus and rheumatoid arthritis. 4<sup>th</sup> European Workshop on Immune-mediated diseases, Cascais, Portugal. November, 18-21 (Poster presentation)

### **Communications in National Conferences**

Vitorino de Almeida, V. et al. Fibrinogen and neutrophil recruitment. 3<sup>rd</sup> IMM PhD Students Meeting, IMM, Lisbon, October 12-13, 2009 (Oral presentation)

### **Invited Lectures and Seminars**

Saldanha, C. “Erythrocyte band 3 protein and hemorheological properties”. 15<sup>th</sup> Conference of the European Society for Clinical Hemorheology and Microcirculation (ESCHM), Pontresina/St.Moritz, Switzerland. June 28 - July 1 2009

Saldanha, C. “Erythrocyte deformability responses to shear stress under external and internal stimuli influences”. 3<sup>rd</sup> Eurosummer School on Biorheology and Symposium on Micro and Nanomechanics and Mechanobiology of Cell, Tissues and Systems. Borovets, Bulgaria. August 29 – September 2, 2009.

### **Advanced Teaching**

Saldanha, C. “Metabolism Integration”. 7<sup>th</sup> Post-graduation Course in Sport Medicine, Sociedade Portuguesa de Medicina Desportiva / Faculdade de Medicina, Universidade de Lisboa. April 17-18, 2009.

Rosario, H.S. “Intravital Microscopy – Going with the Flow”. Bioimaging Nanocourse on Microscopy 2009. Instituto Medicina Molecular, Lisboa. October 27-28, 2009.

### **Public Perception of Science**

Saldanha, C. “Inflammatory process: from the cell to the animal”. Practical training within “Ocupação científica nas ferias 2009” – Program “Ciência Viva”. August 10 – 14, 2009. Co-supervisors: Vanda Vitorino de Almeida and Sofia de Oliveira.

Saldanha, C. (2009) “Quality Promotion in Hemorheology”. Editorial, Bulletin of the Portuguese Society of Hemorheology and Microcirculation, 24; (3):3-4 in [www.hemorreologia.com](http://www.hemorreologia.com).

Saldanha, C. (2009) “Referee Board”. Editorial, Bulletin of the Portuguese Society of Hemorheology and Microcirculation, 24; (3):3-4 in [www.hemorreologia.com](http://www.hemorreologia.com).

Saldanha, C. (2009) “The Erythrocyte Immune Function”. Editorial, Bulletin of the Portuguese Society of Hemorheology and Microcirculation, 24; (2):3-4 in [www.hemorreologia.com](http://www.hemorreologia.com).

Saldanha, C. (2009) “Erythrocyte as an ubiquitous biomarker”. Editorial, Bulletin of the Portuguese Society of Hemorheology and Microcirculation, 24; (1):3-4 in [www.hemorreologia.com](http://www.hemorreologia.com).

### **Thesis Completed**

#### **Master Thesis**

Ana Sofia Pereira Fernandes (2009) Autonomia Funcional nos Indivíduos com Degenerescência Macular Relacionada com a Idade: Avaliação Multifuncional. Masters in Psicogerontology, Faculdade de Medicina da Universidade de Lisboa. Supervisor: Carlota Saldanha.

Paula Alexandra Gomes de Oliveira (2009) Expressão de factores colinérgicos em



monócitos/macrófagos na inflamação. Master in Human Molecular Biology, Faculdade de Ciências da Universidade de Lisboa. Supervisor: Angelo Calado.

**PhD Thesis**

Henrique Baptista Colaço Sobral do Rosário (2009) Matrix metalloproteinase-9 and pancreatic trypsin in the intestinal wall – A contribution to the understanding of intestinal ischemia-reperfusion. Faculdade de Medicina da Universidade de Lisboa. Supervisor: João Martins e Silva and Co-supervisor: Geert Schmid-Schönbein.

## Angiogenesis Unit

### Description and Objectives

Angiogenesis is the formation of new blood vessels from pre-existing ones and is an essential process during development. Nonetheless, it also occurs in adulthood, during wound healing and restoration of blood flow to injured tissues.

Angiogenesis is orchestrated by a variety of pro and anti-angiogenic signals. Their imbalance, promoting either excessive or insufficient angiogenesis, can lead to disease. In cancer, diabetic eye disease and rheumatoid arthritis, excessive angiogenesis feeds diseased tissue and destroys normal tissue. Conversely, insufficient angiogenesis underlies conditions such as coronary heart disease, stroke and delayed wound healing, where inadequate blood-vessel growth leads to poor circulation and tissue death.

The overall goal of the Angiogenesis Unit is to study the molecular and cellular mechanisms that regulate the angiogenic process.

### Head of Unit

Susana Constantino Rosa Santos, PhD, Auxiliary Professor, FMUL

### Research Team

Raquel Nunes Sampaio, PhD	- Post-Doc fellowship, FCT
Ana Inês Domingues	- PhD fellowship, FCT
Inês Sofia Vala	- PhD fellowship, FCT
Ana Catarina Pronto	- Technician, FMUL

### Research Areas

Angiogenesis  
Vascular Biology  
Signal Transduction  
Cellular and molecular Biology

### Major Scientific Achievements

Our overall goal is to study the molecular and cellular mechanisms that regulate the angiogenic process.

In tumor and endothelial cells, we described that VEGFR-2 has a nuclear localization, required for endothelial recovery during wound healing. We are now interested in the role of nuclear VEGFR-2 to identify new mechanisms that may be targeted in situations of wound healing failure or tumor angiogenesis.

Another aim of our unit is to understand the effects of ionizing radiation on tumor angiogenesis, growth and metastasis. Radiotherapy is a widely used local treatment for malignant tumors, characterized by uncontrolled growth and the ability of invading adjacent tissues and metastasize. However, clinical and experimental observations indicate that ionizing radiation might promote a metastatic behavior of cancer cells and that the irradiated host microenvironment might exert tumor-promoting effects. Therefore, a careful analysis of the putative tumor-promoting and pro-metastatic effect of IR is imperative, as radiotherapy is an essential part of cancer treatment.

We are also investigating the effect of the wild-type or mutated Transthyretin (TTR) in the differential response of the hepatic vasculature. The Familial Amyloidotic Polyneuropathy (FAP)

is a disease caused by an abnormal protein, the TTR V30M. Since the liver is the main site of production of TTR, liver transplantation is the only treatment for the disease. However, the patients with FAP have more incidence of hepatic artery thrombosis than other patients. Therefore, it is crucial the study of the cause for this complication in FAP patients after transplantation.

#### **Recent Most Relevant Publications**

Constantino Rosa Santos, S., Miguel, C., Domingues, I., Calado, A., Zhu, Z., Wu, Y. and Dias, S. (2007). VEGF and VEGFR-2 (KDR) internalization is required for endothelial recovery during wound healing. *Exp. Cell Res.* 313: 1561-1574 (Times cited: 16)(Journal IF: 3.948)

Constantino Rosa Santos, S., Vala, I., Miguel, C., Barata, J., Garção, P., Agostinho, P., Mendes, M., Coelho, A., Oliveira, C., Martins e Silva, J. and Saldanha, C. (2007) Expression and subcellular localization of a novel nuclear Acetylcholinesterase protein. *Journal of Biological Chemistry*, 282 (35): 25597-603. (Times cited: 6)(Journal IF: 5.8)

Constantino Rosa Santos, S. and Dias, S. (2004) Internal and external autocrine VEGF/KDR loops regulate survival of subsets of acute leukemia through distinct signaling pathways. *Blood* 103: 3883-3889 (Times cited: 67)(Journal IF: 10.9)

Constantino Rosa Santos, S., Lacronique, V., Bouchaert, I., Monni, R., Bernard, O., Gisselbrecht, S. and Gouilleux, F. (2001) Constitutively active STAT5 variants induce growth and survival of hematopoietic cells through a PI 3-Kinase/AKT dependent pathway. *Oncogene* 20: 2080-2090 (Times cited: 31)(Journal IF: 7.216)

Constantino Rosa Santos, S., Dumon, S., Mayeux, P., Gisselbrecht, S. and Gouilleux, F. (2000) Cooperation between STAT5 and phosphatidylinositol 3-kinase in the IL-3 dependent survival of the bone marrow derived cell line. *Oncogene* 19:1164-1172 (Times cited: 40)(Journal IF: 7.216)

#### **Ongoing Research Projects**

2009/2010 The biological effects of ionizing radiation in the vasculature, Roche Farmacêutica, Novartis Farma, Astellas Farma, Wyeth Lederle Portugal and Merck Sharp & Dohme. Coordinator: S. Constantino

2009/2011 The differential effect of WT and V30M Transthyretin in the hepatic microenvironment and vasculature response, FCT. Coordinator: S. Constantino and R. Perdigoto (PIC/IC/83062/2007).

#### **Communications in International Conferences**

Domingues, I. et al. VEGF and VEGFR-2: New role in the cell nucleus. EMBO Lymphatic and Blood Vasculature from Models to Human Disease Workshop, Helsinki, Finland. June 4-5, 2009 (Poster presentation)

Vala, I. et al. Intriguing effects of ionizing radiation. 9th ESH Euroconference on Angiogenesis, Helsinki, Finland, June 6-7, 2009 (Poster presentation).

#### **Communications in National Conferences**

Domingues, I. et al. VEGFR-2 – New role in the cell nucleus. 3rd IMM PhD Students Meeting, IMM, Lisbon, October 12-13, 2009 (Oral presentation)

Vala, I. et al. Low doses of ionizing radiation promote tumor growth and metastasis by enhancing angiogenesis 3rd IMM PhD Students Meeting, IMM, Lisbon, October 12-13, 2009 (Oral presentation)

### **Invited Lectures and Seminars**

Constantino, S. Undisclosed Effects of Ionizing Radiation in Angiogenesis. Instituto de Medicina Molecular, Lisboa. March 9, 2009.

### **Advanced Teaching**

Constantino, S. "Microenvironment" Biology of Cancer Nanocourse, IMM PhD Programme, IMM Lisboa. April 27-30, 2009.

Constantino, S. organized and lectured the course "Fundamentals of Molecular and Cellular Biology of Cancer. Radiobiology" held at the Radiotherapy Service, Hospital de Santa Maria, February 19 - April 16, 2009.

## Biomembranes Unit

### Description and Objectives

Biological membranes serve not only as biological boundaries but also as dynamic structures essential for several cellular events, both on health and disease. The scope of the Unit is the study of biochemical and biophysical processes occurring at the level of the membranes of human cells and of their viral and bacterial pathogens, even without the involvement of membrane receptors. The Unit is mainly focused on the two steps of the life cycle of enveloped viruses (mainly HIV and dengue virus) that involve biomembranes: the entry of the virus in a target cell and the release of new virus. The questions that we try to answer are related to specific lipid recruitment during viral assembly, virus-target cell interactions conditioning by viral lipid composition and, the role of lipid rafts (heterogeneous membrane areas enriched in specific components) on the virus life cycle and on the action of some anti-HIV drugs. The answering of some of these questions may reveal specific interactions, show how they can be modulated and, hopefully, identify new targets for antiretroviral therapy. Adding to this main focus, we are also involved in collaborative projects on: i) the evaluation of the activity of microbicides that bind to specific component of the membranes of some bacteria; ii) the study of the binding of fibrinogen to platelets and erythrocytes and its relevance as cardiovascular risk factor; and, iii) on the Nanomedicine / Nanotechnology area, the structural characterization of metal nanoparticles conjugated with proteins for biomedical applications (e.g., biosensors).

### Head of Unit

Nuno C. Santos, PhD – Assistant Professor, FMUL

### Research Team

Sónia Gonçalves, PhD	- Researcher, FMUL.
Ivo Martins	- Post-doc fellowship, Marie Curie (EU)
Patrícia Santiago	- Post-doc fellowship, CAPES (Brazil)
Fabiana Carneiro	- Post-doc fellowship, CAPES (Brazil)
Filomena Carvalho	- Assistant, FMUL.
Marco Domingues	- PhD fellowship, FCT
Pedro Matos	- PhD fellowship, FCT
Margarida Rodrigues	- PhD fellowship, FCT
Sofia de Oliveira	- Research fellowship, FCT grant (left in December)
João Abade	- Master Student (left in October 2009).
Teresa Santos	- Technician, FMUL

### Research Areas

Biomembranes  
Molecular Biophysics  
Biochemistry  
Nanomedicine

### Major Scientific Achievements

#### HIV-fusion inhibitors mechanism of action

Enfuvirtide and T-1249 are two HIV-1 fusion inhibitor peptides that bind to gp41 and prevent its fusogenic conformation, inhibiting viral entry into host cells. Previous studies established the relative preferences of these peptides for membrane model systems of defined lipid

compositions. We aimed to understand the interaction of these peptides with the membranes of erythrocytes and peripheral blood mononuclear cells. The peptide behavior toward cell membranes was followed by di-8-ANEPPS fluorescence, a lipophilic probe sensitive to the changes in membrane dipole potential. We observed a fusion inhibitor concentration-dependent decrease on the membrane dipole potential. Quantitative analysis showed that T-1249 has an approximately eight-fold higher affinity towards cells, when compared with enfuvirtide. We also compared the binding towards di-8-ANEPPS labeled lipid vesicles that model cell membranes and obtained concordant results. We demonstrated the distinct enfuvirtide and T-1249 membranotropism for circulating blood cells, which can be translated to a feasible *in vivo* scenario. The enhanced interaction of T-1249 with cell membranes correlates with its higher efficacy, as it can increase and accelerate the drug binding to gp41 in its pre-fusion state.

### **rBPI<sub>21</sub> mechanism of action**

Antimicrobial peptides (AMPs) are important potential alternatives to conventional therapies against bacterial infections. rBPI<sub>21</sub> is a 21 kDa peptide based on the N-terminal region of the neutrophil bactericidal/permeability-increasing protein (BPI). This AMP possesses highly selective bactericidal effects on Gram-negative bacteria and have affinity for lipopolysaccharide (LPS) which is believed to be at the origin of its neutralizing effect of the LPS segregated into the bloodstream. We aim at understanding the molecular bases of rBPI<sub>21</sub> bactericidal and LPS neutralization actions, using biomembrane model systems. Using dynamic light scattering spectroscopy we demonstrate that rBPI<sub>21</sub> promotes aggregation of negatively charged large unilamellar vesicles (LUV), even in the absence of LPS, and LPS aggregates, while for zwitterionic phosphatidylcholine (POPC) LUV the size remains unchanged. The peptide also promotes the fusion (or hemifusion) of membranes containing phosphatidylglycerol (POPG). The aggregation and fusion of negatively charged LUV are peptide concentration-dependent until massive aggregation is reached, followed by sample flocculation/precipitation. Concomitantly, there is a progressive change in the zeta-potential of the LUV systems and LPS aggregates. LUV systems composed of phosphatidylglycerol (POPG) and lipid mixtures with POPG have higher zeta-potential variations than in the absence of POPG. The interaction of rBPI<sub>21</sub> with lipid vesicles is followed by leakage, with higher effect in POPG-containing membranes. LPS aggregation can be related with a decreased toxicity, possibly by facilitating its clearance by macrophage phagocytosis and/or blocking of LPS specific receptor recognition. Our data indicate that rBPI<sub>21</sub> mechanism of action at the molecular level involves the interaction with the LPS of the outer membrane of Gram-negative bacteria, followed by internalization and leakage induction through the (hemi)fusion of the bacterial outer and inner membranes, both enriched in phosphatidylglycerol.

### **Fibrinogen-erythrocyte interaction**

Fibrinogen contributes to erythrocyte hyperaggregation, a vascular risk factor. Glycoprotein  $\alpha_{IIb}\beta_3$  is the specific fibrinogen integrin receptor in platelets. We demonstrate, by force spectroscopy measurements on an AFM, the existence of a single molecule interaction between fibrinogen and an unknown receptor on the erythrocyte membrane, with a lower but comparable affinity relative to platelet binding (fibrinogen-erythrocyte and -platelet average (un)binding forces were 79 and 97 pN, respectively). This receptor is not as influenced by calcium and eptifibatide (an  $\alpha_{IIb}\beta_3$  inhibitor) as the platelet receptor. However, its inhibition by eptifibatide indicates that it is a  $\alpha_{IIb}\beta_3$ -related integrin. Results obtained for Glanzmann thrombastenia (a rare hereditary bleeding disease caused by  $\alpha_{IIb}\beta_3$  deficiency) patients show impaired fibrinogen-erythrocyte binding. Correlation with genetic sequencing data demonstrates that one of the units of the fibrinogen receptor on erythrocytes is a product of the expression of the  $\beta_3$  gene, found to be mutated in these patients.

### **Recent Most Relevant Publications**

Franquelim, H.G., Loura, L.M.S., Santos, N.C. and Castanho, M.A.R.B. (2008) Sifuvirtide screens rigid membrane surfaces. Establishment of a correlation between efficacy and membrane domain selectivity among HIV fusion inhibitor peptides. *J. Am. Chem. Soc.* 130: 6215-6223

(Times cited: 1) (Journal IF: 8.091)

Santiago, P.S., Moura, F., Moreira, L.M., Domingues, M.M., Santos, N.C. and Tabak, M. (2008) Dynamic light scattering and optical absorption spectroscopy study of pH and temperature stabilities of the extracellular hemoglobin of *Glossoscolex paulistus*. *Biophys. J.* 94: 2228-2240 (Times cited: 4) (Journal IF: 4.683)

Veiga, A.S., Santos, N.C., Loura, L.M.S., Fedorov, A. and Castanho, M.A.R.B. (2004) HIV fusion inhibitor peptide T-1249 is able to insert or adsorb to lipidic bilayers. Putative correlation with improved efficiency. *J. Am. Chem. Soc.* 126: 14758-14763 (Times cited: 17) (Journal IF: 8.091)

Santos, N.C., Prieto, M. and Castanho, M.A.R.B. (2003) Quantifying molecular partition into model systems of biomembranes: an emphasis on optical spectroscopic methods. *Biochim. Biophys. Acta* 1612: 123-135. (Times cited: 69) (Journal IF: 4.180)

Santos, N.C., Figueira-Coelho, J., Martins-Silva, J. and Saldanha, C. (2003) Multidisciplinary utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspect. *Biochem. Pharmacol.* 65: 1035-1041 (Times cited: 86)(Journal IF: 4.838)

### Ongoing Research Projects

2007/10 An atomic force microscopy facility devoted to biomedical and biochemical studies at the molecular-level, FCT. Coordinator: M. Castanho (REEQ/140/BIO/2005)

2007/10 Fibrinogen-dependent signalling in microvascular blood cell function – implications of inflammation and cardiovascular disease, FCT. Coordinator: C. Saldanha (PTDC/SAU-OSM/73449/2006)

2007/10 The role of lipid membranes in the dengue virus cell infection, FCT. Coordinator: M. Castanho (PTDC/QUI/69937/2006)

2007/10 Metal- and magnetic nanoparticles functionalized with biological molecules: towards bio-nanoproboscopes, FCT. Coordinator: E. Pereira (PTDC/QUI/64484/2006)

2007/10 Identificação de um marcador de interação eficaz na terapêutica da doença de Alzheimer, Merck Sharp & Dohme Foundation. Coordinator: S. Gonçalves

### Publications in Peer Review International Journals 2009

Domingues, M.M., Castanho, M.A.R.B. and Santos, N.C. (2009) rBPI<sub>21</sub> promotes lipopolysaccharide aggregation and exerts its antimicrobial effects by (hemi)fusion of PG-containing membranes. *PLoS ONE* 4: e8385 (Journal IF: NA)

Carvalho, F.A., de Almeida, J.P., Freitas-Santos, T. and Saldanha, C. (2009) Modulation of erythrocyte acetylcholinesterase activity and its association with G protein-band 3 interactions. *J Membr. Biol.* 228: 89-97 (Journal IF: 2.320)

Justino, G.C., Rodrigues, M., Florêncio, M.H. and Mira, L. (2009) Structure and antioxidant activity of brominated flavonols and flavanones. *J. Mass Spectrom.* 44: 1459-1468 (Journal IF: 3.263)

de Almeida, J.P.L., Carvalho, F.A., Silva-Herdade, A.S., Santos-Freitas, T. and Saldanha C. (2009) Redox thiol status plays a central role in the mobilization and metabolism of nitric oxide in human red blood cells. *Cell Biol. Int.* 33: 268-275 (Journal IF: 1.619)

Domingues, M.M., Lopes, S.C.D.N., Santos, N.C., Quintas, A. and Castanho, M.A.R.B. (2009) Fold-unfold transitions in the selectivity and mechanism of action of the N-terminal fragment of the Bactericidal/Permeability-Increasing protein (rBPI<sub>21</sub>). *Biophys. J.* 96: 987–996 (Journal IF: 4.683)

### Books/Book Chapters

Domingues, M.M. and Santos, N.C. (2009) Laser-light-scattering approach to peptide-membrane interaction, *in* Structure and function of membrane-active peptides, ed. M. Castanho, IUL Press, La Jolla (USA), in press.

Matos, P.M., Franquelim, H.G., Castanho, M.A.R.B. and Santos, N.C. (2009) Fluorescence studies of fluorescent and non-fluorescent peptides interacting with lipid membranes, *in* Topics in Fluorescence Spectroscopy, vol.14 - Protein Fluorescence, ed. C. Geddes, Springer, New York, in press.

### Communications in International Conferences

Abade, J. et al. Microbicide action and lipid selectivity of defensins: comparison between Psd1 and Hnp1 behaviors. EBSA 2009 – 7th European Biophysics Congress, Genoa, Italy, July 11-15, 2009 (Poster presentation). Abstract published in (2009) *Eur. Biophys. J.*, 38, S177.

Cavalho, F.A. et al. Study of the fibrinogen human erythrocyte membrane receptor by AFM. Nanomedicine 2009 – 2nd European Summer School in Nanomedicine, Quinta da Marinha, Portugal, June 12-16, 2009 (Poster presentation)

Carvalho, F.A. et al. Force spectroscopy of the interaction of fibrinogen with erythrocytes and platelets. Biophysical Society's 53rd Annual Meeting, Boston, USA, February 28 – March 4 (Poster presentation). Abstract published in (2009) *Biophys. J.*, 96, 397a.

Carvalho, F.A. et al. Is there a specific erythrocyte membrane receptor for fibrinogen? An atomic force microscopy approach. EBSA 2009 – 7th European Biophysics Congress, Genoa, Italy, July 11-15, 2009 (Poster presentation). Abstract published in (2009) *Eur. Biophys. J.*, 38, S110.

Carvalho, F.A.O., Santiago, P.S., Domingues, M.M., Santos, N.C., Tabak, M. Spectroscopic studies of the stability of the extracellular hemoglobin from *Glossoscolex paulistus* at acid pH. VII Iberoamerican Congress of Biophysics, Búzios (Rio de Janeiro), Brazil, September 30 – October 3, 2009 (Poster presentation)

De Oliveira, S. et al. Fibrinogen and erythrocyte membrane interaction: potential binding sites evaluation. 15th Conference of the European Society for Clinical Hemorheology and Microcirculation (ESCHM), St. Moritz, Switzerland, June 28 – July 1, 2009 (Oral presentation)

Domingues, M.M. et al. rBPI21 aggregates and neutralizes negatively charged lipopolysaccharides and biomimetic membranes. EBSA 2009 – 7th European Biophysics Congress, Genoa, Italy, July 11-15, 2009 (Poster presentation). Abstract published in (2009) *Eur. Biophys. J.*, 38, S161.

Domingues, M.M. et al. The molecular-level mechanism of action of a Gram-negative bacteria-specific antimicrobial peptide. VII Iberoamerican Congress of Biophysics, Búzios (Rio de Janeiro), Brazil, September 30 – October 3, 2009 (Oral presentation)

Franquelim, H.G. et al. Specificity of a HIV fusion inhibitor towards phosphatidylcholine gel phase membranes. EBSA 2009 – 7th European Biophysics Congress, Genoa, Italy, July 11-15, 2009 (Poster presentation). Abstract published in (2009) *Eur. Biophys. J.*, 38, S38. Best posters prize.

Franquelim, H.G. et al. Membrane interactions of HIV fusion inhibitors studied using a biophysical approach. 34th Congress of the Federation of European Biochemical Societies (FEBS), Prague, Czech Republic, July 4-9, 2009 (Poster presentation). Abstract published in (2009) *FEBS J.*, 276, 365-366.

Gomes, I. et al. pH-switched adsorption of cytochrome c to self-assembled monolayers on gold surfaces and nanoparticles. NanoSpain 2009, Zaragoza, Spain, March 9-12, 2009 (Poster presentation)

Gomes, I., et al. pH control of the adsorption of cytochrome c to gold nanostructured surfaces and nanoparticles. NanoBioEurope 2009, Grenoble, France, June 16-18, 2009 (Oral presentation)



presentation)

Matos, P.M. et al. HIV-1 fusion inhibitors-blood cells interaction measurements agree with biomembranes models data. EBSA 2009 – 7th European Biophysics Congress, Genoa, Italy. July 11-15, 2009 (Poster presentation). Abstract published in (2009) Eur. Biophys. J., 38, S191.

Matos, P.M. et al. Human erythrocytes and mononuclear leukocytes are capable of concentrating HIV-1 fusion inhibitor peptides in their membranes. Biophysical Society's 53rd Annual Meeting, Boston, USA. February 28 – March 4, 2009 (Poster presentation). Abstract published in (2009) Biophys. J., 96, 157a.

Matos, P.M. et al. Interaction of HIV-1 fusion inhibitor peptides with erythrocyte and mononuclear lymphocyte membranes. 67th Harden Conference – Decoding the biology of heparan sulphate proteoglycans, Cambridge, UK. March 27 – April 2, 2009 (Poster presentation)

Rodrigues, M. et al. Molecular basis of translocation of novel nucleolar-targeting peptides. EBSA 2009 – 7th European Biophysics Congress, Genoa, Italy, July 11-15, 2009 (Poster presentation). Abstract published in (2009) Eur. Biophys. J., 38, S194.

Santiago, P.S. et al. On the thermal denaturation/dissociation of the giant extracellular hemoglobin of *Glossoscolex paulistus*: dynamic light scattering and optical absorption studies. VII Iberoamerican Congress of Biophysics, Búzios (Rio de Janeiro), Brazil, September 30 – October 3, 2009 (Poster presentation)

### **Invited Lectures and Seminars**

Santos, N.C. Protein-Biomembranes interactions as therapeutic targets. Ciclo de Seminários Professor António Xavier, Faculdade de Ciências da Universidade de Lisboa, Lisbon, Portugal. March 18, 2009.

Santos, N.C. Protein-biomembrane interactions as therapeutic targets. Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Coimbra, Portugal, May 14, 2009.

Santos, N.C. Is there a specific erythrocyte membrane receptor for fibrinogen? An atomic force microscopy approach. Ciência 2009 – Encontro com a Ciência em Portugal, Lisbon, Portugal. July 29-30, 2009.

Santos, N.C. Protein-biomembrane interactions as therapeutic targets. Instituto de Higiene e Medicina Tropical, Lisbon, Portugal, September 28, 2009.

### **Organisation of National and International Conferences**

Santos, N.C. Co-organizer, chairman and lecturer at the VII Iberoamerican Congress of Biophysics, Búzios (Rio de Janeiro), Brazil, September 30 – October 3, 2009.

Santos, N.C. Co-organizer of the 8th Short Course of the Portuguese Biophysical Society – Systems Biology, Santarém, Portugal, October 30 – November 1, 2009.

### **Prizes, Honours and Awards**

Prémio Gulbenkian de Estímulo à Investigação 2009 to Marco M. Domingues.

### **Advanced Teaching**

Carvalho, F.A. “AFM imaging and force spectroscopy practical sessions” IMM PhD Programme, Instituto de Medicina Molecular, Lisbon, May 4-6, 2009.

Domingues, M.M. Dynamic light scattering and zeta-potential practical sessions, Master in Structural and Functional Biochemistry, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Monte da Caparica, October 30, 2009.

## **82 | 2009 IMM Activity Report**

Santos, N.C. “Atomic force microscopy”, Master in Advanced Chemistry and PhD Program, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Coimbra, May 14, 2009.

Santos, N.C. “Force spectroscopy” IMM PhD Programme, Instituto de Medicina Molecular, Lisbon, May 5, 2009.

Santos, N.C. “Force spectroscopy”, Master in Chemistry and Biotechnology, and PhD Program, Instituto Superior Técnico da Universidade Técnica de Lisboa, Lisbon, May 11, 2009.

Santos, N.C. “Light scattering techniques and their Biochemical applications”, Master in Structural and Functional Biochemistry, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Monte da Caparica, October 30, 2009.

Santos, N.C. Organized the Nanomedicine Nanocourse of the IMM PhD Programme, Instituto de Medicina Molecular, Lisbon. May 4-6, 2009.

### **Public Perception of Science**

Rodrigues, M. Volunteer for Science activities. Cientistas ao Palco – Noite dos Investigadores, Lisbon, September 25, 2009.

Santos, N.C. “Membranas biológicas como alvos terapêuticos”. Semana da Ciência e Tecnologia 2009, Escola Secundária Ferreira Dias, Agualva-Cacém, November 27, 2009.

### **Thesis Completed**

#### **Master Thesis**

Ana Sofia Pereira Fernandes (2009) Autonomia Funcional nos Indivíduos com DMI: Avaliação Multifactorial. Mestrado em Psicogerontologia, Faculdade de Medicina da Universidade de Lisboa. Supervisors: Carlota Saldanha and Sónia Gonçalves.

João Almeida Marques Abade (2009) Selectividade membranar de defensina 1 de *Pisum sativum* e péptido 1 de neutrófilos humanos. Mestrado em Biologia Molecular e Genética, Faculdade de Ciências da Universidade de Lisboa. Supervisors: Nuno C. Santos and Sónia Gonçalves.

José Miguel Martins Raimundo (2009) Caracterização da interacção do antibiótico polimixina B com agregados de LPS por espectroscopia de dispersão de luz. Mestrado Integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisor: Nuno C. Santos.

Vasco Madeira Crispim Romão (2009) Avaliação do efeito dos agregados de LPS na velocidade de hemólise. Mestrado Integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisor: Nuno C. Santos.

## Physical Biochemistry Unit

### Description and objectives

The Physical Biochemistry Unit was created in late 2007, mostly with newcomers to the IMM. The goal of the new unit is to unravel the physical principles that govern lipid-peptide interactions, with implications in viral fusion (HIV and Dengue virus are of particular interest), analgesia, novel antibiotics and transfection agents. Peptide-lipid biomembranes interactions are ubiquitous in the living world and a fertile ground for drug discovery and development. At the UBqF we are interested not only in drug targets and drug discovery itself, but also in the molecular-level mechanism of action of drugs that underwent advanced clinical trials and are known for their therapeutic efficacy and safety. More recently, we are focused on the central nervous system and translocation of the blood-brain barrier. The interaction between this barrier membranes and different molecules of clinical interest is now part of our research.

### Head of Unit

Miguel A. R. B. Castanho, Ph.D. – Full Professor, FMUL

### Research team

Ana Dulce A. A. Correia	- Researcher FMUL (left in December)
Ana Salomé Veiga	- Post-doc fellowship Marie Curie
Isa Domingues Serrano	- Post-doc fellowship, FCT
Sónia Sá Santos	- Post-doc fellowship, FCT (since December)
Sónia Troeira Henrique	- Post-doc fellowship Marie Curie
Henri Girão Franquelim	- PhD fellowship, FCT
Manuel N. Melo	- PhD fellowship, FCT
Marta Maria B. Ribeiro	- PhD fellowship, FCT
Sara Matos Santos, MD	- PhD researcher
João Freire	- MD research student
Joana matos	- MD research student
Catarina Cabeçadas	- Undergraduate research student, FCUL (left in September)
David Sousa	- Undergraduate research student, FMUL
Filipa Fernandes	- Undergraduate research student, FMUL
Mafalda neves	- Undergraduate research student, FMUL
Catarina Laborinho	- Technician, FMUL

### Research areas

Analgesic peptides  
 Antimicrobial peptides  
 Blood-brain barrier  
 Cell-penetrating peptides  
 Viral fusion inhibitors

### Major Scientific Achievements

UBqF has an eclectic research team that aims to unravel the basic physical principles that govern the interaction between peptides and lipids. For that purpose, we develop new methodologies customized for our specific needs. Recently, we have developed linear dichroism methods to reveal the orientation of the antifungal polyene antibiotic filipin in lipid bilayers. This discovery was critical to reveal its mode of action, which remained elusive for decades. We also succeeded in devising a kyotorphin derivative (analgesic dipeptide) with improved interaction with lipid

bilayers that was able to cross the Blood-Brain Barrier and remain effective. In addition, the contribute made for the development of methodologies to study and quantify molecular partition into lipid bilayers and lipid bilayer translocation were decisive to establish a link between two so far apart families of peptides: antimicrobial peptides (AMP) and cell-penetrating peptides (CPP). The possibility of a “hybrid” nature for a peptide has been demonstrated for Pep1 while BP100, for instance, was proven to have membrane-translocating capacities, although it is regarded as an AMP. We also perform studies with membrane-active sequences of viral proteins and viral fusion inhibitors. Dengue and HIV are of particular interest to us. It results from our studies that the “fusion peptide” of the proteins exposed by the virus may not be the only sequences responsible for fusion. Viral-host cell fusion seems to be much more complex and appealing than is usually imagined. We also have found a correlation between the efficacy of HIV fusion inhibitors and its membrane-interaction properties.

Descriptive reviews/discussion papers:

- 1) “Joint Determination by Brownian Dynamics and Fluorescence Quenching of Membrane Biomolecules in-Depth Location Profile.”; M. X. Fernandes, J. García de la Torre, M. A. R. B. Castanho (2002) *Anal. Biochem.*, 307, 1-12.
- 2) “Using UV-Vis linear dichroism to study the orientation of molecular probes and biomolecules in lipidic membranes”; M. A. R. B. Castanho, S. Lopes, M. X. Fernandes (2003) *Spectroscopy*, 17, 377-398.
- 3) “Lipid membrane-induced optimization for ligand-receptor docking. Recent tools and insights for the membrane catalysis model”; M. A. R. B. Castanho, M. X. Fernandes (2005) *Eur. Biophysics J.*, 35, 92-103.
- 4) "Cell-penetrating peptides and antimicrobial peptides: how different are they?"; S. T. Henriques, M. N. Melo, M. A. R. B. Castanho (2006) *Biochem. J.* 399, 1–7
- 5) "How to address CPP and AMP translocation? Methods to detect and quantify peptide internalization in vitro and in vivo"; S. T. Henriques, M. N. Melo, M. A. R. B. Castanho (2007) *Molec. Membrane Biol* 24, 173-184.
- 6) “Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations”; M. N. Melo, R. Ferre, M. A. R. B. Castanho (2009) *Nature Rev. Microbiol.* 7, 245-250.

#### **Recent most relevant publications**

- Melo, M.N., Ferre, R. and Castanho, M.A.R.B. (2009) Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat. Rev. Microbiol.* 7: 245-250 (Times cited: 5) (Journal IF: 14.31)
- Franquelim, H.G., Loura, L.M.S., Santos, N.C. and Castanho, M.A.R.B. (2008) Sifuvirtide screens rigid membrane surfaces. Establishment of a correlation between efficacy and membrane domain selectivity among HIV fusion inhibitor peptides. *J. Am. Chem. Soc.* 130: 6215-6223 (Times cited: 1)(Journal IF: 8.091)
- Henriques, S.T., Melo, M.N. and Castanho, M.A.R.B. (2006) Cell-penetrating peptides and antimicrobial peptides: how different are they? *Biochem. J.* 399: 1-7 (Times cited: 49) (Journal IF: 4.371)
- Veiga, A.S., Santos, N.C., Loura, L.M.S., Fedorov, A. and Castanho, M.A.R.B. (2004) HIV fusion inhibitor peptide T-1249 is able to insert or adsorb to lipidic bilayers. Putative correlation with improved efficiency, *J. Am. Chem. Soc.* 126: 14758-14763 (Times cited: 17) (Journal IF: 8.091)
- Santos, N.C., Prieto, M. and Castanho, M.A.R.B. (2003) Quantifying molecular partition into model systems of biomembranes: an emphasis on optical spectroscopic methods, *Biochim. Biophys. Acta* 1565: 29-35 (Times cited: 69) (Journal IF: 4.180)

### Ongoing research projects

2007/2009, An atomic force microscopy facility devoted to biomedical and biochemical studies at the molecular-level, FCT. Coordinator: M. Castanho (Project REEQ/140/BIO/2005)

2007/2010, Targeting kyotorphin derivatives to the brain, FCT. Coordinator: M. Castanho (Project PTDC/SAU-FCF/69493/2006)

2007/2010, The role of lipid membranes on cell infection by Dengue virus, FCT. Coordinator: M. Castanho (Project PTDC/QUI/69937/2006)

2008/2010, Unravelling the mechanism of action of membrane-active peptides combining NMR and optical spectroscopies, CRUP-DAAD. Portuguese Coordinator: M. Castanho (Portuguese-German Joint Action Project A-6/08)

2009/2012, Selected peptides as drug candidates directed to pain and neurodegeneration, European Commission. Coordinator: M. Castanho. (FP7-PEOPLE-2007-3-1-IAPP, Grant no.: 230654)

2009/2012 Elucidating the mechanism of action of cyclotides ultra-stable proteins from plants, European Commission, Portuguese Coordinator: M. Castanho. (PIOF-GA-2008-220318, joint project with the Institute for Molecular Biosciences, The University of Queensland, Australia)

### Publications in Peer Review International Journals 2009

Aguiar, S.I., Pinto, F.R., Nunes, S., Serrano, I., Melo-Cristino, J., Sá-Leão, R., Ramirez, M. and de Lencastre, H. (2010) Increase of Denmark14-230 clone as a cause of pneumococcal infection in Portugal within a background of diverse serotype 19A lineages. *J. Clin. Microbiol.* 48: 101-108 (*Epub October 28, 2009*)(Journal IF: 3.945)

Domingues, M.M., Lopes, S.C.D.N., Santos, N.C., Quintas, A. and Castanho, M.A.R.B. (2009) Fold-unfold transitions in the selectivity and mechanism of action of the N-terminal fragment of the Bactericidal/Permeability-Increasing protein (rBPI<sub>21</sub>). *Biophys. J.* 96: 987-996 (Journal IF: 4.683)

Ferre, R., Melo, M.N., Correia, A.D., Felia, L., Planas, M., Bardaji, E. AND Castanho, M.A.R.B. (2009) The synergistic effect of multiple membrane actions of the cecropin-melittin antimicrobial hybrid peptide BP100. *Biophys. J.* 96: 1815-1827 (Journal IF: 4.683)

Henriques S.T., Craik D.J. (2009) Cyclotides as templates in drug design. *Drug Discovery Today*, In press. (Journal IF 6.618).

Henriques S.T., Pattenden, L. K., Aguilar, M. I., Castanho M.A.R.B. (2009) Fast membrane association is a crucial factor in the peptide pep-1 translocation mechanism: A kinetic study followed by Surface Plasmon Resonance. *Biop.-Peptide Science*, In press. (Journal IF 2.823).

Henriques, S.T. and Craik D.J. (2010) Cyclotides as templates in drug design. *Drug Discov Today*. 15: 57-64 (*Epub October 28, 2009*)(Journal IF 6.618).

Henriques, S.T., Pattenden, L. K., Aguilar, M. I. and Castanho M.A.R.B. (2009) The toxicity of prion protein fragment PrP(106-126) is not mediated by membrane permeabilization as shown by M112W substitution. *Biochemistry* 48: 4198-208 (Journal IF: 3.379)

Kopecka-Pilarczyk, J. and Correia, A.D. (2009) Biochemical response in gilthead seabream (*Sparus aurata*) to in vivo exposure to pyrene and fluorene. *J. Exp. Mar. Biol. Ecol.* 372: 49-57 (Journal IF: 2.074)

Kopecka-Pilarczyk, J. and Correia, A.D. (2009) Biochemical response in gilthead seabream (*Sparus aurata*) to in vivo exposure to a mix of selected PAHs. *Ecotox. Environ. Saf.* 72: 1296-1302 (Journal IF 2.859)

Melo, M.N., Ferre, R. and Castanho, M.A.R.B. (2009) Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat. Rev. Microbiol.* 7: 245-250 (Journal IF 14.31)

Melo, M.N., Sousa, F.J., Carneiro, F.A., Castanho, M.A.R.B., Valente, A.P., Almeida, F.C., Da Poian, A.T. and Mohana-Borges, R. (2009) Interaction of the Dengue virus fusion peptide with membranes assessed by NMR: The essential role of the envelope protein Trp101 for membrane fusion. *J. Mol. Biol.* 392: 736-746 (Journal IF: 4.146)

Ribeiro, M., Serrano, I., and Castanho M. (2009) In vitro Blood-Brain Barrier models – latest advances and therapeutic applications in a chronological perspective. *Mini-Reviews in Medicinal Chemistry*, in press. IF 3,132

Veiga, A.S., Pattenden, L.K., Fletcher, J.M., Castanho, M.A.R.B. and Aguilar, M.I. (2009) Interactions of HIV-1 antibodies 2F5 and 4E10 with a gp41 epitope prebound to host and viral membrane model systems. *ChemBioChem.* 10: 1032-1044 (Journal IF: 3.322)

### **Books / Book chapters**

#### **Books**

Structure and function of membrane-active peptides. Castanho, M. (ed). IUL Publishers (<http://www.iul-press.us/Books/BBT09-Peptides/peptides.html>)

#### **Book Chapters**

Castanho, M. (2009) Membrane-Active Peptides: What after Babylon?, *in* Structure and Function of Membrane-Active Peptide. Castanho, M. (ed). IUL Publishers

Henriques S.T. and Craik D. (2009) Discovery of peptide drugs from natural sources, *in* Peptide Drugs: peptide chemistry and drug design. Dunn, B. (ed). John Wiley & Sons

Henriques, S.T. (2009) Biophysics and the way out of the endosomal vs. non-endosomal dilemma for cell-penetrating peptide pep-1 *in* Structure and Function of Membrane-Active Peptide. Castanho, M. (ed). IUL Publishers

Matos, P.M., Franquelim, H.G., Castanho, M.A.R.B. and Santos N.C. (2009) Fluorescence Studies of Fluorescent and Non-Fluorescent Peptides Interacting with Lipid Membranes, *in* Topics in Fluorescence Spectroscopy, Vol. 14 - Protein Fluorescence. Geddes, C.D. (ed). Springer

Serrano, I., Ribeiro, M. and Castanho M. (2009) Glucose-mediated drug delivery to the Central Nervous System. *SPR Carbohydrate Chemistry*, 36. (in press)

Veiga, A.S. and Franquelim, H.G. (2009) HIV-1 Fusion Inhibitor Peptides: A Cross Correlation between Efficacy and Membrane-Level Activity, *in* Structure and Function of Membrane-Active Peptide. Castanho, M. (ed). IUL Publishers

### **Full Papers in Conference Proceedings**

Ribeiro M.M.B., Correia, A., Heras, M., Bardaji, E., Pinto, A., Pinto, M., Tavares, I., Castanho, M. 2009. A new brain-target analgesic peptide: from biophysics to in vivo proof-of-concept. *Eur. Biophys. J.*, 38 (Suppl): S35-S212. (Journal IF: 2.409)

### **Communications in International Conferences**

Andrade, I. et al. Madelung Disease - Case Report. Post-Graduate Assembly in Anesthesiology, New York, December 11-15, 2009

Correia, A.D. et al. A behavioural fish model of nociception for testing new analgesic drugs. *Amino Acids*, 37, (Suppl): 75-75. (Oral communication)

Craik D.J. et al. Membrane interactions of cyclotides. 7th Gordon Research Conference on Antimicrobial Peptides, Ventura, USA. March, 2009 (Oral presentation)

Estrela, N.L. et al. Does sucrose affect protein's fibrillation behaviour?. 8th International

Conference on Protein Stabilisation, Graz, Austria. April 14-17, 2009. (Poster presentation)

Fragoso, R. et al. Intra-operative thromboembolic complication in a patient under anti-coagulation. Post-Graduate Assembly in Anesthesiology, New York, December 11-15, 2009.

Franquelim, H.G. et al. Membrane intercation of HIV fusion inhibitors studied using a biophysical approach. 34th FEBS Congress, Prague, Czech Republic. July 4-9, 2009 (Poster presentation – also presented at the FEBS Young Scientist Forum)

Franquelim, H.G. et al. Specificity of a HIV fusion inhibitor towards phosphatidylcholine gel phase membranes. 7th EBSA European Biophysics Congress, Genoa, Italy. July 11-15, 2009 (Poster presentation)

Henriques S.T. et al. Affinity for the cell membrane is determinant for cyclotide activity. As shown in a biophysical study with kalata B1 and its analogues. 1st International conference on circular proteins, Heron Island, QLD, Australia. October, 2009 (Oral presentation).

Henriques S.T. et al. How do Cyclotides exert their mode of action? The importance of cell membranes on their diverse activities. 1st International conference on circular proteins, Heron Island, QLD, Australia. October, 2009 (Poster presentation)

Henriques S.T. et al. How do Cyclotides exert their mode of action? The importance of cell membranes on their diverse activities. 21st American Peptide Symposium, Bloomington, Indiana, USA. June 2009 (Oral presentation)

Melo, M.N. et al. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. 7th European Biophysics Congress, Genoa, Italy. July 11-15, 2009 (Poster presentation)

Melo, M.N. et al. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. Young Scientist Program and main conference and 21st IUBMB International Congress and 12th FAOBMB Congress of Biochemistry and Molecular Biology, Shanghai, China, August 2-7, 2009 (Poster presentation)

Ribeiro, M.M.B. et al. A new brain-target analgesic peptide: from biophysics to in vivo proof-of-concept. 7th European Biophysics Congress, Genova, Italy. July 11-15, 2009 (Poster presentation).

Ribeiro, M.M.B. et al. Rationally designing new analgesic peptides: from biophysical screening to in vivo proof-of-concept. 34th FEBS Congress, Prague, Czech Republic, July 4-9, 2009 (Poster presentation – also presented at the FEBS Young Scientist Forum).

Santos, S. et al. Dystrophic Bullous Epidermolysis. Post-Graduate Assembly in Anesthesiology, New York, December 11-15, 2009

Veiga, A.S. and Castanho, M. Cholesterol influence on the interaction of HIV gp41 membrane proximal region-derived peptides with lipid bilayers – a biophysical study. 21st American Peptide Symposium, Bloomington, Indiana, USA. June 7-12, 2009 (Poster presentation)

### Communications in National Conferences

Ribeiro, M.M.B. et al. Validação experimental de uma nova molécula analgésica derivada da Quietorfina. 7º Convénio da Astor, Lisboa, Portugal. January 23, 2009 (Oral communication)

Figueiredo, R. et al. High Sedimentation rate in a low activity case of Rheumatoid Arthritis. Portuguese Institute of Rheumatology Congress, Lisbon.

Figueiredo, R. et al. CINCA Syndrome – a case with an evolution of 37 years. Portuguese Institute of Rheumatology Congress, Lisbon.

Figueiredo, R. et al. Pediatric SAPHO – Typical clinical case in an atypical age. Portuguese Institute of Rheumatology Congress, Lisbon, 2009

Santos S, Andrade I, Hyponatremia after anterior prostate resection, Congress of the Portuguese Society of Anesthesiology, Porto, 2009

### Invited lectures and seminars

Castanho, M. Beating the BBB. (S)low (but effective) throughput screening in the quest for a new analgesic drug. 11<sup>th</sup> Meeting of the Portuguese Society for Neurosciences, Braga, Portugal.

Castanho, M. What's the molecular meaning of MIC when working with AMPs? Lessons from the peptides Omiganan and BP100, University Pierre et Marie Curie, Paris, France.

Castanho, M. "Biomedical research in Portugal. A view from my window", Translational research in Portugal: What are the main obstacles? How to address them? Porto, Portugal.

Castanho, M. The quest for a new analgesic drug. Innovating in drug discovery, Universidade de Aveiro, Portugal.

Castanho, M. "Bioquímica do laboratório ao consultório – descobrir, estudar e desenvolver um novo analgésico", 30<sup>o</sup> Aniversário da Licenciatura em Bioquímica em Coimbra e Portugal, Universidade de Coimbra, Portugal.

Castanho, M. "O papel das Sociedades Científicas: Sociedade Portuguesa de Bioquímica", III Jornadas das Ciências – Ciência: a causa das coisas", Instituto Superior Ciências de Saúde – Norte, Porto, Portugal.

Castanho, M. A experiência de participação de um centro de investigação na Acção Marie Curie IAPP: IMM-Instituto de Medicina Molecular, UL. Sessão de Divulgação 7<sup>o</sup> Programa Quadro de ID&T, Programa People, Lisboa, Portugal.

Castanho, M. Invited panelist at the "Working with your tech transfer office" session of the "UTEN researcher workshop", Lisboa, Portugal.

Santos, S. Course of Pediatric Anesthesia for Nurses, Hospital Fernando Fonseca, Amadora.

Santos, S. Peripheral Nerve Blocks in Orthopaedic Surgery, Hospital Fernando Fonseca, Amadora.

### Organisation of National and International Conferences

Castanho, M. Chairman and organizer of the "Membrane-bioactive peptides in health and Disease" symposium. XXXVIII Annual Meeting of the Brazilian Society for Biochemistry and Molecular Biology, Águas de Lindóia, Brazil. 2009

Castanho, M. Co-chairman of the Organizing Committee I Jornadas Nacionais de Ensino e Divulgação de Bioquímica, Porto. December, 2009

### Prizes, honors and awards

2009 EBSA European Biophysics Congress Poster prize to Henri G. Franquelim.

"UNICA entrepreneurship competition for students and young researchers" – National contest award for best business plan to I. Serrano, M. Ribeiro and M. Castanho

Poster Prize at 21st American Peptide Symposium, (Bloomington, IN, USA) awarded by American Peptide Society to Sónia T. Henriques.

American Peptide Society travel grant (June 2009) to attend the congress: 21th American Peptide Symposium, Bloomington, IN, USA to Sónia T. Henriques

Grünenthal Foundation Award (basic research) - PAIN – (2008 contest edition; awarded 2009) to M. Ribeiro M. Castanho, M. et al.

Citizen of the year; Rotary Club (Santarém, Portugal) 2009 to M. Castanho.

### Patents

WO 2009/123487 A1, World Intellectual Property Organization (WIPO), International Application under the Patent Cooperation Organization (PCT) – Published 8 October 2009



Ribeiro M et al “Compounds for treating pain”

WO 2009/123487 A1, World Intellectual Property Organization (WIPO), International Application under the Patent Cooperation Organization (PCT) – Published 8 October 2009 dos Santos A et al. “Use of compounds in the treatment of tau-induced cytotoxicities”

### **Advanced Teaching**

Castanho M. “The quest for a new analgesic drug. Innovating in drug discovery”, Chemistry PhD Program, University of Aveiro, Portugal, March 11<sup>th</sup> 2009 (open seminar)

Castanho, M. Atomic Force Microscopy; “Post-graduate teaching program for first year Ph.D. students”, Ph.D program, Instituto de Tecnologia Química e Biológica, Univ. Nova de Lisboa, Portugal, 2009.

Castanho, M. “Atomic Force Microscopy” IMM Ph. D. Program – “Nanomedicine course”, Lisbon, Portugal May 4-6, 2009.

Castanho, M. “New careers, new frontiers” course organizer, IMM Ph. D. Program, Lisbon, Portugal, 2009

### **Public perception of science**

Sónia T. Henriques – IMB Science Ambassador (2009), the ambassador program at The University of Queensland, Institute for Molecular Bioscience included training in media interaction, outreach experience with schools, mentoring work experience students, tour and lectures within the IMB for visitors.

## Cell Biology of the Immune System Unit

### Description and Objectives

The general unifying goal of our research unit is to use unbiased functional genomic and genetic tools to identify and characterize human and mouse genes and signaling pathways that regulate central processes of an immune response, their targets and role in an *in vivo* immune response.

### Head of Unit

Luis Ferreira Moita, MD, PhD - Assistant Professor at FMUL

### Principal Investigators

Nina Matova, PhD

### Research Team

Maria Helena Raquel	- Post-Doc fellowship, FCT
Raquel Rodrigues	- Post-Doc fellowship, FCT
Ana Neves-Costa	- Post-Doc fellowship, FCT
Angelo Chora	- Post-Doc fellowship, FCT
Catarina Moita	- PhD fellowship, FCT
Nuno Figueiredo	- MD, PFMA
Richard Staats	- MD, HSM
Susana Moreira	- MD, HSM
Patricia Rodrigues	- Technician fellowship, FCT grant
Rui Freitas	- PhD fellowship, FCT (left in December 2009)
Pedro Alves	- PhD fellowship, FCT (left in December 2009)
Teresa Raquel Pacheco	- Assistant Professor, FMUL (left in April 2009)
Pedro Simões	- Post-Doc fellowship, FCT (left in March 2009)
Nuno Carmo	- PhD Fellowship, FCT (at FFUL)

### Research Areas

Innate Immunity  
Antigen Cross-Presentation  
Alternative Splicing  
Sleep

### Major Scientific Achievements

In collaboration with Dr. Anjana Rao's laboratory at Harvard Medical School we have recently used a short hairpin RNA interference screen to identify heterogeneous ribonucleoprotein L-like (hnRNPLL) as a critical inducible regulator of CD45 alternative splicing (Oberdoerffer et al., *Science*, 2008). Exon array analysis suggested that hnRNPLL acts as a global regulator of alternative splicing in activated T cells and that induction of hnRNPLL during hematopoietic cell activation and differentiation may allow cells to rapidly shift their transcriptomes to favor proliferation and inhibit cell death (Oberdoerffer et al., 2008).

In our own laboratory, we have concluded an shRNA-based screen to identify components of the splicing machinery that are critical regulators of the secretion of IL-1 $\beta$  both through the regulation of the NF- $\kappa$ B pathway and activation of the inflammasome (Moura-Alves, *in preparation*). Similar strategies have been used to identify critical regulators of exosome

secretion (Ostrowski et al., *Nat Cell Biol.* 2010 Jan;12(1):19-30) and antigen cross-presentation (Savina, A. et al., *Immunity.* 2009 Apr 17;30(4):544-55; Moita C. et al., *in preparation*).

#### Recent Most Relevant Publications

Ostrowski, M., Carmo, N.B., Krumeich, S., Fanget, I., Raposo, G., Savina, A., Moita, C.F., Schauer, K., Hume, A.N., Freitas, R.P., Goud, B., Benaroch, P., Hacohen, N., Fukuda, M., Desnos, C., Seabra, M.C., Darchen, F., Amigorena, S., Moita, L.F.\* and Thery, C.\* (2010) Rab27 controls constitutive exosome secretion. *Nat. Cell Biol.* 12: 19-30 \*Joint corresponding authors (Journal IF: 17.774)

Robinson, M., Osorio, F., Rosas, R., Freitas, R., Schweighoffer, E., Groß, O., Verbeek, J.S., Ruland, J., Tybulewicz, V., Brown, G.D., Moita, L.F., Taylor, P.R., and Sousa, C.R (2009) Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. *J. Exp. Med.* 206: 2037-2051 (Journal IF: 15.463)

Savina, A., Peres, A., Cebrian, I., Carmo, N., Moita, C., Hacohen, N., Moita, L.F., and Amigorena, S. (2009) Rac2 controls phagosomal alkalization and crosspresentation selectively in CD8+ dendritic cells. *Immunity* 30: 544-555 (Journal IF: 20.579)

Oberdoerffer, S., Moita, L.F., Neems, D., Freitas, R.P., Hacohen, N. and Rao, A. (2008) Regulation of CD45 alternative splicing by heterogeneous ribonucleoprotein, hnRNPLL. *Science* 321: 686-691 (Journal IF: 28.103)

Moita, L.F.\*, Wang-Sattler, R., Michel, K., Zimmermann, T., Blandin, S., Levashina, E.A. and Kafatos, F.C. (2005) *In vivo* phagocytic screen in *A. gambiae*: new players and conserved pathways of engulfment. *Immunity* 23: 65-73 \*Corresponding author (Journal IF: 20.579)

#### Ongoing Research Projects

2009/ 2011 Immunological basis for the association of hypersomnic sleep disorders and cardiovascular disease, FCT. Coordinator: L.F. Moita (PIC/IC/82991/2007)

2007/2010 Genetic dissection of innate immune processes using RNA interference, FCT. Coordinator: L.F. Moita (PTDC/SAU-MII/78333/2006)

2007/2010 Synthetic pathogens for integrated biophysical and genetic dissection of antigen presentation, FCT. Coordinator: L.F. Moita (PTDC/SAU-MII/69280/2006)

2006/2009: Synthetic pathogens for the integrated biophysical and genetic dissection of antigen presentation. Human Frontier Science Program. Coordinator: L.F. Moita (HFSP - RGY0058/2006-C)

2006/2009: Technology Transfer From American Institutions to the Institute of Molecular Medicine for the Improvement of Competitiveness and Scientific Excellence in Biomedicine in the Areas of Cancer, Infectious and Immunological Research, FLAD – Fundação Luso-Americana para o Desenvolvimento. Coordinator: L.F. Moita

#### Publications in Peer Review International Journals 2009

Savina, A., Peres, A., Cebrian, I., Carmo, N., Moita, C., Hacohen, N., Moita, L.F. and Amigorena, S. (2009) Rac2 controls phagosomal functions and crosspresentation selectively in CD8+ dendritic cells. *Immunity* 30: 544-555 (Journal IF: 20.579)

Robinson, M.J., Osorio, F., Rosas, M., Freitas, R., Schweighoffer, E., Groß, O., Verbeek, J.S., Ruland, J., Tybulewicz, V., Brown, G.D., Moita, L.F., Taylor, P.R. and Reis e Sousa, C. (2009) Dectin-2 is a Syk-coupled pattern recognition receptor crucial for Th17 responses to fungal infection. *J. Exp. Med.* 206: 2037-2051 (Journal IF: 15.612)

Ostrowski. M., Carmo, N.B., Krumeich, S., Fanget, I., Raposo, G., Savina, A., Moita, C.F., Schauer, K., Hume, A.N., Freitas, R.P., Goud ,B., Benaroch, P., Hacohen, N., Fukuda, M.,

Desnos, C., Seabra, M.C., Darchen, F., Amigorena, S. and Moita, L.F.\*, Thery, C.\* (2009) Rab27 controls constitutive exosome secretion. *Nat. Cell Biol.* 12: 19-30 \*Corresponding authors (Journal IF: 17.623)

### Communications in International Conferences

Freitas, R.P. Synthetic pathogens for integrated biophysical and genetic dissection of antigen presentation. Keystone Meeting on Dendritic Cells, Banff, Canada. March 29 – April 3, 2009 (Poster presentation)

Lanca, T. Identification of tumour-associated antigens underlying the anti-lymphoma activity of human gamma-delta t cells. 2<sup>nd</sup> European Congress of Immunology, Berlin, Germany. September 12-16, 2009 (Poster presentation)

Lanca, T. The NKG2D ligand ULBP1 determines lymphoma and leukemia cell susceptibility to human gd T cell cytotoxicity. Tri-Society Annual Conference of the International-Cytokine-Society/International-Society-of-Interferon-and-Cytokine-Research/Society-of-Leukocyte-Biology, Lisbon, Portugal. October 17-21, 2009 (Poster presentation)

Moita, C. Genetic dissection of antigen cross-presentation pathways. Keystone Meeting on Dendritic Cells, Banff, Canada. March 29 – April 3, 2009 (Poster presentation)

Moita, L.F. Genetic Dissection of antigen cross-presentation pathways. GRC on Antigen Cross-Presentation, Lucca, Italy. June 14-19, 2009 (Oral Presentation)

Moita, L.F. Synthetic pathogens for integrated biophysical and genetic dissection of antigen presentation. HFSP meeting, Tokyo, Japan. June 1-4, 2009 (Poster presentation)

Moita, L.F. Synthetic pathogens for integrated biophysical and genetic dissection of antigen presentation. GRC on Antigen Cross-Presentation, Lucca, Italy. June 14-19, 2009 (Poster Presentation)

Moura-Alves, P. M. Tuberculosis activates the inflammasome leading to processing and release of IL-1b from macrophages. Keystone Meeting on Dendritic Cells, Banff, Canada. March 29 – April 3, 2009 (Poster presentation)

Moura-Alves, P. Systematic identification of splicing factors with a role in IL-1b secretion by shRNA screening. Keystone Meeting on Dendritic Cells, Banff, Canada. March 29 – April 3, 2009 (Poster presentation)

Moura-Alves, P. Systematic Identification of splicing factors with a role in IL-1b secretion by shRNA screening. 2<sup>nd</sup> European Congress of Immunology, Berlin, Germany. September 12-16, 2009 (Poster presentation)

Neves-Costa, A. Roles of the putative epigenetic regulator TET1 in innate immune responses. Tri-Society Annual Conference of the International-Cytokine-Society/International-Society-of-Interferon-and-Cytokine-Research/Society-of-Leukocyte-Biology, Lisbon, Portugal. October 17-21, 2009 (Poster presentation)

Raquel, H. Identification of genes involved in unconventional protein secretion pathways. Tri-Society Annual Conference of the International-Cytokine-Society/International-Society-of-Interferon-and-Cytokine-Research/Society-of-Leukocyte-Biology, Lisbon, Portugal. October 17-21, 2009 (Poster presentation)

Savina, A. Rac2 controls phagosomal functions and crosspresentation selectively in CD8+ dendritic cells. GRC on Antigen Cross-Presentation, Lucca, Italy. June 14-19, 2009 (Poster presentation)

### Invited Lectures and Seminars

Moita, L.F. RNAi dissection of splicing regulation in innate and adaptive immune responses. Interdisciplinary Focus Meeting (EURASNET) on “Splicing in Cell Biology”. IMM, Lisboa,

Portugal. October 5-6, 2009

Moita L.F. RNAi dissection of splicing regulation in innate and adaptive immune responses. 5<sup>th</sup> Iberian EMBL alumni association meeting. IBMC, Porto, Portugal. October 2, 2009

Moita, L.F. Implicações Clínicas da Investigação Básica nas Doenças Pulmonares. Pneumology department, HSM, Lisboa, Portugal. May 20, 2009

### **Organisation of National and International Conferences**

Moita, L.F. organizer of Workshop Pathways of Drug Discover. Instituto de Medicina Molecular, Lisboa, Portugal. October 23, 2009

Moita, L.F. Co-organizer of Interdisciplinary Focus Meeting (EURASNET) on “Splicing in Cell Biology”. IMM, Lisboa, Portugal. October 5-6, 2009

Moita, L.F. Coordinator of IMM seminar series.

### **Advanced Teaching**

Moita, L.F. RNAi-based Dissection of Immune Response Signaling Pathways. PhD program for Medical Doctors, Faculdade de Ciências Médicas, Universidade Nova de Lisboa. October 31, 2009

Moita, L.F. The bioethical limits of research. Curso de Mestrado em Bioética (7<sup>a</sup> edição) - Módulo XII “Investigação Bioética em Serviços de Saúde” (Responsável Prof. Paulo Costa). Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal. June 25, 2009

Moita, L.F. RNAi-based Dissection of Immune Response Signaling Pathways. GABBA courses - Workshop on Gene Expression: From Genes to Systems. Faculdade de Ciências da Universidade de Lisboa, Portugal. April 15, 2009

Moita, L.F. “RNAi”, Gene Expression module of the “Doctoral Program for Physicians - PFMA” held at the Instituto de Medicina Molecular/IMM, Lisbon, October 7, 2009.

Moita, L.F. RNAi-based Dissection of Immune Response Signaling Pathways. PhD program for Medical Doctors, Faculdade de Ciências Médicas da Universidade Nova de Lisboa, Lisbon. October 31, 2009

## Cancer Biology Unit

### Description and Objectives

The Cancer Biology Unit aims to understand the cooperative role of cell-autonomous alterations and microenvironmental cues for the development of cancer, especially hematological malignancies of lymphoid origin. Our work characterizes the cellular and molecular mechanisms implicated in the acquisition of a selective advantage by malignant/leukemic cells over their normal counterparts, focusing on the dissection of signaling pathways activated by cell-intrinsic events and extra-cellular factors. To do so, we integrate different biochemical, cellular and molecular biology techniques and *in vitro* and *in vivo* models. The basic and pre-clinical research performed in the lab is translation-oriented and complemented by active ongoing collaborations with clinicians. Ultimately, our research strategy aims to identify and characterize molecular targets for the development of novel, more selective therapeutic tools against cancer.

### Head of Unit

João Taborda Barata, PhD

### Research Team

Ana Gírio, PhD	– Post-Doc fellowship, FCT
Leonor Sarmento, PhD	– Post-Doc fellowship, FCT (since September 2009)
Ana Silva	– PhD fellowship, FCT (left in August 2009)
Bruno Cardoso	– PhD fellowship, FCT
Catarina Henriques	– PhD fellowship, FCT
Leila Martins	– PhD fellowship, FCT
Nádia Correia	– PhD fellowship, FCT
Milene Silva	– Research fellowship, FCT grant
Daniel Ribeiro	– Mastet student
Marie Tazreiter	– Short-term trainee, Master student (March-June)
Priscila Zenatti	– Short-term trainee, PhD student (February-May)

### Research Areas

Oncobiology  
Signal transduction  
Cellular and molecular biology

### Major Scientific Achievements

We have shown that interleukin-7 (IL-7), a cytokine produced by bone marrow and thymic stromal cells, promotes viability and proliferation of T-cell acute lymphoblastic leukemia (T-ALL) by activating PI3K activity, which is mandatory for Bcl-2 upregulation, p27<sup>kip1</sup> downregulation, Rb hyperphosphorylation, mitochondrial homeostasis, Glut1 expression and glucose utilization (Blood 98:1524, J Exp Med 200:659).

In addition to the fact that IL-7 stimulation upregulates the activity of PI3K, we found that PI3K/Akt pathway is constitutively hyperactivated in most (>85%) primary T-ALL cases we analyzed. This appears to be mainly due to posttranslational, nondeletional inactivation of the phosphatase and tumor suppressor PTEN, which is the principal negative regulator of PI3K/Akt pathway. Overexpression and hyperactivation of the ubiquitous kinase CK2 in T-ALL cells results in phosphorylation and stabilization of PTEN with concomitant functional abrogation of PTEN phosphatase activity. Furthermore, high levels of reactive oxygen species contribute to

PTEN oxidation and consequent inactivation. These two mechanisms lead to PTEN inhibition in T-ALL cells in the absence of PTEN gene alterations that normally characterize other tumors. Interestingly, treatment with PI3K or CK2 inhibitors induced T-ALL cell death without significantly affecting normal T-cell precursors (J Clin Invest 118:3762). These data suggest that inhibition of CK2 may have therapeutic potential in T-ALL. With this goal in mind, we tested the efficacy of CK2 antagonists combined with gamma-secretase inhibitors (GSIs), which block Notch activity, in targeting T-ALL cells. Notch activating mutations are very common in T-ALL and have been reported to lead to PTEN transcriptional downregulation. Therefore, we reasoned that CK2 inhibitors and GSIs would cooperate in activating PTEN in leukemia cells. Indeed, our data demonstrated that combined inhibition of CK2 and Notch leads to cooperative effects in blocking cell proliferation and viability of T-ALL cells (Haematologica doi:10.3324/haematol.2009.011999) . Interestingly, despite the importance of PTEN posttranslational inactivation, our collaboration with Drs. Yunes and Brandalise at Centro Infantil Boldrini, Campinas, SP, Brazil, revealed that T-ALL patients harboring exon 7 PTEN mutations may be at increased risk of relapse (Leukemia doi: 10.1038/leu.2009.209).

In parallel we continued to try and understand the functional relevance of microenvironmental factors for leukemia progression and the molecular mechanisms involved. Our effort resulted in the identification of mTOR as a key molecule for IL-4-mediated growth and cell cycle progression of T-ALL cells (Leukemia 23:206-208).

Finally, we actively collaborated with other IMM Units, participating for instance in the characterization of the signaling pathways responsible for the expansion of particular subsets of normal naïve T-cells (Blood 113: 2999) or involved in the activation of  $\gamma\delta$  T-cells stimulated by phospho-antigens with capacity to promote  $\gamma\delta$  T-cell anti-tumoral effects (PLoS ONE 4:e5657).

Current projects in the lab include the dissection of IL-7-mediated pathways involved in T-cell leukemia and normal T-cell development, and the analysis of the functional and molecular effects of the overexpression of particular putative oncogenes in human hematopoietic stem/progenitor cells. We are also expanding our studies to other hematological malignancies, especially chronic lymphocytic leukemia. We continue to collaborate with other IMM Units and external groups in a number of projects related to different aspects of cancer research.

### Recent Most Relevant Publications

Silva, A. , Yunes, J.A., Cardoso, B.A. , Martins, L.R., Jotta, P.Y., Abecasis, M., Nowill, A.E., Leslie, N.R., Cardoso, A.A. and Barata, J.T. (2008) PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. J. Clin. Invest. 118: 3762-3774 (Times cited: 7)(Journal IF: 16.559)

Barata, J.T., Silva, A., Brandão, J.G., Nadler, L.M., Cardoso, A.A. and Boussiotis, V.A. (2004) Activation of PI3K is indispensable for Interleukin 7-mediated viability, proliferation, glucose use, and growth of T cell acute lymphoblastic leukemia cells. J. Exp. Med. 200: 659-669 (Times cited: 46)(Journal IF: 15.463)

Barata, J.T., Boussiotis, V.A., Yunes, J.A., Ferrando, A.A., Moreau, L.A., Veiga, J.P., Sallan, S.E., Look, A.T., Nadler, L.M. and Cardoso, A.A. (2004) IL-7-dependent human leukemia T-cell line as a valuable tool for drug discovery in T-ALL. Blood 103: 1891-1900 . (Times cited: 14)(Journal IF: 10.432)

Barata, J.T., Cardoso, A.A., Nadler, L.M. and Boussiotis, V.A. (2001) Interleukin-7 promotes survival and cell cycle progression of T-cell acute lymphoblastic leukemia cells by down-regulating the cyclin-dependent kinase inhibitor p27kip1. Blood 98: 1524-1531 (Times cited: 59)(Journal IF: 10.432)

### Ongoing Research Projects

2007/10 Overexpression of Oncogenes in Normal Human Hematopoietic Stem Cells: Impact on T-cell Leukemia, FCT. Coordinator: J.T. Barata (PTDC/SAU-OB/69974)

## 96 | 2009 IMM Activity Report

2007/2010 Causes and Prevention of Childhood Leukaemia, Children with Leukaemia Charity Grant. Coordinator: J.T. Barata

2009/2012 Regulation of ZAP-70 and PTEN activity and stability in chronic lymphocytic leukemia: prognostic and therapeutic implications, FCT. Coordinator: J.T. Barata (PIC/IC/83193)

2009/2012 Clinical implications of activation of PI3K/Akt signaling pathway in primary adult acute lymphoblastic leukemia, FCT. Coordinator: J. Lacerda (PIC/IC/83023)

### Publications in Peer Reviewed International Journals 2009

Silva, A.\*, Jotta, P.Y.\*, Silveira, A.B., Ribeiro, D., Brandalise, S.R., Yunes, J.A. and Barata, J.T. (2009) Regulation of PTEN by CK2 and Notch1 in primary T-cell acute lymphoblastic leukemia: rationale for combined use of CK2- and gamma-secretase inhibitors. *Haematologica (Epub December 16, 2009)* \* Co-first authors (Journal IF: 5.978)

Jotta, P.Y., Ganazza, M.A., Silva, A., Viana, M.B., da Silva, M.J., Zambaldi, L.J.G., Barata, J.T., Brandalise, S.R. and Yunes, J.A. (2009) Negative prognostic impact of PTEN mutation in pediatric T-cell acute lymphoblastic leukemia. *Leukemia 24: 239-242 (Epub October 15, 2009)*(Journal IF: 8.634)

Correia, D.V., d'Orey, F., Cardoso, B.A., Lança, T., Grosso, A.R., deBarros, A., Martins, L.R., Barata, J.T. and Silva-Santos, B. (2009) Highly active microbial phosphoantigen induces rapid yet sustained MEK/Erk- and PI-3K/Akt-mediated signal transduction in anti-tumor human  $\gamma\delta$  T-cells. *PLoS ONE 4: e5657* (Journal IF: NA)

Azevedo, R.I.\*, Soares, M.V.D.\*, Barata, J.T., Tendeiro, R., Serra-Caetano, A., Victorino, R.M.M. and Sousa, A.E. (2009) IL-7 sustains CD31 expression in human naive CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. *Blood 113: 2999-3007* \*Co-first authors (Journal IF: 10.432)

Cardoso, B.A.\*, Martins, L.R.\*, Santos, C.I.\*, Nadler, L.M., Boussiotis, V.A., Cardoso, A.A. and Barata, J.T. (2009) Interleukin-4 stimulates proliferation and growth of T-cell acute lymphoblastic leukemia cells by activating mTOR signaling. *Leukemia 23: 206-208* \*Co-first authors (Journal IF: 8.634)

### Communications in International Conferences

Martins, L.R. et al. Targeting CK2 Overexpression as a Novel Therapeutic Tool in Chronic Lymphocytic Leukemia. The American Society of Hematology 51st Annual Meeting, New Orleans, LA, USA. December 5-8, 2009 (Oral presentation)

Silva, A. et al. IL-7 accelerates human T-cell leukemia progression in vivo. Tri-Society Annual Conference, Cellular and Cytokine Interactions in Health and Disease, Lisbon, Portugal, October 18-21, 2009 (Poster presentation)

Silva, A. et al. Crosstalk between PI3K-Akt/PKB pathway and Intracellular Reactive Oxygen Species is Essential for IL-7-mediated Viability of Leukemia T-cells. Keystone Symposium on PI 3-Kinase Signaling in Disease (Z3), Olympic Valley, CA, USA. April 22 – 27, 2009 (Poster presentation)

### Communications in National Conferences

Silva, A. Et al. Crosstalk between PI3K-Akt/PKB pathway and Intracellular Reactive Oxygen Species is Essential for IL-7-mediated Viability of Leukemia T-cells. SINAL 2009 – 3rd National Meeting on Signal Transduction, Universidade de Aveiro, Portugal, May 29-30, 2009 (Oral presentation)

Cardoso, B.A. et al. HDAC inhibitors in T-ALL: is TAL1 important? SINAL 2009 – 3<sup>rd</sup> National



Meeting on Signal Transduction, Universidade de Aveiro, Portugal, May 29-30, 2009 (Poster presentation)

#### **Invited Lectures and Seminars**

Barata, J.T. Does IL-7/IL-7R-mediated signaling promote human T-cell leukemia? SINAL 2009 – 3rd National Meeting on Signal Transduction, Universidade de Aveiro, Portugal, May 29-30, 2009

Barata, J.T. Plenary session – Beyond the gene: PTEN inactivation in human T cell leukemia. XI Meeting of Sociedade Ibérica de Citometria (Iberian Society of Cytometry), Covilhã, Portugal. May 7-9, 2009

Barata, J.T. Inactivation of PTEN in primary T-cell acute leukemia: beyond the canons. WKZ – Wilhelmina Children's Hospital, University Medical Center, Utrecht, The Netherlands, April 16, 2009.

Barata, J.T. PTEN inactivation in primary T-cell acute leukemia: more than the canons. Max Delbruck Center for Molecular Medicine, Berlin, Germany. February 9, 2009.

#### **Organisation of National and International Conferences**

Barata, J.T. Co-Chair, Day 2 session, SINAL 2009 – 3rd National Meeting on Signal Transduction, Universidade de Aveiro, Portugal. May 29-30, 2009.

#### **Prizes, Honours and Awards**

Prémio Pulido Valente de Ciência / Pulido Valente Science Prize 2009 - Translational Molecular Oncology to Ana Silva for the work PTEN Posttranslational Inactivation and Hyperactivation of the PI3K/Akt Pathway Sustain Primary T Cell Leukemia Viability. Journal of Clinical Investigation 2008, 118: 3762.

#### **Patents**

Provisional patent application on the therapeutic use of CK2 inhibitors for treatment of chronic lymphocytic leukemia.

#### **Advanced Teaching**

Barata, J.T. Organizer and lecturer of Biology of Cancer Nanocourse. IMM PhD Programme, IMM, Lisbon, Portugal. April 27-30, 2009.

Barata, J.T. Signal Transduction Aberrations in Cancer and 'Novel' Concepts in Cancer Signal Transduction. Advanced Course on Oncobiology, BEB PhD Program of the Center for Neuroscience and Cell Biology (CNC), Universidade de Coimbra, Portugal March 17, 2009.

#### **Public Perception of Science**

Barata, J.T. Participant in the Round Table on Perspectives of Oncologic Treatment, Module I – Oncobiology, ENJOY Med meeting, IMM, Lisbon, Portugal. November 7, 2009.

## Thesis Completed

### Master Thesis

Daniel Ribeiro (2009) The role and therapeutic potential of regulators and effectors of PI3K/Akt pathway in T-cell leukemia. Faculdade de Ciências da Universidade de Lisboa. Supervisor: J.T. Barata

## Clinical and Translational Oncology Research Unit

### Description and Objectives

The Clinical and Translational Oncology Research Unit studies mechanisms of tumor invasion and progression in metastatic setting particularly in organs such as bone and liver. We aim to contribute for the identification of molecular markers representative as risk factor for metastases occurrence and possible therapeutic targets. The research will be performed mostly in human neoplastic tissue and results are to be correlated with clinical information.

We also aim to develop translational research in the area of target enzymes for antineoplastic agents, namely topoisomerase inhibitors, and contribute to gain insight into the mechanisms that modulate the cytotoxicity of drugs that target topoisomerase I and II. Within the same scope we are involved in the identification of mechanisms of resistance to biotherapy in neuroendocrine tumors and sensitivity to endocrine treatments in breast cancer.

### Head of Unit

Luis Costa, MD, PhD – Director of the Oncology Division, HSM; Auxiliary Professor, FMUL

### Research Team

Teresa Raquel Pacheco	– Auxiliary Professor, FMUL
Ana Cristina Santos	– Auxiliary Investigator, FMUL
Sandra Casimiro, PhD	– Post-Doc fellowship, FCT
Tânia Rodrigues, MD	– Oncology fellow
Joana Ribeiro, MD	– Oncology fellow
Isabel Fernandes, MD	– Oncology fellow
Inês Luís, MD	– Oncology fellow
Irina Alho	– PhD fellowship, FCT
Joana Costa	– PhD fellowship, FCT
Ricardo Pires	– Technician fellowship, FCT grant

### Research Areas

Mechanisms of invasion and progression of solid tumors in bone.

Mechanisms of invasion and progression of colo-rectal cancer in liver.

Modulation of sensitivity to antineoplastic agents such as topoisomerase inhibitors, somatostatin analogues and aromatase inhibitors.

### Major Scientific Achievements

#### Bone Metastatic Disease

NTX normalization at 3 months of bisphosphonate therapy is predictive of overall survival and skeletal related events in breast cancer with bone metastases. In contrast to NTX, ICTP is not affected by bisphosphonate therapy and is generated from non-osteoclastic origins. In this study we analyzed whether ICTP was predictive of NTX normalization at 3 months of therapy and clinical outcome. We concluded that ICTP might be a useful biomarker to predict which patients will not normalize levels of NTX with bisphosphonate therapy. Both, ICTP and NTX are significant prognostic biomarkers in breast cancer.

Both collagen fragments (NTX and ICTP) were synthesized and have been tested as potential growth factors for human cancer cell lines. This hypothesis could explain the strong prognostic factor attributed to NTX and ICTP in bone metastases patients. We presented at last CIBD the first results of these experiments.

Cancer-induced bone destruction is associated with increased osteoclastogenic activity induced by tumor cells, and an unbalanced bone remodelling cycle that favours bone osteolysis. In this process the RANK-RANKL signalling is crucial for both osteoclastogenesis and osteoclastic activity. Furthermore, breast cancer cells have been shown to also express the RANK receptor and to increase migration towards RANKL stimuli, however it is not known if RANK - RANKL interaction stimulates breast cancer cell to invade and degrade bone collagen. It is our goal to determine if RANKL increases the ability of breast cancer cells to invade a type I collagen matrix and degrade bone collagen. Our data suggest that RANKL can trigger an increase in expression of MMP1 by breast cancer cells expressing RANK, contributing to invasion via degradation of matrix proteins directly by the cancer cells.

More recently we analyzed the expression of MMP1 and DKK1 in 96 bone metastases samples. We found that MMP1 levels in serum of these patients do correlate with survival.

### **Human Tumor Sample Biobank**

In the context of bone metastatic disease and colorectal cancer ongoing projects we successfully implemented a human tumor sample biobank, resulting from a strong cooperation between IMM and HSM. The team enrolled in the collection of human tumor samples comprises clinicians (Oncologists, Pathologists, Surgeons) and life sciences researchers (Biologists, Biochemists), together committed in providing the samples necessary for the prosecution of these projects goals. A well established network, starting at the Surgery/Oncology Department and ending in the UIOC laboratory, has now been able to collect a total of 217 fresh frozen human tumor samples (175 from colorectal cancer; 25 bone metastases and 17 other sites of metastases). Our collaboration expanded to Hospital Pulido Valente.

### **Recent Most Relevant Publications**

Costa, L. and Major, P.P. (2009) Effect of bisphosphonates on pain and quality of life in patients with bone metastases. *Nat. Clin. Prac. Oncol.* 6: 163-174 (Journal IF: 9.113)

Lipton, A., Campbell-Baird, C., Harvey, H., Kim, C., Demers, L. and Costa, L. (2010) Phase I trial of Zoledronic acid + Imatinib mesylate (Gleevec) in patients with bone metastases. *Am. J. Clin. Oncol.* 33: 75-78 (Journal IF: 1.792)

Casimiro, S., Guise, T.A. and Chirgwin, J. (2009) The critical role of the bone microenvironment in cancer metastases. *Mol. Cel. Endoc.* 310: 71-81 (Times cited: 1)(Journal IF: 3.611)

Vitor, R.F., Esteves, T., Marques, F., Raposinho, P., Paulo, A., Rodrigues, S., Rueff, J., Casimiro, S., Costa L. and Santos, I. (2009) (99mTc)-Tricarbonyl complexes functionalized with anthracenyl fragments: synthesis, characterization and evaluation of their radiotoxic effects in murine melanoma cells. *Cancer Biother. Radiopharm.* 24: 1-13 (Journal IF: 1.318)

Aapro, M., Abrahamsson, P. A., Body, J. J., Coleman, R. E., Colomer, R., Costa, L., Crino, L., Dirix, L., Gnani, M., Gralow, J., Hadji, P., Hortobagyi, G. N., Jonat, W., Lipton, A., Monnier, A., Paterson, A. H. G., Rizzoli, R., Saad, F. and Thurlimann, B. (2008) Guidance on the use of bisphosphonates in solid tumours: recommendations of an international expert panel. *Ann. Oncol.* 19: 420-422 (Times cited:43)(Journal IF: 4.935)

### **Ongoing Research Projects**

2007/2010 Mechanisms of invasion and progression of cancer in bone: Role of matrix metalloproteinases, FCT. Coordinator: L. Costa (SFRH/BPD/34801/2007)

2007/2010 Molecular Markers of Bone Metastasis, NOVARTIS (Partially funded). Coordinator: L. Costa.

2009/2012 The importance of LMW-PTP in the progression of bone metastatic disease and therapeutic response, FCT. Coordinator: L. Costa (SFRH/BD/44716/2008)

2008/2010 Analysis of RANK/RANKL and MMPs in clinical samples of solid tumors bone

metastases, AMGEN. Coordinator: L. Costa

2009/2012 Prognostic Signatures in Colorectal Cancer, FCT. Coordinator: L. Costa (PIC/IC/82821/2007)

2008/2012 Stromal-epithelial interactions in rectal cancer: Role of Chemoradiotherapy- induced senescence in clinical outcome, FCT. Coordinator: L. Costa (SFRH/BD/45219/2008)

2009/2010 Metastatic neuroendocrine carcinoma: regulation of akt and 4ebp1 phosphorylation by mtor and clinical response to somatostatin analogues, Novartis (partially funded). Coordinator: L. Costa, I. Fernandes and T. Pacheco.

2009/2010 Detection of Aromatase in Breast Cancer Metastases in Humans, Astra Zeneca (partially funded). Coordinators: L. Costa, J. Ribeiro and L. Correia (Pathology Division).

2009/2010 Study of Gastric cancer FFPE HER (Human Epidermal Growth Factor Receptor) Family Dimerization products and correlation with stage and outcomes, Sanofi Aventis and Penn State University funded. Coordinators: I. Luis and L. Costa.

### Publications in Peer Review International Journals 2009

Casimiro, S., Guise, T.A. and Chirgwin, J. (2009) The critical role of the bone microenvironment in cancer metastases. *Mol. Cel. Endoc.* 310: 71-81 (Journal IF: 3.611)

Vitor, R.F., Esteves, T., Marques, F., Raposinho, P., Paulo, A., Rodrigues, S., Rueff, J., Casimiro, S., Costa L. and Santos, I. (2009) (99m)Tc-tricarbonyl complexes functionalized with anthracenyl fragments: synthesis, characterization, and evaluation of their radiotoxic effects in murine melanoma cells. *Cancer Biother. Radiopharm.* 24: 1-13 (Journal IF: 1.318)

Costa, L. and Major, P.P. (2009) Effect of bisphosphonates on pain and quality of life in patients with bone metastases. *Nat. Clin. Prac. Oncol.* 6: 163-174. (Journal IF: 9.113)

Lipton, A., Campbell-Baird, C., Harvey, H., Kim, C., Demers, L. and Costa, L. (2010) Phase I trial of Zoledronic acid + Imatinib mesylate (Gleevec) in patients with bone metastases. *Am. J. Clin. Oncol.* 33: 75-78 (*E-pub July 31, 2009*)(Journal IF: 1.792)

### Publications in Peer Review National Journals 2009

Caiado, J., Rodrigues, T., Pedro, E., Costa, L. and Barbosa, M.P. (2009) Dessensibilização a fármacos em oncologia: Experiência de um Serviço de Imunoalergologia. *Rev Port Imunoalergologia* 17: 57 – 74

### Communications in International Conferences

Casimiro, S. et al. Type I collagen telopeptides NTx and ICTP stimulate breast cancer cells MDA-231-BO2 proliferation *in vitro*". VIII International Meeting on Cancer Induced Bone Diseases, , Arlington, Washington, USA. October 28-31, 2009 (Poster presentation)

Costa, L. et al. Serum type I collagen C-Telopeptide (ICTP) predicts bone resorption level (NTX) normalization, skeletal-related events (SREs) and overall survival (OS) in breast cancer (BC) patients treated with bisphosphonates (BP). VIII International Meeting on Cancer Induced Bone Diseases, , Arlington, Washington, USA. October 28-31, 2009 (Poster presentation)

Rodrigues, T.A. et. al. Involvement of chemokine receptors CXR4, CCR7, CCR6, and CXCR5 in colorectal cancer metastasis. 2009 Gastrointestinal Cancers Symposium, San Francisco, USA. January 15-17, 2009 (Poster presentation)

Costa, L. and Guise, T. Emerging recommendations for the prevention of cancer treatment-induced bone loss in women with breast cancer. ECCO 15 and 34th ESMO Multidisciplinary Congress, Berlin, Germany. September 20-24, 2009 (Poster presentation)

Luis, I. et al. Interaction between bisphosphonates and taxanes in patients with metastatic bone

disease. ECCO 15 and 34th ESMO Multidisciplinary Congress, Berlin, Germany. September 20-24, 2009 (Poster presentation)

Luis, I. et al. Clinical characteristics of patients with lung cancer and metachronous or synchronous tumors with other localizations. ECCO 15 and 34th ESMO Multidisciplinary Congress, Berlin, Germany. September 20-24, 2009 (Poster presentation)

### **Communications in National Conferences**

Tato-Costa, J. et al. New approaches for cellular senescence detection: focusing in formalin-fixed paraffin-embedded tissues. 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon, Portugal. October 12-13, 2009 (Poster presentation)

Alho, I. et al Low molecular weight protein tyrosine phosphatase (LMW-PTP) isoforms in the regulation of metastatic bone disease". 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon, Portugal. October 12-13, 2009 (Poster presentation)

Costa, L. Papel da Supressão ovárica em doentes pré-menopausa com cancro da mama. VII Congresso Nacional de Senologia, Vila Moura. Novembro 15, 2009 (Oral presentation)

Costa, L. Best Of da Terapêutica Médica no Cancro da Mama em 2009. VII Congresso Nacional de Senologia, Vila Moura. Novembro 15, 2009 (Oral presentation)

Costa, L. Antitumoral effect of bisphosphonates in urological tumors. XI Workshop on Oncological Urology, Espinho, Portugal. May 16, 2009 (Oral presentation)

### **Invited Lectures and Seminars**

Costa, L. "Established Benefits of Bisphosphonates in Breast Cancer and Prostate Cancer", ZENITH meeting a CME-certified meeting. Seville, Spain. January 24, 2009

Costa, L. "Cancer Metastases: is bone a useful paradigm", IMM Seminar, Lisboa. July 6, 2009.

Costa, L. et al. "Cancro do Cólon após Cirurgia: risco de recidiva e opções. Discussão de dois casos e apresentação de um projecto multidisciplinar". Sessão Anatomo-Clínica do HSM e FMUL, Lisboa. Abril 16, 2009

### **Organisation of National and International Conferences**

Organizer of the Oncology Series at IMM.

### **Advanced Teaching**

Costa, L. "Invasion and Metastases" Programa de Doutoramento em Ciências da Saúde. da FMUC. Coimbra, Portugal, December 3, 2009

Costa, L. "Farmacogenética e terapêutica do cancro; novos alvos terapêuticos" Curso de farmacogenética clínica Pré jornadas de genética da Sociedade Portuguesa de Genética (SPG). FMUL, Lisboa. Abril 27, 2009.

Costa, L. "Terapêutica Hormonal do Cancro da Mama". Curso de Pós Graduação em Senologia – Universidade Lusófona, Lisboa. February 13, 2009

## Embryonic Development of Vertebrates Unit

### Description and Objectives

The design of the vertebrate body plan involves the creation of asymmetries between the left and the right side of the lateral plate mesoderm, in order to correctly position the internal organs. In addition, it also comprises the control of symmetry between the left and the right side of the presomitic mesoderm to ensure the symmetric formation of the somites and therefore the correct allocation of symmetric body structures such as the axial skeleton and skeletal muscles. We would like to understand the cellular and molecular mechanisms that control the left-right (LR) asymmetric placement of internal organs and the bilateral symmetric formation of musculoskeletal elements in vertebrates. In addition we are interested in make the bridge between the fundamental developmental processes that we have been studying with the mechanisms that have to be activated during regeneration upon severe injury.

### Head of Unit

Leonor Saúde, PhD – Invited Auxiliary Professor, FMUL

### Research Team

Susana Pascoal, PhD	- Post-Doc fellowship, FCT
Rita Fior, PhD	- Post-Doc fellowship, FCT
Susana Lopes, PhD	- Post-Doc fellowship, FCT
Raquel Lourenço	- PhD fellowship, FCT
Raquel Mendes	- PhD fellowship, FCT
Sofia Azevedo	- PhD fellowship, FCT
Patrícia Matos	- Technician fellowship, FCT grant
Joana Lima	- Master Student, FCUL
Andreia Pinto	- Technician fellowship, FCT grant (left in August 2009)

### Research Areas

Left-right asymmetry  
Somitogenesis  
Regeneration  
Ciliogenesis

### Major Scientific Achievements

#### 1. Notch signalling promotes left-right asymmetry through ciliary length control

The importance of cilia in embryonic development and adult physiology is emphasized by human ciliopathies. However, the signalling pathways behind cilia formation are poorly understood. We show that Notch signalling is a key pathway for cilia length control. In deltaD zebrafish mutants, cilia length is reduced and the expression of the ciliogenic factor foxj1a is downregulated in the Kupffer's vesicle. Conversely, when Notch signalling is hyperactivated, cilia length increases and foxj1a expression is upregulated. The short cilia found in deltaD mutants reduce the fluid flow velocity inside the Kupffer's vesicle, whereas the increase in ciliary length does not seem to affect flow velocity. Our results reveal that DeltaD links ciliary length control and fluid flow velocity with transcriptional activation of laterality genes. In addition, our deltaD mutant analysis discloses an uncoupling between gut and heart laterality. This work was submitted for publication.

#### 2. Mesogenin switches mesodermal progenitors into presomitic mesoderm cells by negative

### **feed back into the Wnt/Ntl loop and coordination of cell movement**

Somites are metameric structures that are laid down rhythmically and sequentially from the presomitic mesoderm (PSM) and will give rise to the vertebral column and skeletal muscles. The PSM is a tissue that is highly dynamic: at the same time that the anterior part is forming the somites the posterior end is growing, with new cells constantly entering from the tailbud. In order to make a complete body axis, progenitors in the tailbud region have to be maintained until the end of somitogenesis and the rate at which cells differentiate has to be controlled. It has been shown recently that maintenance of progenitors depends on the positive feedback loop between WNT and Brachyury/Ntl (Martin and Kimelman, 2008), however what controls the rate of differentiation of tailbud progenitors into PSM remains unclear. In this study we investigated the role of mesogenin (msgn) in this transition in the zebrafish embryo. Our results show that msgn has two major roles in PSM development: in the gastrula specifies the germ ring domain of future PSM and restricts the axial/adaxial domains, and later in the tailbud msgn is important for the switch from tailbud progenitors to PSM, by a negative feedback into the WNT/Ntl loop. In addition, we show that msgn is important for the internalization movement of tailbud cells into PSM, controlling in this way axis elongation and as a consequence somite size. This work is being prepared for publication.

### **3. A non-conserved role for *dmrt2* in left-right establishment in vertebrates**

We concluded the analysis of *dmrt2* homozygous mutant embryos and showed that there are no LR defects, neither at the lateral plate mesoderm nor at the presomitic mesoderm. In addition, we also found that *dmrt2* is not expressed in the mouse node and we could not find any other member of this family expressed in the murine node. We concluded that the expression of *dmrt2* has not been conserved during evolution underlying the different functions that this gene plays between the zebrafish and the mouse. This work is being prepared for publication. This work is being prepared for publication

### **Recent Most Relevant Publications**

Saúde, L., Lourenço, R., Gonçalves, A. and Palmeirim, I. (2005) *terra* is a left-right asymmetry gene required for left-right synchronization of the segmentation clock. *Nat. Cell Biol.* 7: 918-992 (Times cited: 13)(Journal IF: 17.774)

Freitas, C., Rodrigues, S., Saúde L. and Palmeirim I. (2005) Running after the clock. *Int. J. Dev. Biol.* 49: 317-322 (Times cited: 9)(Journal IF:2.830)

### **Ongoing Research Projects**

2007/2009 Identification of the direct target genes of Terra using a ChIP-on-chip approach, FCT. Coordinator: L. Saúde (PTDC/SAU-OB/71596/2006)

2007/2010 Functional study of the molecular clock during vertebrate limb development, FCT. Coordinator: L. Saúde (PTDC/SAU-OB/64628/2006)

### **Communications in International Conferences**

Mendes, R. and Saúde L. The role of N-cadherin during chick heart morphogenesis. 16th International Society of Developmental Biologists Congress, Edinburgh, UK. September 6-10, 2009 (Poster presentation)

Pascoal, S. and Saúde L. The role of cilia during pectoral fin formation. 16th International Society of Developmental Biologists Congress, Edinburgh, UK. September 6-10, 2009 (Poster presentation)

Azevedo, S. et al. Regeneration in zebrafish caudal fin: the role of telomerase. 16th International Society of Developmental Biologists Congress, Edinburgh, UK. September 6-10, 2009 (Poster presentation)



Lopes, S.S. et al. A novel role for Notch signalling in left-right determination through ciliary length control. 16th International Society of Developmental Biologists Congress, Edinburgh, UK. September 6-10, 2009 (Poster presentation)

Lourenço, R. et al. The role of Terra in vertebrates. 6th European Zebrafish Genetics and Development Meeting, Rome, Italy. July 15-19, 2009 (Poster presentation)

Fior, R. et al. The role of mesogenin in mesoderm formation. LRI Annual Symposium 2009, London, UK. June 17-19, 2009 (Poster presentation)

Lopes, S.S. et al. Notch signalling promotes left-right asymmetry by controlling cilia size in zebrafish. Biophysical Mechanisms of Development – IGC Workshop Series, Oeiras, Portugal. May 26-27, 2009 (Poster presentation)

Pascoal, S. and Saúde, L. The role of Notch signalling pathway in regeneration. Interdisciplinary Approaches in Fish Skeletal Biology, Tavira, Portugal. April 27-29, 2009 (Poster presentation)

### **Invited Lectures and Seminars**

Saúde, L. Symmetry versus Asymmetry decisions in the vertebrate embryo. Centro Andaluz de Biología del Desarrollo (27 Feb 2009), Seville, Spain

### **Advanced Teaching**

Fior, R. "Notch talking" PhD Programme in Integrative Biomedical Sciences, Instituto Gulbenkian de Ciência, Oeiras. December 2, 2009

Lopes, S. "Left-right determination in vertebrates" PhD Programme in Integrative Biomedical Sciences, Instituto Gulbenkian de Ciência, Oeiras. December 2, 2009

Pascoal, S. "Cilia in development and disease" PhD Programme in Integrative Biomedical Sciences, Instituto Gulbenkian de Ciência, Oeiras. December 2, 2009

Saúde, L. "Cell synchronization in development" PhD Programme in Integrative Biomedical Sciences, Instituto Gulbenkian de Ciência, Oeiras. December 2, 2009

Saúde, L. "How to prepare an original and competitive research proposal" Programa Gulbenkian de Formação Médica Avançada. IMM, Lisbon. October 15, 2009

Saúde, L. "Left-right determination in vertebrates" GAABA PhD Program. CADBD, Seville, Spain. February 27, 2009

## Gene Expression & Biophysics Unit

### Description and Objectives

In biology several important processes occur at spatial dimensions currently beyond the reaches of conventional light microscopy. Our laboratory is concerned with biological questions at this scale as they are related to gene expression. As such the study of RNA transcription, metabolism and transport are active areas of study in the lab, as well as the development and innovation of technology to study these problems. Dynamic multi-molecular complexes in the eukaryotic cell nucleus remain well outside the resolution of most optical microscopy techniques. The direct observation of these events at the single molecule level, is currently the subject of intense research. Intrinsic to RNA transcription are modifications to the nuclear architecture and the dynamic repositioning of chromosomal loci, as well as the interplay of several ribonucleic proteins (RNPs). These events remain opaque at the single RNA transcript level, and questions such as the timing and spatial position of interactions of these RNPs with RNA, the number of genes targeted in the transcriptional process at a single spatial location, the splicing and the export of RNA, remain unresolved. Further how the stochastic nature gene expression is affected by nuclear architecture are also important questions we are interested in our lab. Understanding and visualizing these processes has implications not only for our understanding of basic biology, but extends to mechanisms in development and disease.

### Head of Unit

Musa M. Mhlanga, Ph.D from New York University School of Medicine

### Research Team

Ricardo Henriques	- PhD fellowship, FCT
Catarina Barata	- Technitian (left in December)

### Research Areas

RNA transport  
Super-resolution  
Nuclear Architecture,  
Gene Expression

### Major Scientific Achievements

My research has focused on harnessing the power of nucleic acids to understand the most fundamental processes in cell biology. In 1996, at the beginning of my graduate school career (at The Rockefeller University and then at New York University School of Medicine), molecular beacons had just been developed in the laboratory of Fred R. Kramer and Sanjay Tyagi. Nucleic acid probes possess extraordinary abilities in their capability to identify their complements. I took advantage of this property by developing in vitro methods used to investigate gene organization and function and in the diagnosis of disease. This led to my development of the now routine technique of spectral genotyping using real-time PCR and molecular beacons. This work was subsequently published in *Science* and *Methods*, and is highly cited.

Nucleic acid probes, such as molecular beacons that fluoresce upon hybridization to their nucleic acid complements, offer great promise in being able to visualize nucleic acid dynamics in living cells. Indeed in vivo techniques to investigate nucleic acid metabolism, distribution and function have thus far been the exclusive domain of protein biology. In order to fully grasp the diverse roles nucleic acids play in living cells it is critical to be able to track and image the distribution

of DNA and RNA molecules in their living contexts; as green fluorescent protein and its variants that fluoresce in diverse colors allow us to image the dynamics of proteins. This was an extremely difficult goal to achieve and I spent four years of my graduate work overcoming numerous obstacles to be able to use molecular beacons to follow native mRNA in living cells. Working with fellow graduate student Diana Bratu, we were able to show the dynamics of oskar mRNA in the living *Drosophila* oocyte. In addition, I also developed a separate technique to be able to image gene expression and nuclear cytoplasmic transport in mammalian cells by exploiting the tRNA export pathway. These novel developments were published in the journals PNAS, Nucleic Acid Research and Nature Protocols.

First as an NSF post-doctoral fellow and now as a Research Associate in the laboratory of Dr. Ulf Nehrbass at the Institut Pasteur, I have put the ability to follow gene expression in real-time at the center of approaches in understanding how RNA is transported and transcribed. Extending my previous work on imaging oskar mRNA in *Drosophila* I have sought to understand the roles of nuclear processing and cytoplasmic events in the transport and localization of oskar. Efficient mRNA transport in eukaryotes requires highly choreographed relationships between nuclear and cytoplasmic proteins. For oskar mRNA, the *Drosophila* posterior determinant, these spatio-temporal requirements remain opaque during its multi-step transport process. I have been able to covisualize oskar mRNA with Staufen protein *in vivo*, for the first time. I have found oskar to be present in particles distinct from Staufen throughout its transport, contrary to current models. I have also shown the role of a nuclear protein in remodeling and aggregating oskar in the nucleus. This process appears to be necessary for long-range transport of mRNA. An important part of this study is the development and use of image analysis techniques to be able to understand the biophysical processes involved in mRNA/protein transport. With the help of physicists and mathematicians I have developed image analysis algorithms for this purpose as well as to determine levels of interaction between RNA and proteins. In a related project, I have also collaborated with the group of Dr. Philippe Sansonetti to develop systems/synthetic biology approaches to visualize the expression of host immune genes concurrent with bacterial infection. These approaches will form the basis for future scientific direction in my work. I have attached a short confidential summary of this work. Further we are using this as a platform for visual high-throughput (high content) screening here at the Institut Pasteur and at our sister institute Institut Pasteur-Korea to screen libraries of compounds. We are currently screening for compounds that target *Plasmodium falciparum* (malaria), HIV, tuberculosis and various cancers. This work is also the subject of intellectual property filings and submissions.

#### Recent Most Relevant Publications

Henriques, R. and Mhlanga, M.M. (2009) PALM and STORM: What hides beyond the Rayleigh limit? *Biotechnol. J.* 4: 846-857 (Times cited: 3)(Journal IF: NA)

Mhlanga, M.M., Bratu, D.P., Genovesio, A., Rybarska, A., Chenouard, N., Nehrbass, U. and Olivo-Marin, J.C., (2009) *In Vivo* Colocalisation of oskar mRNA and Trans-Acting Proteins Revealed by Quantitative Imaging of the *Drosophila* Oocyte *PLoS One* 4: e6241 (Times cited: 1)(Journal IF: NA)

Cabal, G., Enninga, J. and Mhlanga, M.M. (2007) Single molecule tracking of mRNA and proteins, in 'Imaging Cellular and Molecular Biological Function' (Springer)

Mhlanga, M.M., Vargas, D.Y., Fung, C.W., Kramer, F.R. and Tyagi, S. (2005) tRNA-linked molecular beacons for imaging mRNAs in the cytoplasm of living cells. *Nucleic Acids Res.* 33: 1902-1912 (Times cited: 48)(Journal IF: 6.878)

Bratu, D.P., Cha, B.J., Mhlanga, M.M., Kramer, F.R. and Tyagi, S. (2003) Visualizing the distribution and transport of mRNAs in living cells. *Proc. Nat. Acad. Sci. USA* 100: 13308-13313 (Times cited: 120)(Journal IF: 9.380)

### Ongoing Research Projects

2008/2012 Development of Super-resolution microscopy hardware and software tools for use in PALM/STORM. Andor. Coordinator: M Mhlanga

### Publications in Peer Review International Journals 2009

Henriques, R., Mhlanga, M.M. (2009) PALM and STORM: What hides beyond the Rayleigh limit? *Biotechnol. J.* 4: 846 – 857 (Times cited: 3)(Journal IF: NA)

Mhlanga, M.M., Bratu, D.P., Genovesio, A., Rybarska, A., Chenouard, N., Nehrbass, U. and Olivo-Marin, J.C. (2009) “In vivo” colocalisation of oskar mRNA and trans-acting proteins revealed by quantitative imaging of the *Drosophila* oocyte *PLOSOne* 4: e6241 (Times cited: 1)(Journal IF: NA)

### Books/Book Chapters

Barreiro, L.B., Henriques, R. and Mhlanga, M.M. (2009) High-throughput SNP Genotyping: Combining Tag SNPs and Molecular Beacons, *in* *Methods in Molecular Biology, Single Nucleotide Polymorphisms* Komar, A.A. (ed). Humana/Springer

### Invited Lectures and Seminars (2009)

Mhlanga, M.M. Biotechnology Conference, Dailan, China.

Mhlanga, M.M. Beijing Genomics Institute, Beijing, China.

Mhlanga, M.M. International Genomics Conference, Shenzhen, China.

Mhlanga, M.M. ICS-UNIDO, Trieste, Italy.

Mhlanga, M.M. Witswatersrand Univ, Johannesburg South Africa.

Mhlanga, M.M. Univ. of KZN, Durban, South Africa.

Mhlanga, M.M. Johns Hopkins Univ. Baltimore MD, USA.

Mhlanga, M.M. Public Health Research Institute, Newark, NJ USA.

### Patents

PCT/EP2008/062204 WO2009/065635 A1 Single cell based reporter assay to monitor gene expression patterns with high spatio-temporal resolution. Applicant: Institut Pasteur [FR/FR]; Inventors: Mhlanga, M. [US/FR]; Enninga, J. [DE/FR]; Sansonetti, P. [FR/FR]; Nehrbass, U. [DE/FR].

### Advanced Teaching

Mhlanga, M. "PALM/STORM imaging" EMBO course on Light Microscopy in Living Cells, Instituto Gulbenkian de Ciências, Oeiras. June 14-22, 2009.

Mhlanga, M. Organized and lectured at the microscopy course “Innovative Microscopy to Understand Fundamental Biology” held at CSIR, Pretoria, South Africa. May 16-22, 2009.

## Immunology & Infectious Diseases Programme

**Programme Coordinators:** Mário Ramirez, José Moniz Pereira, Rui Victorino

### **Heads of Unit:**

Ana Espada de Sousa, MD, PhD

João Eurico da Fonseca, MD, PhD

João Pedro Simas, DVM, PhD

Mário Ramirez, PhD

Maria Mota, PhD

Luis Graca, MD, PhD

Bruno Silva-Santos, PhD

Maria Ermelinda Camilo, MD, PhD

Henrique Veiga-Fernandes, PhD

Gunnar Rudolf Mair, PhD

Luísa Figueiredo, PhD

Elsa Anes, PhD, FFUL

João Gonçalves, PhD, FFUL

### **Principal Investigators:**

Ana Espada de Sousa, Rui Victorino, M<sup>a</sup>.  
Conceição Santos, João Lacerda

João Eurico da Fonseca, Helena Canhão

João Pedro Simas

Mário Ramirez, José Melo Cristino, Thomas  
Hänscheid, João Carriço

Maria Mota, Miguel Prudêncio

Luis Graca

Bruno Silva-Santos

Maria Ermelinda Camilo, Helena Cortez Pinto,  
Isabel Monteiro-Grillo, Paula Ravasco

Henrique Veiga-Fernandes

Gunnar Rudolf Mair

Luísa Figueiredo, PhD

Elsa Anes

João Gonçalves, Inês Soeiro



## Clinical Immunology Unit

### Description and Objectives

The Clinical Immunology Unit (UIC)'s research focuses on human T cell homeostasis and immune regulation with the ultimate goal of identifying new strategies for immunological reconstitution and targets for immune-based therapies.

An important part of UIC's research effort is centred on HIV/AIDS immunopathogenesis mainly through the study of HIV-2, a naturally attenuated form of HIV infection.

UIC prioritizes the "bedside to the bench" approach and, given the transversal nature of Clinical Immunology, allows for the association of physician/clinical researchers from different medical specialties in parallel with basic researchers.

### Head of Unit

Ana Espada de Sousa, MD, PhD – Researcher, FMUL

### Principal Investigators

Rui M. M. Victorino, MD, PhD. – Professor of Medicine and Clinical Immunology, FMUL and Director of the Medicine 2 Department, HSM

M. Conceição Santos, Pharm.D., Ph.D. – Researcher, FMUL

João Lacerda, MD, PhD – Associate Professor, FMUL, Consultant Heamatologist, HSM

Íris Caramalho, BSc, PhD – Staff Scientist; IMM

Maria Soares, BSc, PhD – Staff Scientist, IMM (affiliated to Flow Cytometry Unit)

### Research Team

Russell B. Foxall, B.Sc., MSc, PhD – Post-Doc Fellowship, FCT

Ana Isabel Lopes, MD, PhD – Assistant Professor, FMUL, Consultant Paediatrician, HSM

Francisca Fontes, MD, PhD – Assistant Professor, FMUL, Consultant Internist, HSM

Carlos Ferreira, MD, PhD – Assistant Professor, FMUL, Consultant Internist, HSM

Susana Lopes da Silva, MD, MSc – Immunoallergologist, HSM

José Gonçalo Marques, MD – Consultant Paediatrician, HSM, Lecturer, FMUL,

Helena Nunes Cabaço, BSc – PhD Fellowship, FCT

Rui S. Soares, BSc, MSc – PhD Fellowship, FCT

Adriana S. Albuquerque, MSc – PhD Fellowship, FCT

Rita Azevedo, BSc – PhD Fellowship, FCT (currently at Imperial College, London)

Rita Tendeiro, BSc – PhD Fellowship, FCT

Ana Rita Barbosa, BSc – PhD Fellowship, FCT

Rita Cavaleiro, BSc, MSc, PhD – PhD Fellowship, FCT (left in November)

Paula Matoso, BSc, MSc – Research Fellowship, FCT

Maria Fátima Duarte, MD – Resident in Immunoallergology, HSM (left in February)

Maria Isabel Mascarenhas, MD – Resident in Immunoallergology, HSM (left in August)

Sofia Pinto Luz, MD – Resident in Immunoallergology, HSM (since April)

Maria João Paes, MD – Resident in Immunoallergology, HPV (since September)

Joana Vaz – Graduate Student in Microbiology, FMUL (left in June)

## Research Areas

Human T cell homeostasis  
 Immune regulation  
 HIV/AIDS immunopathogenesis, particularly through the study of HIV-2 infection.  
 Primary immunodeficiencies  
 Immunological reconstitution.

## Major Scientific Achievements

### ***Major Depletion of Plasmacytoid Dendritic Cells in HIV-2 Infection, an Attenuated Form of HIV Disease***

Plasmacytoid dendritic cells (pDC) provide an important link between innate and acquired immunity, mediating their action mainly through IFN- $\alpha$  production. pDC suppress HIV-1 replication, but there is increasing evidence suggesting they may also contribute to the increased levels of cell-apoptosis and pan-immune activation associated with disease progression. In spite of having a clinical spectrum similar to that of HIV-1, HIV-2 infection is characterized by a strikingly lower viremia and a much slower rate of CD4 decline and AIDS progression, irrespective of disease stage. We reported a similar marked reduction in circulating pDC levels in untreated HIV-1 and HIV-2 infections in association with CD4 depletion and T cell activation, in spite of the undetectable viremia found in the majority of HIV-2 patients. Moreover, the same overexpression of CD86 and PD-L1 on circulating pDC was found in both infections irrespective of disease stage or viremia status. Our observation that pDC depletion occurs in HIV-2 infected patients with undetectable viremia indicates that mechanisms other than direct viral infection determine the pDC depletion during persistent infections. However, viremia was associated with an impairment of IFN- $\alpha$  production on a per pDC basis upon TLR9 stimulation. These data support the possibility that diminished function in vitro may relate to prior activation by HIV virions in vivo, in agreement with our finding of higher expression levels of the IFN- $\alpha$  inducible gene, MxA, in HIV-1 than in HIV-2 individuals. Importantly, serum IFN- $\alpha$  levels were not elevated in HIV-2 infected individuals. In conclusion, our data in this unique natural model of “attenuated” HIV immunodeficiency contributed to the understanding of pDC biology in HIV/AIDS pathogenesis, showing that in the absence of detectable viremia a major depletion of circulating pDC in association with a relatively preserved IFN- $\alpha$  production does occur.

### ***IL-7 Sustains CD31 Expression in Human Naïve CD4+ T Cells and Preferentially Expands the CD31+ Subset in a PI3K-Dependent Manner.***

The CD31+ subset of human naïve CD4+ T cells is thought to contain the population of cells that have recently emigrated from the thymus, whilst their CD31- counterparts have been proposed to originate from CD31+ after homeostatic cell division. Naïve T cell maintenance is known to involve homeostatic cytokines such as IL-7. However, the role that this cytokine has in the homeostasis of naïve CD4+ T cell subsets defined by CD31 expression has not been previously investigated. We provided evidence that IL-7 exerts a preferential proliferative effect on CD31+ naïve CD4+ T cells from adult peripheral blood as compared to the CD31- subset. IL-7-driven proliferation did not result in loss of CD31 expression, suggesting that CD31+ naïve CD4+ T cells can undergo cytokine-driven homeostatic proliferation whilst preserving CD31 expression. Furthermore, IL-7 sustained or increased CD31 expression even in non-proliferating cells. Both proliferation and CD31 maintenance were dependent on the activation of phosphoinositide 3-kinase (PI3K) signalling. Taken together, our data suggest that during adulthood, CD31+ naïve CD4+ T cells are maintained by IL-7 and that IL-7-based therapies may exert a preferential effect on this population.

### ***Foxp3 induction in human and murine thymus precedes the CD4+ CD8+ stage but requires early T cell receptor expression.***

The thymus generates a T cell lineage dedicated to immune regulation, “naturally-occurring” regulatory T cells, best specified by the forkhead family transcription factor Foxp3. We conducted a parallel study in humans and mice where we dissected the earliest stages of Foxp3



induction during thymocyte development. By analyzing a large collection of 21 human thymuses we show that Foxp3 can be consistently detected in CD4 immature single positive thymocytes that precede the CD4(+)CD8(+) (double positive, DP) stage. The reduced levels of CD3 expression found at this stage of human thymocyte development raise the question of TCR requirement for Foxp3 induction. We further show that, in mice, Foxp3 expression was also detected in pre-DP thymocytes of TCR $\alpha$ -sufficient but not in TCR $\alpha$ -deficient animals, genetically demonstrating the TCR-dependence of Foxp3 expression at pre-DP stages of T cell development.

#### Recent Most Relevant Publications

Cavaleiro, R., Baptista, A.P., Soares, R.S., Tendeiro, R., Foxall, R.B., Gomes, P., Victorino, R.M. and Sousa, A.E. (2009) Major depletion of plasmacytoid dendritic cells in HIV-2 infection, an attenuated form of HIV disease. *PLoS Pathog.* 5: e1000667 (Journal IF: 9.125)

Azevedo, R.I., Soares, M.V., Barata, J.T., Tendeiro, R., Serra-Caetano, A., Victorino, R.M. and Sousa, A.E. (2009) IL-7 sustains CD31 expression in human naive CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. *Blood* 113: 2999-3007 (Journal IF: 10.432)

Albuquerque, A.S., Cortesão, C.S., Foxall, R.B., Soares, R.S., Victorino, R.M. and Sousa, A.E. (2007) Rate of increase in circulating IL-7 and loss of IL-7R $\alpha$  expression differ in HIV-1 and HIV-2 infections: two lymphopenic diseases with similar hyper immune-activation but distinct outcomes. *J. Immunol.* 178: 3252-3259 (Times cited: 16)(Journal IF: 6.000)

Sousa, A.E., Carneiro, J., Meier-Schellersheim, M., Grossman, Z., and Victorino, R.M. (2002) CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J. Immunol.* 169: 3400-3406 (Times cited: 175)(Journal IF: 6.000)

Grossman, Z., Meier-Schellersheim, M., Sousa, A.E., Victorino, R.M. and Paul W.E. (2002) CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat. Med.* 8: 319-321 (Times cited: 224)(Journal IF: 27.553)

#### Ongoing Research Projects

2007/2010 HIV-2 infection and the thymus, FCT. Coordinator: A.E. Sousa (PTDC/SAU-MII/66248/2006)

2007/2010 HIV/AIDS Program IMM-FCG, Fundação Calouste Gulbenkian. Coordinator: A.E. Sousa

2008/2010 Cellular and Molecular Response of HIV-2 and HIV-1 Infection to Integration Modulation, Merck & Co., Inc.. Coordinator: A.E. Sousa

2008/2011 Immune Reconstitution after Unrelated and Haplotype-Mismatched Stem Cell Transplantation, FCT. Coordinator: M. Soares (PTDC/SAU-MII/67662/2006)

2009/2011 Long-term impact of thymectomy performed in early infancy, FCT. Coordinator: R.M.M. Victorino (PIC/IC/83068/2007)

2009/2012 Clinical implications of activation of PI3K/Akt signaling pathway in primary adult acute lymphoblastic leukemia, FCT. Coordinator: João F. Lacerda (PIC/IC/83023/2007)

2009/2012 Lymphoid Tissues in HIV-2 Infected patients, FCT. Coordinator: Ana E Sousa (PIC/IC/82712/2007)

#### Publications in Peer Review International Journals 2009

Azevedo, R.I.\*, Soares, M.V.\*, Barata, J.T., Tendeiro, R., Serra-Caetano, A., Victorino, R.M.,

## 114 | 2009 IMM Activity Report

Sousa, A.E. (2009) IL-7 sustains CD31 expression in human naïve CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. *Blood* 113: 2999-3007 \*joint first authors (Journal IF: 10.9)

Cavaleiro, R., Baptista, A.P., Foxall, R.B., Victorino, M. and Sousa, A.E. (2009) Dendritic cell differentiation and maturation in the presence of HIV type 2 envelope. *AIDS Res. Hum Retroviruses* 25: 425-431 (Journal IF: 2.024)

Cavaleiro, R., Baptista, A.P., Soares, R.S., Tendeiro, R., Foxall, R.B., Gomes, P., Victorino, M. and Sousa, A.E. (2009) Major Depletion of Plasmacytoid Dendritic Cells in HIV-2 Infection, an Attenuated Form of HIV Disease. *PLoS Pathog* 5: e1000667 (Journal IF: 9.125)

Gonçalves-Sousa, N., Ribot, J.C., deBarros, A., Correia, D.V., Caramalho, I. and Silva-Santos B. (2010) Inhibition of murine gammadelta lymphocyte expansion and effector function by regulatory alphabeta T cells is cell-contact-dependent and sensitive to GITR modulation. *Eur. J. Immunol.* 40: 61-70 (*Epub October 28, 2009*)(Journal IF: 4.865)

Nunes-Cabaço, H., Ribot, J.C., Caramalho, I., Serra-Caetano, A., Silva-Santos, B. and Sousa, A.E. (2009) Foxp3 induction in human and murine thymus precedes the CD4+ CD8+ stage but requires early TCRalpha expression. *Immunol. Cell Biol.* *In press* (Journal IF: 3.859)

Rodrigues-Alves, R., Lopes, A., Pereira-Santos, M.C., Lopes-Silva, S., Spínola-Santos, A., Costa, C., Branco-Ferreira, M., Lidholm, J. and Pereira-Barbosa, M. (2009). Clinical, Anamnestic and Serological Features of Peach Allergy in Portugal. *Int. Arch. Allergy Immunol.* 149: 65-73 (Journal IF: 2.160)

### Publications in Peer Review National Journals 2009

Pedro, E. and Pereira Santos, M.C. (2009) Controvérsias em Imunoalergologia. Venenos, alergia sem IgE. *Revista Portuguesa de Imunoalergologia*, XVII (1):7-11.

Sokolova, A., Costa, A.C., Pereira Barbosa, M., Trindade, J.C., Bento, L., Pereira Santos, M.C. (2009) Microarray-immuno solid-phase allergen chip na avaliação do perfil de sensibilização às proteínas do leite de vaca. *Revista Portuguesa de Imunoalergologia*, XVII (4):325-342.

### Books/Book Chapters

Victorino R.M.M. (2009) A Investigação no Hospital, *in* Governação dos Hospitais. Campos, L., Borges M. and Portugal, R. (eds). Ministério da Saúde. Casa das Letras.

### Communications in International Conferences

Albuquerque, A.S. et al. Immunological reconstitution following thymic transplantation of a nude/SCID patient. European Society for Immunodeficiencies (ESID) Summer School, Slovenia. September 2-6, 2009 (Oral presentation)

Azevedo, R.I. et al. Il-7 Sustains CD31 Expression in Human Naïve CD4+ T Cells and Preferentially Expands the CD31+ Subset in a PI3K-Dependent Manner. 2nd European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Barbosa, R. et al. Common variable immunodeficiency: relationship between IL-17 producing CD4 T cells and the imbalances of the B- and T-cell populations. 2nd European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Costa, A.C. et al. Development of fish tolerance: changes in sensitization profile identified by immunoblotting. XXVIII Congress of the European Academy of Allergy and Clinical Immunology, Warsaw, Poland. June 2009 (Oral presentation)

Costa, A.C. et al. The use of recombinant fish parvalbumins (rCypc1, rGadc1) in the diagnosis and prognosis of fish allergic patients. XXVIII Congress of the European Academy of Allergy and Clinical Immunology, Warsaw, Poland. June 2009 (Oral presentation)

Ferreira, C. et al. Autoimmune Lymphoproliferative Syndrome: a rare cause of splenomegaly. XVIII Porto Cancer Meeting - 1st Joint Meeting Bordeaux-Porto, Portugal, April 2009 (Poster presentation)

Foxall, R.B. et al. Frequency of cycling regulatory T cells in two AIDS-associated diseases with distinct clinical outcomes: HIV-1 and HIV-2 infections. 2nd European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Geraldo Dias, J. et al. Comparative study of diagnostic methods for allergic disease in adults. XXVIII Congress of the European Academy of Allergy and Clinical Immunology, Warsaw, Poland. June 2009 (Poster and oral presentation)

Luz, S. et al. Walnut and or peanut allergy. XXVIII Congress of the European Academy of Allergy and Clinical Immunology, Warsaw, Poland. June 2009 (Poster and oral presentation)

Pereira da Silva, S. et al. Clinical characterisation and EUROclass classification in a common variable immunodeficiency cohort. XXVIII Congress of the European Academy of Allergy and Clinical Immunology, Warsaw, Poland. June 2009 (Poster and oral presentation).

Soares, R. et al. Cell-associated viral burden in HIV-2 versus HIV-1 immunodeficiencies. Fourth International Workshop on HIV Persistence during Therapy, St. Martin, West Indies. December 8-11, 2009 (Poster presentation)

Tendeiro, R. et al. Evidence for increased replicative rate of the naïve CD4+ T cell pool during HIV-2 infection, a naturally occurring attenuated form of HIV disease. 2nd European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

#### Communications in National Conferences

Albuquerque, A.S. et al. Foxp3+ CD4 T cells in human nude SCID, FOXP1 deficiency: overrepresentation at presentation and kinetics of recovery following HLA mismatched thymic transplantation. XXXV Reunião Anual da Sociedade Portuguesa de Imunologia. Lisboa. September 28-30, 2009 (Oral presentation)

Albuquerque, A.S. Foxp3+ CD4 T cells in human nude SCID, FOXP1 deficiency: overrepresentation at presentation and kinetics of recovery following HLA mismatched thymic transplantation. 3rd IMM PhD Students Meeting, Lisbon. October 12-13, 2009 (Oral presentation)

Barbosa, R. et al. Common variable immunodeficiency: relationship between IL-17 producing CD4 T cells and the imbalances of the B- and T-cell populations. 3rd IMM PhD Students Meeting, Lisbon. October 12-13, 2009 (Poster presentation)

Barbosa, R. et al. Common variable immunodeficiency: relationship between IL-17 producing CD4 T cells and the imbalances of the B- and T-cell populations. XXXV Reunião Anual da Sociedade Portuguesa de Imunologia. Lisboa. September 28-30, 2009 (Poster presentation)

Caramalho, I. et al. Deciphering human thymic regulatory T cell development. XXXV Reunião Anual da Sociedade Portuguesa de Imunologia. Lisboa. September 28-30, 2009 (Poster presentation)

Costa, A.C. et al. Aquisição de tolerância às proteínas do peixe: alteração do padrão de sensibilização identificado por “immunoblotting”. Portuguese Society for Immunoallergology and Clinical Immunology XXX Annual Meeting, Albufeira. October 1-4, 2009 (Oral presentation)

Costa, A.C. et al. Caracterização clínico-laboratorial de doentes com urticária crónica autoimune. Portuguese Society for Immunoallergology and Clinical Immunology XXX Annual Meeting, Albufeira. October 1-4, 2009 (Oral presentation)

Costa, A.C. et al. Diagnóstico de urticária crónica autoimune- contribuição do Teste de Activação dos Basófilos e avaliação por citometria de fluxo. Portuguese Society for Immunoallergology and Clinical Immunology XXX Annual Meeting, Albufeira. October 1-4,

2009 (Oral presentation)

Foxall, R.B. et al. Frequency of cycling regulatory T cells in two AIDS-associated diseases with distinct clinical outcomes: HIV-1 and HIV-2 infections. XXXV Reunião Anual da Sociedade Portuguesa de Imunologia. Lisboa. September 28-30, 2009 (Poster presentation)

Luz, S. et al. Alergia a noz e/ou amendoim- padrões clinico-laboratoriais. Portuguese Society for Immunoallergology and Clinical Immunology XXX Annual Meeting, Albufeira. October 1-4, 2009 (Poster presentation)

Mascarenhas, I. et al. Reacção imediata a anestésicos locais: contribuição diagnóstica dos métodos “in vitro”. Portuguese Society for Immunoallergology and Clinical Immunology XXX Annual Meeting, Albufeira. October 1-4, 2009 (Oral presentation)

Silva, S.L. et al. Autoimmune lymphoproliferative syndrome diagnosed after splenectomy due to refractory thrombocytopenia: Immunological characterization of a family with ALPS due to a Fas mutation. 1º Congresso Nacional de Auto-imunidade / XV Reunião Anual de NEDAI, Torres Vedras. April 23-25, 2009 (Poster presentation)

Silva, S.L. et al. Autoimunidade na Imunodeficiência Comum Variável. 1º Congresso Nacional de Auto-imunidade / XV Reunião Anual de NEDAI, Torres Vedras. April 23-25, 2009 (Oral presentation)

Silva, S.L. et al. Severe autoimmune manifestations in association with major T-Cell disturbances: A diagnostic and treatment challenge. 1º Congresso Nacional de Auto-imunidade / XV Reunião Anual de NEDAI, Torres Vedras. April 23-25, 2009 (Oral presentation)

Tendeiro, R. Evidence for increased replicative rate of the naïve CD4+ T cell pool during HIV-2 infection, a naturally occurring attenuated form of HIV disease. XXXV Reunião Anual da Sociedade Portuguesa de Imunologia. Lisboa. September 28-30, 2009 (Poster presentation)

Tendeiro, R. et al. Evidence for increased replicative rate of the naïve CD4+ T-cell pool during HIV-2 infection, a naturally occurring attenuated form of HIV disease. 3rd IMM PhD Students Meeting, Lisbon. October 12-13, 2009 (Oral presentation)

### Invited Lectures and Seminars

Caramalho, I. Regulatory T cells mediate diabetes protection in LPS-treated NOD mice. I Meeting of the “Núcleo de Doenças Auto-imunes da Clínicas Universitárias das Medicinas I e II”. HSM, FMUL, IMM, Lisboa, Portugal. October 23-24, 2009.

Caramalho, I. Regulatory T cells mediate diabetes protection in LPS-treated NOD mice. I National Congress on Autoimmunity. Torres Vedras, Portugal. April 24-25, 2009.

Cavaleiro, R. Dendritic cells in HIV-1 and HIV-2 infections. XI Congresso da Sociedade Ibérica de Citometria, Covilhã, Portugal. May 7-9, 2009.

Foxall, R. CD25 Expression in HIV infection “more than Tregs”. XI Congresso da Sociedade Ibérica de Citometria, Covilhã, Portugal. May 7-9, 2009.

Pereira Santos, M.C. Alergénios recombinantes-vantagens e limitações. Reunião da Primavera da Sociedade Portuguesa de Alergologia e Imunologia Clínica, Viseu, Portugal. March 28, 2009.

Pereira Santos, M.C. Inflamação, alergia e vírus. XVI Jornadas de Pediatria–Clínica Universitária do HSM, Lisboa, Portugal, November 11-14, 2009.

Pereira Santos, M.C. Reacções adversas a fármacos-Teste de activação de basófilos: contribuição no diagnóstico. Simpósio: Actualidades em Imunoalergologia - Sociedade Portuguesa de Alergologia e Imunologia Clínica, Setúbal, Portugal. May 15-16, 2009.

Silva, S. Cuidados ao doente imunodeprimido – A criança com Imunodeficiência Primária. XVI Jornadas de Pediatria – Departamento da Criança e da Família do HSM. Lisboa, November 11-14, 2009.

Silva, S. Imunodeficiência Comum Variável. III Jornadas do Departamento de Pediatria do

Hospital Fernando Fonseca. Lisboa. March 19-20, 2009.

Soares, M. Telomere Length by Flow FISH as a tool to assess immune replicative senescence. XI Congresso da Sociedade Ibérica de Citometria, Covilhã. Portugal, May 7-9, 2009.

Sousa, A.E. Novas perspectivas na patogénese da SIDA: implicações clínicas. Ciclo de Sessões de Pós-graduação das Clínicas Universitárias de Medicina I e II HSM/FMUL. Lisboa, Portugal. November 4, 2009

Sousa, A.E. Understanding AIDS pathogenesis through HIV-2 infection. X Jornadas do Núcleo de Estudos da Infecção VIH da Sociedade Portuguesa de Medicina Interna, Vilamoura, Portugal. June 20, 2009.

### **Organisation of National and International Conferences**

Sousa, A.E., Caramalho, I. and Lopes da Silva, S. co-organized the “XXXV Annual Meeting of the Portuguese Society for Immunology”, IMM, Lisboa, Portugal. September 28-30, 2009.

UIC’s Scientific Retreat. Convento da Arrábida. Serra da Arrábida, Portugal, November 5-7, 2009.

### **Prizes, Honours and Awards**

Award of Excellence in Immunology Dr. Mário Arala Chaves to Tendeiro, R., Soares, M., Baptista, A.P., Cavaleiro, R., Foxall, R.B., Soares, R., Gomes, P., Victorino, R.M.M. and Sousa AE at the XXXV Reunião Anual da Sociedade Portuguesa de Imunologia for the poster “Evidence for increased replicative rate of the naïve CD4+ T cell pool during HIV-2 infection, a naturally occurring attenuated form of HIV disease”

Schering- Plough –2º prémio das Comunicações orais to Costa, A.C., Melo, A., Palma-Carlos, A., Pereira Santos, M.C. at the Portuguese Society for Immunoallergology and Clinical Immunology XXX Annual Meeting for the oral presentation “Diagnóstico da urticária crónica autoimune-contribuição do teste de activação dos basófilos e avaliação por citometria de fluxo”.

### **Advanced Teaching**

Sousa, A.E. Organizer of the “Immunology Module” VII Master Course on Emergent Infections, Faculdade de Medicina Universidade de Lisboa. November 16, 23, 30, 2009.

Albuquerque, A.S. and Tendeiro, R. “Immunology: Laboratorial Lessons” VII Mestrado em Doenças Infecciosas Emergentes, Faculdade de Medicina Universidade de Lisboa. November 16, 23, 30, 2009.

Caramalho, I. “Monitoring antigen-specific immune responses” VII Mestrado Doenças Infecciosas Emergentes, Faculdade de Medicina Universidade de Lisboa. November 30, 2009.

Tendeiro, R. “Vacinas” VII Mestrado em Doenças Infecciosas Emergentes, Faculdade de Medicina da Universidade de Lisboa. November 30, 2009.

Sousa, A.E. “Imunopatogénese da Infecção HIV/SIDA” VII Master Course on Emergent Infections, , Faculdade de Medicina da Universidade de Lisboa. November 23, 2009.

Pereira Santos, M.C. “Diagnóstico de reacções adversas a fármacos”, Curso Pós-graduado – Reacções adversas a fármacos: imunopatologia e clínica, Universidade Lusófona, Lisboa. October-November 2009.

Sousa, A.E. “Imunodeficiência associada à infecção VIH” Immunology Course. Faculdade de Medicina ICBAS, Porto. June 4, 2009.

Sousa, A.E. “Seminário da Clínica à Biologia Molecular”, Instituto Superior de Ciências da Saúde. Braga, February, 2009.

Cavaleiro, R. “Vacinas” VI Mestrado em Doenças Infecciosas Emergentes, Faculdade de Medicina da Universidade de Lisboa. February 2, 2009.

Sousa, A.E. “Imunologia e Infecção” VI and VII Master Course on Emergent Infections, Faculdade de Medicina Universidade de Lisboa. January 19 and November 16, 2009.

Foxall, R. “Evaluation of Antigen-specific responses” VI Mestrado Doenças Infecciosas Emergentes. Faculdade de Medicina, Lisboa. January 26 and February 2, 2009.

Sousa, A.E. “Imunodeficiências” VI Master Course on Emergent Infections, Faculdade de Medicina Universidade de Lisboa. January 26, 2009.

Sousa, A.E. Organizer of the “Immunology Module” VI Master Course on Emergent Infections, Faculdade de Medicina Universidade de Lisboa. January 19, 26 and February 2, 2009.

### **Thesis Completed**

#### **PhD Thesis**

Rita Jorge Dias Cavaleiro (2009) Modulação de Células Dendríticas e Monócitos pelo Vírus da Imunodeficiência Humana Tipo 2, Faculdade de Medicina da Universidade de Lisboa. Supervisor: A. Espada de Sousa and Co-supervisor: R.M.M. Victorino

Russell Bourne Foxall (2009) HIV-specific responses, T-cell activation and regulatory T-cells in AIDS pathogenesis: lessons from the HIV-2 model, Faculdade de Medicina da Universidade de Lisboa. Supervisor: A. Espada de Sousa and Co-supervisor: R.M.M. Victorino

Catarina S. Cortesão (2009) Studies on a human model and a murine model of immunodeficiency: HIV-2 infection and TACI deficiency, Faculdade de Medicina da Universidade de Lisboa. Co-supervisors: A. Espada de Sousa and M. Cascalho.

#### **Master Thesis**

Ana Célia Lopes Costa Hipólito (2009) “Diagnóstico de urticária crónica autoimune- estudo de activação dos basófilos por citometria de fluxo”, Mestrado em Imunologia Médica, Faculdade de Medicina da Universidade de Lisboa. Supervisor: Maria Conceição Santos.

#### **Specialiaty in Immunoallergology**

Clinical Immunology Laboratory training period for the final degree  
(Ordem dos Médicos/Ministry of Health)

Maria Fátima Duarte, M.D. – Centro Hospitalar de Lisboa Norte - Hospital de Santa Maria

Maria Isabel Mascarenhas, M.D. – Centro Hospitalar de Lisboa Norte - Hospital de Santa Maria

#### **Diploma Thesis**

Rute Machado (2009) “O tratamento antiretroviral da infecção pelo HIV-2”, Trabalho Final do Mestrado Integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisor: A. Espada de Sousa.

## Rheumatology Research Unit

### Description and Objectives

The Rheumatology Research Unit (RRU) results from a partnership between the Instituto de Medicina Molecular/Faculdade de Medicina da Universidade de Lisboa and the Rheumatology Department of the Santa Maria Hospital. Basic scientists and clinicians work together promoting translational research and clinical excellence in the field of Rheumatology.

Our specific research aims are the study of the pathogenesis of inflammatory joint diseases (Rheumatoid Arthritis (RA), Juvenile Idiopathic Arthritis, Spondyloarthropaties and Systemic Lupus Erythematosus (SLE)) and bone disorders (such as osteoporosis) in order to characterize potential tools for early diagnosis and prognosis and potential targets for novel therapies.

Ongoing research projects are devoted to the study of neutrophils, monocytes and B cells in very early arthritis, to the analysis of the effects of inflammation on atherogenesis in RA and SLE and to the relevance of TNF polymorphisms in the prognosis and pharmacogenetics of several joint diseases. We are also involved in research of bone quality in cooperation with Instituto Superior Técnico. All these research efforts are based on a dual approach which includes human samples and animal models of rheumatic diseases. Finally, we are involved in clinical trials, development of registries (in collaboration with the Portuguese Society of Rheumatology) and epidemiological studies, in providing services to the community, including expertise advisory to governmental institutions, and in postgraduate teaching.

The final long term objective of the RRU is the future recognition as an European League Against Rheumatism Centre of Excellence in Rheumatology.

### Head of Unit

João Eurico Fonseca, MD, PhD – Auxiliary Professor, Faculdade de Medicina da Universidade de Lisboa (FMUL); Rheumatologist, Rheumatology Department, Santa Maria Hospital (HSM); Lisbon, Portugal

### Principal Investigators

Helena Canhão, MD, PhD – Auxiliary Professor, Faculdade de Medicina da Universidade de Lisboa (FMUL); Rheumatologist, Rheumatology Department, Santa Maria Hospital (HSM); Lisbon, Portugal

### Research Team

Pamela Weinmann, PhD	- Post-Doc fellowship, FCT
Saba Abdulghani, PhD	- Post-Doc fellowship, Private funding
Rita Moura	- PhD fellowship, FCT (currently in University College London)
Joana Caetano-Lopes	- PhD fellowship, FCT (co-supervision Helsinki University)
Rita Cascão,	- PhD fellowship, FCT
Luis Santos	- PhD fellowship, FCT (co-supervision with IST/UTL)
José Teles	- PhD fellowship, FCG (currently in Lund University)
Maria José Santos, MD	- PhD student (Hospital Garcia da Orta)
Ana Filipa Mourão, MD	- PhD fellowship, FCT MD/PhD program - Hospital Egas Moniz
Elsa Sousa, MD	- PhD student, Assistant FMUL, HSM (currently in the Amsterdam Academic Medical Centre)
Inês Perpétuo	- MSc student
Ana Farinho	- MSc student
Ana Lopes	- MSc student
Inês Aleixo	- Research student, FCT grant

Bruno Vidal	- Assistant and clinical trials coordinator, FCT grant
Ana Rodrigues, MD	- HSM
Joaquim Pereira, MD	- Assistant FMUL, HSM
Diana Fernandes	- Research student, IEFPP/IMM fellowship
Marketa Fojtíková, MD	- PhD student, EULAR fellowship

### Research Areas

- Joint inflammatory diseases - pathogenesis, early diagnosis, prognosis and pharmacogenetics
- Inflammatory cells and Inflammation
- Bone biology, structure and biomechanics
- Epidemiology of Rheumatic Diseases, Cohorts, Registries and Databases - Recommendations and Guidelines

### Major Scientific Achievements

The main achievements of the Rheumatology Research Unit in 2009 were:

- 1- Establishing TNF alpha promoter polymorphisms at position -308 as prognostic markers in Ankylosing Spondylitis (AS) and Juvenile Idiopathic Arthritis (JIA).
- 2- Describing the degradation of bone biomechanical properties in an arthritis animal model.
- 3- Coordinating the elaboration of national practical guide for the management of RA with biological agents.
- 4- Contribution for the elaboration of international guidelines on the use of Methotrexate and Recommendations for diagnosis and monitoring of undifferentiated arthritis (3e Initiative).
- 5- Coordinating the elaboration and establishment of the national databases and registries of rheumatic diseases treated with biotechnology drugs.
- 6- Coordination of the Portuguese Rheumatology Biobank, a part of the IMM biobank.
- 7- Participation in multinational clinical trials as national coordinators allowing refractory patients to have access to the state of art in biotechnological therapies.
- 8- The Bial Prize for Clinical Medicine 2008 was awarded to JE Fonseca and collaborators in May 2009. This is the most prestigious Portuguese prize in the field of clinical research.
- 9- Establishment of a research network with the Harvard Medical School based on the study of genetic and biochemical markers of RA in an American and Portuguese rheumatoid patients cohorts.
- 10- Creation of a network of biologists, biomedical engineers and physicians, sharing a common mission, goals and values, which allow the ongoing pattern of a highly interactive work that is leading the translational research in the field of Rheumatology in Portugal.

### Recent Most Relevant Publications

Ligeiro, D., Fonseca, J.E., Abade, O., Abreu, I., Cruz, M., Nero, P., Cavaleiro, J., Teles, J., Trindade, H., Caetano, J.M. and Branco, J. (2007) Influence of human leucocyte antigen-DRB1 on the susceptibility to rheumatoid arthritis and on the production of anti-cyclic citrullinated peptide antibodies in a Portuguese population. *Ann. Rheum. Dis.* 66: 246-248 (Times cited: 2)(Journal IF: 7.188)

Fonseca, J.E., Cavaleiro, J., Teles, J., Sousa, E., Andreozzi, V.L., Antunes, M., Amaral-Turkman, M.A., Canhão, H., Mourão, A.F., Lopes, J., Caetano-Lopes, J., Weinmann, P., Sobral, M., Nero, P., Saavedra, M.J., Malcata, A., Cruz, M., Melo, R., Braña, A., Miranda, L., Patto, J.V., Barcelos, A., da Silva, J.C., Santos, L.M., Figueiredo, G., Rodrigues, M., Jesus, H., Quintal, A., Carvalho, T., da Silva, J.A., Branco, J. and Queiroz, M.V. (2007) Contribution for new genetic markers of rheumatoid arthritis activity and severity: sequencing of the tumor necrosis factor-alpha gene



promoter. *Arthritis Res. Ther.* 9: R37 (Times cited: 5)(Journal IF: 4.485)

Fonseca, J.E., Cortez-Dias, N., Francisco, A., Sobral, M., Canhão, H., Resende, C., Castelão, W., Macieira, C., Sequeira, G., Saraiva, F., Pereira da Silva, J.A., Carmo-Fonseca, M. and Viana Queiroz, M. (2005) Inflammatory cell infiltrate and RANKL/OPG expression in rheumatoid synovium– comparison with other inflammatory arthropathies and correlation with outcome. *Clin. Exp. Rheumatol.* 23: 185-192 (Times cited: 12)(Journal IF: 2.364)

Fonseca, J.E., Carvalho, T., Cruz, M., Nero, P., Sobral, M., Mourão, A.F., Cavaleiro, J., Abreu, I., Carmo-Fonseca, M. and Branco, J.C. (2005) Polymorphism at position –308 of the tumor necrosis factor alpha gene and rheumatoid arthritis pharmacogenetics. *Ann. Rheum. Dis.* 64: 793-794 (Times cited: 22)(Journal IF: 7.188)

Fonseca, J.E., Edwards, J.C., Blades, S. and Goulding, N.J. (2002) Macrophage subpopulations in rheumatoid synovium: reduced CD163 expression in CD4+ T lymphocyte-rich microenvironments. *Arthritis Rheum.* 46: 1210-1216 (Times cited: 20)(Journal IF: NA)

### Ongoing Research Projects

2007/2010 Effects of inflammation on bone and cartilage: collagen pattern and biomechanical properties, FCT. Coordinator: J.E. Fonseca (PTDC/SAU-BEB/65992/2006)

2008/2010. The role of neutrophils in the initiation and perpetuation of Rheumatoid Arthritis, Portuguese Society of Rheumatology Coordinator: J.E. Fonseca

2009/2011 Cardiovascular risk and inflammation- contribution to the etiopathogenesis of atherosclerosis and cardiovascular disease, FCT. Coordinator: J.E. Fonseca (PIC/IC/82920/2007)

2009/2011 Evaluation of bone quality and its relation to osteoporotic fracture risk, Associação Portuguesa de Osteoporose. Coordinator: H. Canhão

2009/2012 The role of B cells in early Rheumatoid Arthritis, Portuguese Society of Rheumatology. Coordinator: J.E. Fonseca

2010/2012. Development of carbon monoxide releasing molecules for the treatment of rheumatoid arthritis (COMRA), European Union FP7 call Program "Research for the benefit of SMEs" – Call identifier: FP7-SME-2008-1. General Coordinator: N Arantes-Oliveira. Local Coordinator: J.E. Fonseca. Proposal No: 230629

2006/ National and International Guidelines for the diagnosis and treatment of rheumatic diseases, Portuguese Society of Rheumatology. Coordinators: J.E. Fonseca, H. Canhão and M.J. Santos

Research Projects involving National and International Databases and Registries. Cooperation with Institutional Health Organizations. Coordinator: J.E. Fonseca

Fostering the knowledge and quality of National Rheumatology

### Publications in Peer Review International Journals 2009

Abdulghani, S., Caetano-Lopes, J., Canhão, H. and Fonseca, J.E. (2009) Biomechanical effects of inflammatory diseases on bone-rheumatoid arthritis as a paradigm. *Autoimmun. Rev.* 8: 668-671 (Journal IF: 5.371)

Caetano-Lopes, J., Canhão, H. and Fonseca, J.E. (2009) Osteoimmunology – the immune regulation of bone. *Autoimmun. Rev.* 8: 250-255 (Journal IF: 5.371)

Caetano-Lopes, J., Nery, A.M., Canhão, H., Duarte, J., Cascão, R., Rodrigues, A., Perpétuo, I., Abdulghani, S., Amaral, P.M., Sakaguchi, S., Kontinen, Y.T., Graça, L., Vaz, M.F. and Fonseca J.E. (2009) Chronic arthritis leads to disturbances in the bone collagen network. *Arthritis Res. Ther. In press* (Journal IF: 4.49)

Caetano-Lopes, J., Nery, A.M., Henriques, R., Canhão, H., Duarte, J., Amaral, P.M., Vale, M.,

Moura, R.A., Pereira, P.A., Weinmann, P., Abdulghani, S., Souto-Carneiro, M., Rego, P., Monteiro, J., Sakagushi, S., Queiroz, M.V., Konttinen, Y.T., Graça, L., Vaz, M.F. and Fonseca, J.E. (2009) Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. *Clin. Exp. Rheumatol.* 27: 475-482 (Journal IF: 2.366)

Cascão, R., Rosário, H.S., Souto-Carneiro, M.M. and Fonseca, J.E. (2009) Neutrophils in Rheumatoid Arthritis: more than simple final effectors. *Autoimmun. Rev. In press* (Journal IF: 5.371)

Fonseca, J.E., Canhão, H., Tavares, N.J., Cruz, M., Branco, J. and Queiroz, M.V. (2009) Persistent low grade synovitis without erosive progression in magnetic resonance imaging of rheumatoid arthritis patients treated with infliximab over 1 year. *Clin. Rheumatol.* 28: 1213-1216 (Journal IF: 1.559)

Fonseca, J.E., Santos, M.J., Canhão, H. and Choy, E. (2009) Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmun. Rev.* 8: 538-542 (Journal IF: 5.371)

Moura, R., Weinmann, P., Pereira, P., Caetano-Lopes, J., Canhão, H., Sousa, E., Mourão, A.F., Rodrigues, A.M., Queiroz, M.V., Souto-Carneiro, M.M., Graça, L. and Fonseca JE (2009) Alterations on peripheral blood B cell subpopulations in very early arthritis patients. *Rheumatol. In press* (Journal IF: 4.136)

Mourão, A.F., Amaral, M., Caetano-Lopes, J., Isenberg, D. (2009) An analysis of joint replacement in patients with systemic lupus erythematosus. *Lupus* 18: 1298-1302 (Journal IF: 2.244)

Mourão, A.F., Caetano-Lopes, J., Costa, P., Canhão, H., Santos, M.J., Pinto, P., Brito, I., Nicola, P., Cavaleiro, J., Teles, J., Sousa, A., Gomes, J.M., Branco, J., da Costa, J.T., Pedro, J.G., de Queiroz M.V. and Fonseca, J.E. (2009) Tumor necrosis factor-alpha -308 genotypes influence inflammatory activity and TNF-alpha serum concentrations in children with juvenile idiopathic arthritis. *J Rheumatol.* 36: 837-842 (Journal IF: 3.151)

Mourão, A.F., Rustin, M.H.A. and Isenberg, D. (2009) Exacerbation of psoriatic skin lesions in 3 patients with psoriatic arthritis receiving anti-tumour necrosis factor-alpha therapy. *Clin. Exp. Rheumatol. In press* (Journal IF: 2.366)

Pimentel-Santos, F.M., Ligeiro, D., Matos, M., Mourão, A.F., Sousa, E., Pinto, P., Ribeiro, A., Sousa, M., Barcelos, A., Godinho, F., Cruz, M., Fonseca, J.E., Guedes-Pinto, H., Trindade, H., Evans, D.M., Brown, M.A. and Branco, J.C. (2009) Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. *Clin. Exp. Rheumatol.* 27: 800-806 (Journal IF: 2.366)

Polido-Pereira, J., Ferreira, D., Rodrigues, A.M., Nascimento, C., Costa, P., Almeida, M., da Silva, J.E., Simão, C., Stone, R., Ramos, F., Neto, A., da Costa, J.C., Melo-Gomes, J., Gomes-Pedro, J., Viana-Queiroz, M., Canhão, H. and Fonseca, J.E. (2009) Rituximab use in pediatric autoimmune diseases: four case reports. *Ann. NY Acad. Sci.* 1173: 712-720 (Journal IF: 2.303)

Pons-Estel, B.A., Sánchez-Guerrero, J., Romero-Díaz, J., Iglesias-Gamarra, A., Bonfa, E., Borba, E.F., Shinjo, S.K., Bernatsky, S., Clarke, A., García, M.A., Marcos, J.C., Duarte, A., Berbotto, G.A., Scherbarth, H., Marques, C.D., Onetti, L., Saurit, V., Souza, A.W., Vellozo, E., Catoggio, L.J., Neira, O., Burgos, P.I., Ramirez, L.A., Molina, J.F., De La Torre, I.G., Silvariño, R., Manni, J.A., Durán-Barragán, S., Vilá, L.M., Fortin, P.R., Calvo-Alén, J., Santos, M.J., Portela, M., Esteva-Spinetti, M.H., Weisman, M., Acevedo, E.M., Segami, M.I., Gentiletti, S.B., Roldán, J., Navarro, I., Gonzalez, E., Liu, J.M., Karlson, E.W., Costenbader, K.H., Wolfe, F. and Alarcón, G.S. (2009) Validation of the Spanish, Portuguese and French versions of the Lupus Damage Index questionnaire: data from North and South America, Spain and Portugal. *Lupus* 18: 1033-1052 (Journal IF: 2.244)

Santos, M.J., Vinagre, F., Nero, P., Barcelos, F., Barcelos, A., Rodrigues, A.M., de Matos, A.A., Silva, C., Miranda, L., Capela, S., Marques, A., Branco, J. and da Silva, J.C. (2009) Predictors of damage progression in Portuguese patients with systemic lupus erythematosus. *Ann. NY Acad. Sci.* 1173: 822-888 (Journal IF: 2.303)

Sillat, T., Pollanen, R., Caetano-Lopes, J., Porola, P., Ma, G., Korhonen, M. and Konttinen, Y.T. (2009) Intracrine androgenic apparatus in human bone marrow stromal cells. *J. Cell. Mol. Med.* 13: 3296-3302 (Journal IF: 5.114)

Sousa, E. and Isenberg, D. (2009) Treating lupus: from serendipity to sense, the rise of the new biologicals and other emerging therapies. *Best Pract. Res. Clin. Rheumatol.* 23: 563-574 (Journal IF: 2.088)

Sousa, E., Caetano-Lopes, J., Pinto, P., Pimentel, F., Teles, J., Canhão, H., Rodrigues, A., Resende, C., Mourão, A.F., Ribeiro, C., Pinto, T.L., Rosa, C.M., da Silva, J.A., Branco, J., Ventura, F., Queiroz, M.V. and Fonseca, J.E. (2009) Ankylosing spondylitis susceptibility and severity - contribution of TNF gene promoter polymorphisms at positions -238 and -308. *Ann. NY Acad. Sci.* 1173: 581-588 (Journal IF: 2.303)

Suarez-Gestal, M., Calaza, M., Endreffy, E., Pullmann, R., Ordi-Ros, J., Domenico Sebastiani, G., Ruzickova, S., Santos, M.J., Papasteriades, C., Marchini, M., Skopouli, F.N., Suarez, A., Blanco, F.J., D'Alfonso, S., Bijl, M., Carreira, P., Witte, T., Migliaresi, S., Gomez-Reino, J.J., Gonzalez, A.; European Consortium Of SLE DNA Collections (2009) Replication of recently identified systemic lupus erythematosus genetic associations: a case control study. *Arthritis Res. Ther.* 11: R69 (Journal IF: 4.49)

Vaz, C.C., Couto, M., Medeiros, D., Miranda, L., Costa, J., Nero, P., Barros, R., Santos, M.J., Sousa, E., Barcelos, A. and Inês, L. (2009) Undifferentiated connective tissue disease: a seven-center cross-sectional study of 184 patients. *Clin. Rheumatol.* 28: 915-921 (Journal IF: 1.559)

Vinagre, F., Santos, M.J., Prata, A., Canas Silva, J. and Santos, A.I. (2009) Assessment of salivary gland function in Sjögren's Syndrome: the role of Salivary Gland Scintigraphy. *Autoimmun. Rev.* 8: 672-676 (Journal IF: 5.371)

Visser, K., Katchamart, W., Loza, E., Martinez-Lopez, J.A., Salliot, C., Trudeau, J., Bombardier, C., Carmona, L., van der Heijde, D., Bijlsma, J.W., Boumpas, D.T., Canhão, H., Edwards, C.J., Hamuryudan, V., Kvien, T.K., Leeb, B.F., Martín-Mola, E.M., Mielants, H., Müller-Ladner, U., Murphy, G., Østergaard, M., Pereira, I.A., Ramos-Remus, C., Valentini, G., Zochling, J. and Dougados, M. (2009) Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. *Ann. Rheum. Dis.* 68: 1086-1093 (Journal IF: 7.188)

### Publications in Peer Review National Journals 2009

Baeten, D., van Hagen, M., Magro, F., Filipe, P. and Fonseca, J.E. (2009) Immune-mediated inflammatory diseases: a new multidisciplinary concept. *Acta Reumatol. Port.* 34: 457-459

Canhão, H., Santos, M.J., Costa, L., Bogas, M., Mourão, A.F., Machado, P., Fonseca, J.E., Silva, J.A.; Multinational 3E Initiative (2009) Portuguese recommendations for the use of methotrexate in the treatment of rheumatoid arthritis. *Acta Reumatol. Port.* 34: 78-95

Cascão, R., Rosário, H.S. and Fonseca, J.E. (2009) Neutrophils: warriors and commanders in immune mediated inflammatory diseases. *Acta Reumatol. Port.* 34: 313-326

Fonseca, J.E., Silva, J.A., Canhão, H., Santos, M.J., Barcelos, A., Inês, L., Costa, M.L., Rodrigues, M., Bernardo, A., Cordeiro, A., Cravo, A.R., Ribeiro, A., Teixeira, A., Malcata, A., Faustino, A., Ribeiro, C., Nour, D., Araújo, D., Sousa, E., Mariz, E., Ramos, F., Vinagre, F., Ventura, F.S., Sequeira, G., Santos, H., Branco, J.C., Ramos, J., Santo, J.E., Costa, J.A., da Silva, J.A., Miranda, L., Sampaio, L., Afonso, M.C., Saavedra, M.J., Cunha, M.I., Queiroz, M.V., Couto, M., Pinto, P., Valente, P., Abreu, P., Figueira, R.O., Ramiro, S., Nóvoa, T., Bernardes, M.; Grupo de Estudos de Artrite Reumatóide da Sociedade Portuguesa de Reumatologia (2009) Practical guide for the use of biotechnological therapies in Rheumatoid Arthritis. *Acta Reumatol. Port.* 34: 395-399

Rodrigues, A., Caetano-Lopes, J., Nery, A., Sousa, E., Polido-Pereira, J., Vale, M., Amaral, P., Romeu, J.C., Viana Queiroz, M., Monteiro, J., Vaz, M.F., Fonseca, J.E. and Canhão, H. (2009)

Evaluation of bone mechanical strength and fracture risk assessment (Frax) in patients with hip joint replacement surgery. *Acta Reumatol. Port.* 34: 504-510

Santos, M.J. and Fonseca, J.E. (2009) Metabolic Syndrome, inflammation and atherosclerosis - The role of adipokines in health and in systemic inflammatory rheumatic diseases. *Acta Reumatol. Port.* 34: 590-598

### **Communications in International Conferences 2009**

Aleixo, I.P. et al. Comparison of trabecular bone biomechanical and structural properties between men and women with fragility fractures. 36th American Society for Bone and Mineral Research Congress, Denver, USA. September 11-15, 2009 (Poster presentation)

Bogas, M. et al. Methotrexate treatment in rheumatoid arthritis: best practice in clinical remission, common infection and tuberculosis. Results from a systematic literature review. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Caetano-Lopes, J. et al. Chronic arthritis leads to disturbances in the bone collagen network. 36th American Society for Bone and Mineral Research Congress, Denver, USA. September 11-15, 2009 (Poster presentation)

Caetano-Lopes, J. et al. Chronic arthritis leads to disturbances in the bone collagen network. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Cascão, R. et al. Cytokine network in the first 6 weeks of rheumatoid arthritis onset. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Dias, M. et al. Permeability analysis of scaffolds for bone tissue engineering. 1st International Conference on Tissue Engineering, Leiria, Portugal. July 9-11, 2009 (Oral presentation)

Fernandes, D. The LTA 252 A/G polymorphism might be a marker for rheumatoid arthritis susceptibility. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Figueiredo, R. et al. Tumor necrosis factor gene -308 position influences the response to TNF antagonist therapy in ankylosing spondylitis patients. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Gonzalez, A. et al. Weak association of Systemic Lupus Erythematosus clinical features with susceptibility alleles. American College Rheumatology Annual Meeting, Philadelphia, USA. October 18-21, 2009 (Poster presentation)

Lino, M.M. et al. Ultrasonographic, Structural and Biomechanical Analysis of Tendons. 1st International Conference on Tissue Engineering, Leiria, Portugal. July 9-11, 2009 (Oral presentation)

Machado, P. et al. How should we manage methotrexate dosing after clinical remission? A systematic literature review. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Miranda, S. et al. The secondary immune response is dependent of a normal production of reactive oxygen species by the NADPH-oxidase complex. 29th European Workshop for Rheumatology Research, Warsaw, Poland. February 26-28, 2009 (Oral presentation)

Mourao, A.F. et al. How should we screen for and treat tuberculosis in a patient under methotrexate? A systematic literature review. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Nicola, P.J. et al. The Portuguese registry of rheumatic diseases: the strategy behind merging the electronic medical record, research databases and patients' registries. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Padovan, E. et al. Loss of monocyte specialization in rheumatoid arthritis. Adaptive Immunity and the Pathogenesis of Rheumatic Diseases, Genoa, Italy. September 24-27, 2009 (Poster presentation)

Perpétuo, I. et al. Cytokine profile in rheumatoid arthritis patients under different therapies. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Polido-Pereira, J.M. et al. Tuberculosis safety monitoring of the use of monoclonal antibodies against TNF in Portuguese rheumatoid arthritis patients based on an electronic medical chart and registry (BioReportAR): experience of a single centre. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Rodrigues, A. et al. Safe use of etanercept in an ankylosing spondylitis patient with a kidney transplant treated with tacrolimus and micophenolate mophetil. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Rodrigues, A. et al. Seven years of experience with biological treatment in juvenile idiopathic arthritis. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Santos, L. A DXA validation of a bone remodeling model for the assessment of bone quality. Mathematical Aspects of Imaging, Modeling and Visualization in Multiscale Biology, Austin, Texas, USA. March 31- April 4, 2009 (Poster presentation)

Santos, L. et al. An approach towards patient-specific bone remodelling simulation in osteoporosis. The fourth issue of the International Conference on Computational Bioengineering, Bertinoro, Italy. September 16-18, 2009 (Poster presentation)

Santos, M.J. et al. Cardiovascular risk may not be increased in women with mild lupus. American College Rheumatology Annual Meeting, Philadelphia, USA. October 18-21, 2009 (Poster presentation)

Santos, M.J. et al. Hemorheological changes associated with systemic lupus erythematosus and rheumatoid arthritis. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Santos, M.J. et al. Interleukin-6 promoter -174 G/C polymorphism is associated with disease severity and cardiovascular risk in lupus patients. 4th European Workshop on Immune Mediated Inflammatory Diseases, Lisbon, Portugal. November 18-20, 2009 (Poster presentation)

Santos, M.J. et al. The incidence of cardiovascular events among rheumatoid arthritis patients in Portugal. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Suarez-Gestal, M. et al. Replication of recently identified Systemic Lupus Erythematosus genetic associations: a case control study (CL). Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Oral presentation)

Vinagre, F. et al. Switching anti-TNF agents in juvenile idiopathic arthritis. Annual European Congress of Rheumatology EULAR, Copenhagen, Denmark. June 10-14, 2009 (Poster presentation)

Weinmann, P. et al. The secondary immune response is dependent of a normal production of reactive oxygen species by the NADPH-oxidase complex. 2nd European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Oral presentation)

#### **Communications in National Conferences 2009**

Silva, S.L. et al. Severe autoimmune manifestations in association with major T-cell disturbances: a diagnostic and treatment challenge. Primeiro Congresso Nacional de Autoimunidade. Lisbon, Portugal. April 23-25, 2009 (Oral presentation)

### Invited Lectures and Seminars 2009

Canhao, H. Abatacept - um novo mecanismo de acção no tratamento da artrite reumatoide. Reunião Instituto Português de Reumatologia, Lisbon, Portugal. November 13, 2009.

Canhao, H. Artrites juvenis. XII Jornadas da Liga Portuguesa Contra as Doenças Reumáticas, Lisbon, Portugal. October 9, 2009.

Canhao, H. Como diagnosticar, monitorizar e tratar a artrite indiferenciada. Reunião Nacional Recomendações para o diagnóstico e terapêutica da artrite indiferenciada, Penha Longa, Sintra, Portugal. May 29-30, 2009.

Canhão, H. Cryopyrin-associated periodic syndromes. Reunião Síndromes auto-inflamatórias, Lisbon, Portugal, November 2, 2009.

Canhao, H. Novas terapêuticas para a Osteoporose. VIII Jornadas do Aparelho Locomotor, Braga, Portugal. October 31, 2009.

Canhao, H. Practical examination of the joints and periarticular injections. IV European Rheumatology Course for Clinical Practitioners, Lisbon, Portugal. October 9-10, 2009.

Canhão, H. Segurança do Denosumab, Lisbon, Portugal. November 6, 2009.

Canhao, H. Swollen, red knee in children. Jornadas de Ortopedia Infantil. Lisbon, Portugal. January 24, 2009.

Canhao, H. Viabilização da investigação em Reumatologia, biobanco e acesso a financiamento. Artrite Reumatóide: Um paradigma para novas estratégias terapêuticas, investigação clínica e gestão de cuidados de saúde, Lisbon, Portugal. May 15, 2009.

Canhao, H. Vitamin D and its applications. III European Rheumatology Course for Clinical Practitioners. Budapest, Hungary. March 13, 2009.

Fonseca JE. Boas práticas de gestão do Hospital de Dia. Artrite Reumatóide: Um paradigma para novas estratégias terapêuticas, investigação clínica e gestão de cuidados de saúde, Lisbon, Portugal. May 15, 2009.

Fonseca, J.E. 5 perguntas e 5 respostas sobre agentes biológicos. Primeiro Encontro Multidisciplinar de Gastroenterologia: Gastroenterologia e Reumatologia, Lisbon, Portugal. October 29-30, 2009.

Fonseca, J.E. Aspectos imunológicos da artrite inicial, Primeiro Congresso Nacional de Auto-imunidade, Lisbon, Portugal. April 23-25, 2009.

Fonseca, J.E. Bases de dados da SPR. Jornadas de Outono da Sociedade Portuguesa de Reumatologia, Lisbon, Portugal. October 3-5, 2009.

Fonseca, J.E. Bases de dados da SPR. Primeira Reunião de Internos de Reumatologia da Sociedade Portuguesa de Reumatologia, Torres Vedras, Portugal. June 19-20, 2009.

Fonseca, J.E. Citocinas e dor. Simpósio “Dor no aparelho locomotor” da Sociedade Portuguesa de Reumatologia, Porto, Portugal. April 3-5, 2009.

Fonseca, J.E. Devemos repensar a terapêutica da artrite reumatóide? XVII Jornadas Internacionais do Instituto Português de Reumatologia, Lisbon, Portugal. December 10-11, 2009.

Fonseca, J.E. Strontium ranelate better efficacy on bone microarchitecture. 9th Meeting of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis, Athens, Greece. March 18-21, 2009.

Fonseca, J.E. Terapêuticas biotecnológicas no Lúpus Eritematoso Sistémico – uma visão pessoal. 1ª Reunião do Núcleo de Doenças Auto-imunes das Clínicas Universitárias das Medicinas I e II, Lisbon, Portugal. October 22-24, 2009.

Fonseca, J.E. Tratamento da Artrite Psoriática – dos fármacos actuais aos biotecnológicos. II Fórum de Espondilartropatias, Aveiro, Portugal. May 9, 2009.

Fonseca, J.E. Uma nova visão das doenças reumáticas inflamatórias: um exemplo de interacção

da biologia celular e molecular com a clínica. Sessão do Hospital do Santo Espírito, Angra do Heroísmo, Portugal. July 10, 2009.

Santos, M.J. Artrite Reumatóide Inicial. Desafios à gestão integrada da doença. II Summit Reumatologia, Monte Real, Portugal. December 5, 2009.

Santos, M.J. Doença cardiovascular na Artrite Reumatóide: que importância, como controlar os seus riscos? VIII Jornadas do Aparelho Locomotor, Braga, Portugal. October 31, 2009.

Santos, M.J. Em caso de resposta inadequada a um primeiro agente biológico (anti-TNF): mudança de classe terapêutica versus manutenção na mesma classe. Artrite Reumatóide: um paradigma para novas estratégias, investigação clínica e gestão de cuidados de saúde, Lisbon, Portugal. May 15, 2009.

Santos, M.J. Osteoporose. XVI Jornadas do Serviço de Medicina Interna do Hospital S Bernardo, Setúbal, Portugal. October 12, 2009.

Santos, M.J. Valor diagnóstico e prognóstico dos auto-anticorpos no Lúpus Eritematoso Sistémico. 4º Workshop Diagnóstico Laboratorial em Autoimunidade, Lisbon, Portugal. November 16, 2009.

### **Organisation of National and International Conferences 2009**

Canhao, H. Organizer and lecturer of the “Reunião Nacional Recomendações para o diagnóstico e terapêutica da artrite indiferenciada”, Penha Longa, Sintra, Portugal. May 29-30, 2009.

Fonseca, J.E. Organizer and lecturer of “Artrite Reumatóide: Um paradigma para novas estratégias terapêuticas, investigação clínica e gestão de cuidados de saúde”, Lisbon, Portugal. May 15, 2009.

Fonseca, J.E. and Canhao, H. Organizers and Course Directors. of “IV European Rheumatology Course for Clinical Practitioners”, Lisbon, Portugal. October 9-10, 2009.

Fonseca, J.E. Organizer and lecturer of the “4th European Workshop on Immune Mediated Inflammatory Diseases”, Lisbon, Portugal. November 18-20, 2009.

Fonseca, J-E. Co-organizer of the “12.º Workop “Educação pela Ciência”, Lisbon, Portugal. December 10, 2009.

### **Prizes, Honours and Awards**

Bial Prize for Clinical Medicine 2008 awarded to Fonseca, J.E., Canhão, H., Santos, M.J., Mourão, A.F., Sousa, E., Caetano Lopes, J., Moura, R., Weinmann, P., Pereira da Silva, J.A., Branco, J. and Viana Queiroz, M. for the work “Uma nova visão das doenças reumáticas inflamatórias: um exemplo de interação da biologia celular e molecular com a clínica.”

Associação Portuguesa de Osteoporose/ Merck Sharp awarded to Rodrigues, A.M., Caetano Lopes, J., Aleixo, I., Vidal, B., Perpétuo, I., Sousa, E., Pereira, J., Pereira, M.F., Romeu, J.C., Vaz, M.F., Viana Queiroz, M., Monteiro, J., Fonseca, J.E. and Canhão, H. for the project "Avaliação da qualidade do osso de epífises femorais e correlação com os factores de risco clínicos de fractura em doentes submetidos a artroplastia da anca".

### **Advanced Teaching**

Canhao, H. Fisiopatologia, diagnóstico e terapêutica da osteoporose. Practical Course for Pharmacists. Funchal, Madeira. May 22, 2009.

Canhao, H. Multinational recommendations for undifferentiated arthritis. Video recording for Rheumatologists. Paris, France. December 11, 2009.

Fonseca, J.E. Osteopénia, osteoporose e risco de fracturas, II Jornadas da Mulher e da Criança, Lisbon, Portugal. May 7-9, 2009.

Fonseca, J.E. Towards a safe remission. Clinical challenge in inflammatory diseases annual course & masterclass. Cascais, Portugal. November 17-18, 2009.

Fonseca, J.E. Progressos em terapêuticas farmacológicas. Medicamentos biológicos – Reumatologia. IV Workshop Progressos em terapêuticas farmacológicas. Medicamentos biológicos. Lisbon, Portugal. November 25, 2009.

### **Public Perception of Science**

Fonseca, J.E. participated, during 2009, in 1 TV program (RTP1) to discuss the burden of Inflammatory Joint Diseases.

Fonseca, J.E. participated in several news paper interviews related to the Bial Prize, May, 2009.

Participation in "Ciência Viva" in July and September, receiving a total of 6 students.

Perpétuo, I. and UIR participated in a TV program about "Ciência Viva".

Participation in a National Meeting to promote Science and Research "Cientistas ao Palco - Noite dos investigadores 2009" with a booth "O Restaurante IMM". September 25, 2009.

Participation in "Encontro de Estudantes de Medicina - EnjoyMed" to divulge Unit Research Projects. November 7, 2009.

### **Thesis Completed 2009**

#### **Master Thesis**

Catarina Ferreira (2009) "Caracterização da resposta funcional dos neutrófilos de sangue de doentes com artrite em fase inicial" Master in Biology, Faculdade de Ciências da Universidade de Lisboa. Supervisors: P. Weinmann and M. Telhada.

André Saraiva (2009) "Biomechanical comparison of female and male bone" Master on Biomedical Engineering, Instituto Superior Técnico da Universidade Técnica de Lisboa and Faculdade de Medicina da Universidade de Lisboa. Supervisors: J.E. Fonseca and F. Vaz.

Inês Perpétuo (2009) "Avaliação do comportamento dos osteoclastos em doentes com artrite reumatóide sob diversas terapêuticas" Master in Biology, Faculdade de Ciências da Universidade de Lisboa. Supervisors: H. Canhão and M. Telhada.

Raquel Marques (2009) "BioRePortAP, a primeira análise" Master in Medicine, Faculdade de Medicina da Universidade de Coimbra. Supervisors: J.E. Fonseca and M.J. Salvador.



## Viral Pathogenesis Unit

### Description and Objectives

The overall aim is to gain an understanding of basic molecular mechanisms afforded by herpesviruses. Our motivation is that this approach will increase our understanding of viral pathogenesis and also reveal potential strategies for therapeutic intervention. The model system consists of infection of laboratory mice with MuHV-4 that establishes latent infection in B cells. Using this model we have recently identified and characterized viral proteins with immunomodulatory functions. Presently we are focusing our efforts in two ORFs designated M2 and ORF73. We have confirmed that both ORFs are important for the establishment of latency, and thus have initiated studies to reveal the role that these proteins play during *in vivo* infection. We have shown that M2 modulates B cell receptor induced antigen triggered activation, and that ORF73 modulates NF- $\kappa$ B transcriptional activity.

### Head of Unit

João Pedro Simas, PhD - Associate Professor, Faculdade de Medicina, Universidade de Lisboa

### Research Team

Marta Miranda, PhD	- Post-Doc fellowship, FCT
Sofia Marques, PhD	- Post-Doc fellowship, FCT
Teresa Carlos, PhD	- Post-Doc fellowship, FCT
Lénia Rodrigues, PhD	- Post-Doc fellowship, FCT
Jérémie Decalf	- Post-Doc fellowship, FCT
Filipa Lopes	- PhD fellowship, FCT
Marta Alenquer	- PhD fellowship, FCT
Cristina Silva	- PhD fellowship, FCT

### Research Areas

Viral Pathogenesis

### Major Scientific Achievements

The pathogenesis of persistent viral infections depends critically on long-term viral loads. Yet what determines these loads is largely unknown. We showed that a single CD8<sup>+</sup> T cell epitope sets the long-term latent load of a lymphotropic gamma-herpesvirus, Murid herpesvirus-4 (MuHV-4). The MuHV-4 M2 latency gene contains an H2-Kd -restricted T cell epitope, and wild-type but not M2- MuHV-4 was limited to very low level persistence in H2d mice. Mutating the epitope anchor residues increased viral loads and re-introducing the epitope reduced them again. Like the Kaposi's sarcoma-associated herpesvirus K1, M2 shows a high frequency of non-synonymous mutations, suggesting that it has been selected for epitope loss. *In vivo* competition experiments demonstrated directly that epitope presentation has a major impact on viral fitness. Thus, host MHC class I and viral epitope expression interact to set the long-term virus load.

Host colonisation by lymphotropic gammaherpesviruses depends critically on the expansion of viral genomes in GC B cells. Yet, host and virus molecular mechanisms involved in driving such proliferation remain largely unknown. We showed that the mORF73 protein encoded by the MuHV-4 inhibits host nuclear factor-kappa B (NF- $\kappa$ B) transcriptional activity through poly-ubiquitination and subsequent proteasomal-dependent nuclear degradation of the NF- $\kappa$ B family member p65/RelA. The mechanism involves the assembly of an ElonginC/Cullin5/SOCS

(suppressors of cytokinesignalling)-like complex, mediated by an unconventional viral SOCS-box motif present in mORF73. Functional deletion of this SOCS-box motif ablated NF- $\kappa$ B inhibitory effect of mORF73, suppressed MuHV-4 expansion in GC B cells and prevented MuHV-4 persistent infection in mice. These findings demonstrate that viral inhibition of NF- $\kappa$ B activity in latently infected GC centroblasts is critical for the establishment of a gammaherpesvirus persistent infection, underscoring the physiological importance of proteasomal degradation of RelA/NF- $\kappa$ B as a regulatory mechanism of this signaling pathway.

### Recent Most Relevant Publications

Milho, R., Smith, C.M., Marques, S., Alenquer, M., May, J.S., Gillet, L., Gaspar, M., Efstathiou, S., Simas, J.P.\* and Stevenson PG\* (2009). In vivo Imaging of Murid Herpesvirus-4 infection. *J. Gen. Virol.* 90: 21-32 \* joint corresponding authors (Journal IF: 3.092)

Stevenson, P.G., Simas, J.P. and Efstathiou, S. (2009) Immune control of mammalian gamma-herpesviruses: lessons from murid herpesvirus-4. *J. Gen. Virol.* 90: 2317-2330 (Journal IF: 3.092)

Rodrigues, L., Filipe, J., Seldon, M.P., Fonseca, L., Anrather, J., Soares, M.P. and Simas, J.P. (2009) Termination of NF-kappaB activity through a gammaherpesvirus protein that assembles an EC5S ubiquitin-ligase. *EMBO J.* 28: 1283-1295 (Journal IF: 8.295)

Marques, S., Alenquer, M., Stevenson, P.G. and Simas, J.P. (2008) A single CD8+ T cell epitope sets the long-term latent load of a murid herpesvirus. *PLoS Pathog.* 4: e1000177 (Journal IF: 9.125)

Pires de Miranda, M., Alenquer, M., Marques, S., Rodrigues, L., Lopes, F., Bustelo, X.R. and Simas, J.P. (2008) The Gammaherpesvirus m2 protein manipulates the Fyn/Vav pathway through a multidocking mechanism of assembly. *PLoS One* 3: e1654 (Journal IF: NA)

### Ongoing Research Projects

2008/2010 Herpesvirus-encoded microRNAs as novel regulators of host gene expression: molecular function during latent infection in B-lymphocytes, FCT. Coordinator: P. Simas (PTDC/SAU-MII/65017/2006)

2010/2012 Gammaherpesvirus latent infection: immunological synapse modulation, FCT. Coordinator: P. Simas (PTDC/SAU-MII/099314/2008)

### Publications in Peer Review International Journals 2009

Stevenson, P.G., Simas, J.P. and Efstathiou, S. (2009) Immune control of mammalian gamma-herpesviruses: lessons from murid herpesvirus-4. *J. Gen. Virol.* 90: 2317-2330 (Journal IF: 3.092)

Carlos, T.S., Young, D.F., Schneider, M., Simas, J.P. and Randall, R.E. (2009) Parainfluenza virus 5 genomes are located in viral cytoplasmic bodies whilst the virus dismantles the interferon-induced anti-viral state of cells. *J. Gen. Virol.* 90: 2147-2156 (Journal IF: 3.092)

Rodrigues, L., Filipe, J., Seldon, M.P., Anrather, J., Soares, M.P. and Simas, J.P. (2009) Termination of NF- $\kappa$ B activity via a gherpesvirus protein that assembles an EC5S ubiquitin-ligase. *EMBO J.* 28: 1283-1295 (Journal IF: 8.295)

Milho, R., Smith, C.M., Marques, S., Alenquer, M., May, J.S., Gillet, L., Gaspar, M., Efstathiou, S. and Simas, J.P.\*, Stevenson PG\* (2009) "In vivo" Imaging of Murid Herpesvirus-4 infection. *J. Gen. Virol.* 90: 21-32 \*Joint corresponding authors (Journal IF: 3.092)

## Molecular Microbiology and Infection Unit

### Description and Objectives

The main objective of the Unidade de Microbiologia Molecular e Infecção is to understand the dynamics of populations of bacterial pathogens and how they respond to selective forces. Current work focuses on characterizing the effect of antimicrobial use and human vaccination on the bacterial population. Investigations are also exploring the relationships between commensal and disease causing populations of the same bacterial pathogen with the aim of identifying particularly successful clones at causing disease as well as successful colonizers for further characterization. A strong bioinformatics effort in the area of data warehousing, the development of user interfaces, data analysis and visualization tools is ongoing. The development of novel laboratory methodologies for the diagnosis of infectious diseases is also an active area of research.

### Head of Unit

Mário Ramirez, PhD – Associate Professor, FMUL

### Principal Investigators

José Melo Cristino, MD, PhD – Full Professor, FMUL

Thomas Hänseid, MD, PhD - Assistant Professor, FMUL

João Carriço, PhD - Assistant Investigator, FMUL

### Research Team

Sandra Aguiar	- PhD fellowship, FCT
Maria João Frias	- PhD fellowship, FCT
Elisabete Martins	- PhD fellowship, FCT
Margarida Carrolo	- PhD fellowship, FCT
Rosangela Frita	- PhD fellowship, FCT

### Research Areas

Population biology and epidemiology  
 Interactions of malaria and other infectious diseases  
 Novel diagnostic tools for diagnosis and sensitivity testing  
 Bioinformatics

### Recent Most Relevant Publications

Aguiar, S.I., Serrano, I., Pinto, F.R., Melo-Cristino, J. and Ramirez, M. (2008) The presence of the pilus locus is a clonal property among pneumococcal invasive isolates. *BMC Microbiol.* 8: 41 (Times cited: 9)(Journal IF: 2.877)

Hänseid, T., Egan, T.J. and Grobusch, M.P. (2007) Haemozoin: from melatonin pigment to drug target, diagnostic tool, and immune modulator. *Lancet Infect. Dis.* 7: 675-685 (Times cited: 12)(Journal IF: 13.165)

Martins, E.R., Pessanha, M.A., Ramirez, M., Melo-Cristino, J. and the Portuguese Group for the Study of Streptococcal Infections (2007) Analysis of Group B streptococcal isolates from infants and pregnant women in Portugal revealing two lineages with enhanced invasiveness. *J. Clin. Microbiol.* 45: 3224-3229 (Times cited: 10)(Journal IF: 3.945)

Carriço, J.A., Silva-Costa, C., Melo-Cristino, J., Pinto, F.R., de Lencastre, H., Almeida, J.S. and Ramirez, M. (2006) Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. J. Clin. Microbiol. 44: 2524-2532 (Times cited: 13)(Journal IF: 3.945)

Pinho, M.D., Melo-Cristino, J., Ramirez, M.; Portuguese group for the study of streptococcal infections. (2006) Clonal relationships between invasive and noninvasive lancefield group C and G Streptococci and *emm*-specific differences in invasiveness. J. Clin. Microbiol. 44: 841-846 (Times cited: 7)(Journal IF: 3.945)

### Ongoing Research Projects

2009/2011 Implications for therapy and disease presentation of the changes triggered by the use of the pneumococcal 7-valent conjugate vaccine, FCT. Coordinator: M. Ramirez (PIC/IC/83065/2007)

2009/2011 CAREPNEUMO - Combating antibiotic resistance pneumococci by novel strategies based on in vivo and in vitro host-pathogen interactions Coordinator: M. Ramirez (FP7-HEALTH-2007-223111)

2009/2011 Does malaria increase susceptibility to tuberculosis? The role of malaria-pigment induced immune-suppression, FCT. Coordinator: Thomas Hanscheid (PIC/IC/83214/2007)

2008/2011 Population and genomic consequences of vaccination against *Streptococcus pneumoniae*, FCT. Coordinator: M. Ramirez (PTDC/SAU-ESA/64888/2006)

2008/2011 A computational biology approach using *Streptococcus pneumoniae* as a model towards an integrative view of bacteria/host interactions and evolution, FCT. Coordinator: M. Ramirez (PTDC/SAU-ESA/71499/2006)

2008/2011 *Streptococcus pyogenes* invasive disease and antimicrobial resistance – two faces of a changing pathogen, FCT. Coordinator: J. Melo-Cristino (PTDC/SAU-ESA/72321/2006)

2007/2010 Caracterização microbiológica e molecular de *Streptococcus spp.* patogénicos e sua importância na profilaxia e terapêutica das infeções humanas em Portugal, Fundação Calouste Gulbenkian. Coordinator: J. Melo-Cristino

### Publications in Peer Review International Journals 2009

Pamplona, A., Hanscheid, T., Epiphany, S., Mota, M.M., Vigário, A.M. (2009) Cerebral malaria and the hemolysis/methemoglobin/heme hypothesis: shedding new light on an old disease. Int. J. Biochem. Cell Biol. 41: 711-716 (Journal IF: 4.178)

Hänscheid, T., Valadas, E. (2009) Diagnosis of cryptosporidiosis using PCR or auramine O with LED fluorescent microscopy: which end of the stick? Acta Trop. 109: 247-248 (Journal IF: 1.707)

Earnshaw, S., Monnet, D.L., Duncan, B., O'Toole, J., Ekdahl, K., Goossens, H., European Antibiotic Awareness Day Technical Advisory Committee, and European Antibiotic Awareness Day Collaborative Group (2009) European Antibiotic Awareness Day, 2008 - the first Europe-wide public information campaign on prudent antibiotic use: methods and survey of activities in participating countries. Euro. Surveill. 14: 19280 (Journal IF: NA)

Carrolo, M., Pinto, F.R., Melo-Cristino, J. and Ramirez, M. (2009) Pherotypes are driving genetic differentiation within *Streptococcus pneumoniae*. BMC Microbiol. 9: 191 (Journal IF:2.877)

Frias, M.J., Melo-Cristino, J. and Ramirez, M. (2009) The autolysin LytA contributes to efficient bacteriophage progeny release in *Streptococcus pneumoniae*. J. Bacteriol. 191: 5428-5440. (Journal IF:3.636)

Francisco, A.P., Bugalho, M., Ramirez, M. and Carriço, J.A. (2009) Global optimal eBURST

analysis of multilocus typing data using a graphic matroid approach. BMC Bioinformatics 10: 152 (Journal IF:3.781)

#### Communications in International Conferences

Martins, E.R. et al. Pili are a clonal property of *Streptococcus agalactiae*. 19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Helsinki, Finland. May 16-19, 2009.

Silva-Costa, C. et al. *In vitro* susceptibility to tigecycline and to an extended panel of antimicrobials of Gram positive and Gram negative isolates in Portugal. 19th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Helsinki, Finland. May 16-19, 2009.

Aguiar, S.I. et al. Pili distribution among invasive *Streptococcus pneumoniae* in Portugal after the introduction of the 7-valent conjugate vaccine. 9th European Meeting on the Molecular Biology of the Pneumococcus (Europneumo), Bern, Switzerland. June 4-7, 2009.

Carrolo, M. et al. Sexual preferences are driving genetic differentiation within *Streptococcus pneumoniae*. 9th European Meeting on the Molecular Biology of the Pneumococcus (Europneumo), Bern, Switzerland. June 4-7, 2009.

Pinto, F.R. et al. Improving the analysis of pneumococcal multi-strain array CGH data. 9th European Meeting on the Molecular Biology of the Pneumococcus (Europneumo), Bern, Switzerland. June 4-7, 2009.

#### Communications in National Conferences

Francisco, A. et al. Exploring the relationship of strains characterized by sequence based typing methods using goeBURST algorithm. Microbiotec 2009, Vilamoura, Portugal. November 28-30, 2009.

Carriço, J.A. et al. New developments for evaluating the congruence between microbial typing methods. Microbiotec 2009, Vilamoura, Portugal. November 28-30, 2009.

Carriço, J.A. et al. [www.comparingpartitions.info](http://www.comparingpartitions.info) - a web-tool for quantitative assessment of classification agreement. Jornadas de Bioinformática 2009, Lisboa, Portugal. November 3-6, 2009.

Francisco, A. et al. goeBURST software: a scalable platform for the data analysis, integration and visualization of sequence based typing methods data. Jornadas de Bioinformática 2009, Lisboa, Portugal. November 3-6, 2009.

#### Invited Lectures and Seminars

Hänscheid, T. Microscopic methods for the diagnosis of TB & Laboratory Infrastructures for TB work in Africa. CANTAM meeting, Brazzaville, Republic of Congo. February 2009.

Hänscheid, T. Microscopic methods for the diagnosis of TB & Laboratory Infrastructures for TB work in Africa. CANTAM meeting, Yaounde, Camarões. May 2009.

Hänscheid, T. New colorations and Microscopic methods for the diagnosis of TB. TB Workshop, Lambaréné. Gabão. December 2009.

Carriço, J.A. Bacterial clone definition using microbial typing methods: Advantages and caveats. Microbiotec 2009, Vilamoura, Portugal. November, 28-30.

#### Organisation of National and International Conferences

Carriço, J.A. served on the Scientific and Organizing Committees of Jornadas de Bioinformática

2009, Lisboa, Portugal. November 3-6, 2009.

### **Prizes, Honours and Awards**

Best poster award in Bioinformatics, Comparative Genomics and Evolution. Microbiotec 2009 to Carriço, J.A., Severiano, A., Pinto, F., Melo-Cristino, J. and Ramirez, M. “New developments for evaluating the congruence between microbial typing methods”

### **Advanced Teaching**

Melo Cristino, J., Hänscheid, T. and Ramirez, M. Lecturers and Organizers of the module “Microbiology”. Master Course in Emerging Infectious Diseases 2008-2009, Faculdade de Medicina da Universidade de Lisboa

### **Thesis Completed**

#### **Master Thesis**

Teresa Maria Monteiro Maurício Lopes Meira (2009) Estudo da resistência aos anti-maláricos – desenvolvimento de um novo teste de sensibilidade Plasmodium spp., baseado na detecção da hemozoína. Master in Biochemistry, Faculdade de Ciências da Universidade de Lisboa. Supervisors: T. Hänscheid and M.M. Meireles .

## Malaria Unit

### Description and Objectives

Malaria, caused by *Plasmodium* species transmitted between *Anopheles* mosquitoes and humans, continues to be a major devastating disease and global public health problem. Annually, more than 300-400 million people worldwide develop malaria and nearly 1 million children die. There is no highly effective, deployable malaria vaccine, and the prevalence of drug-resistant malaria is increasing throughout Africa, Asia and South America which has been associated with increasing morbidity and mortality.

Today's efforts towards the control of malaria are limited by our current understanding of the biology of *Plasmodium* and of the complex relationships between the three components of the malaria triad—the human, the mosquito, and the parasite. Despite work in each of these systems advancing rapidly in the last decade, the malaria science community is now at a turning point where many believe the success of current and future control efforts will depend on making significant advances in our understanding of the biology of malaria. A potential approach to malaria control is to target mechanisms crucial for the development of *Plasmodium* and/or the pathology caused by its infection. This requires detailed knowledge of the complex host cell-*Plasmodium* interaction. The Malaria Unit is focusing its efforts on the study of mechanisms used by *Plasmodium*-induced host factors important for the establishment and control of a malaria infection and the pathology associated with it. Determining the host proteins that either promote infections or help ward them off would not only shed light on the basic mechanisms of malaria infection but also provide potential new targets for therapy.

### Head of Unit

Maria M. Mota, MSc, PhD - Principal Investigator, Laboratório Associado, IMM

### Principal Investigators

Miguel Prudêncio, PhD - Staff Scientist, IMM-FCT

### Research Team

Ana Pamplona, PhD	– Post-Doc fellowship, FCT (left in July)
Ana Margarida Vigário, PhD	– Post-Doc, Invited researcher from Univ. da Madeira
Claúdia Cunha, PhD	– Post-Doc fellowship, FCT
Eliana Real, PhD	– Post-Doc fellowship, EMBO
Ghislain Cabal, PhD	– Post-Doc, fellowship, Marie Curie
Kirsten Hanson, PhD	– Post-Doc, fellowship, Marie Curie
Peter Liehl, PhD	– Post-Doc fellowship, INSERM (FCT until March)
Vanessa Zuzarte Luís, PhD	– Post-Doc, Fellowship, EMBO (FCT until August)
Carina Santos	– PhD fellowship, FCT
Maurice Itoe	PhD fellowship, Marie Curie
Patricia Inácio	– PhD fellowship, FCT
Sílvia Portugal	– PhD fellowship, FCT
Fernanda Baptista	– Laboratory and Insectary manager, fellowship, HHMI
Ana Parreira	– Laboratory and Insectary technician, fellowship, FCT grant

## Research Areas

Host-*Plasmodium* cellular and molecular Interactions

*Plasmodium* biology

Malaria pathogenesis

Anti-malarial intervention

## Major Scientific Achievements

Following their deposition in the skin of their vertebrate host, by *Anopheles* female mosquitoes, *Plasmodium* sporozoites must travel to the liver where they traverse and infect hepatocytes. Once there, each sporozoite develops and replicates into thousands of merozoites that are then released in the bloodstream, infecting red blood cells (RBCs) and causing all the malaria associated pathology.

Our unit has been focused on revealing the role of host factors not only in the establishment of *Plasmodium* sporozoites inside hepatocytes (liver stage) but also on the establishment of malaria-associated pathology (blood stage). The Malaria Unit of the IMM has been at the forefront of liver stage infection research, identifying mammalian host molecules that influence *Plasmodium* liver infection. We have pioneered the use of high-throughput RNA interference (RNAi) to identify host genes involved in liver infection by *Plasmodium* parasites (Rodrigues *et al.*, 2008. *Cell Host and Microbe*; Prudêncio *et al.*, 2008. *PLoS Pathogens*). Recently, we carried out a microarray study that showed that *Plasmodium* development inside the hepatocyte induces the expression of host genes that may provide the parasite with the nutrients required for its replication (Albuquerque *et al.* 2009. *BMC Genomics*). Moreover, we have developed new reliable and expedite methods to measure hepatic infection by *Plasmodium* with increasing efficiency and cost-effectiveness (Prudêncio *et al.*, 2008. *Cell Microbiol.*; Ploemen *et al.*, 2009. *PLoS ONE*).

Importantly, although the liver and the blood stages constitute natural sequential steps of *Plasmodium*'s life cycle, they are frequently seen as independent entities. Nevertheless, these stages of *Plasmodium* infection may frequently occur simultaneously in endemic areas. Indeed, after infecting hepatocytes for approximately a week, *Plasmodium* can infect RBCs for days or even weeks after that. Thus, a new infection can be initiated while the parasites from the previous one are still replicating inside erythrocytes. We have recently asked what would be the impact of an ongoing blood stage infection on a subsequent *Plasmodium* sporozoite liver infection. Using a rodent model of infection, we showed that ongoing blood stage infections impair the growth of *Plasmodium* sporozoites in hepatocytes and that secondary liver infections can only be established after a previous blood stage has been reduced below a certain threshold. This effect is parasite density dependent and so phenotypically akin to quorum sensing. We eliminate *Plasmodium*-secreted factors, host cell survival and innate or adaptive immunity as explanations for this observation. Instead, we show that parasitized red blood cells increase host expression of the iron regulatory hormone hepcidin, which preferentially sequesters iron in macrophages. Reducing iron availability to infected hepatocytes *in vivo* limits *Plasmodium* sporozoite proliferation. Finally we demonstrate how this niche protection resulting from the inter-kingdom interaction between *Plasmodium* and its host provides a rationale for epidemiological observations from human malarial infections that were previously unexplained. Most of the work summarised above has been published in medium- to high-impact international journals. Those that have not yet been published have been submitted for publication.

## Recent Most Relevant Publications

Albuquerque, S.S., Carret, C., Grosso, A.R., Tarun, A.S., Peng, X., Kappe, S.H., Prudêncio, M. and Mota, M.M. (2009) Host cell transcriptional profiling during malaria liver stage infection reveals a coordinated and sequential set of biological events. *BMC Genomics* 10: 270 (Journal IF: 3.926)

Prudêncio, M. and Lehmann, M.J. (2009) Illuminating the host - how RNAi screens shed light



on host-pathogen interactions. *Biotechnol. J.* 4: 826-837 (Journal IF: NA)

Rodrigues, C.D., Hannus, M., Prudêncio, M., Martin, C., Gonçalves, L.A., Portugal, S., Epiphanio, S., Akinc, A., Hadwiger, P., Jahn-Hofmann, K., Röhl, I., van Gemert, G.J., Franetich, J.F., Luty, A.J.F., Sauerwein, R., Mazier, D., Koteliensky, V., Vornlocher, H.P., Echeverri, C.J. and Mota, M.M. (2008) Host scavenger receptor SR-BI plays a dual role in the establishment of malaria parasite liver infection. *Cell Host Microbe* 4: 271-282 (Times cited: 11)(Journal IF: 7.436)

Epiphanio, S., Mikolajczak, S.A., Gonçalves, L.A., Pamplona, A., Portugal, S., Albuquerque, S., Goldberg, M., Rebelo, S., Anderson, D.G., Akinc, A., Vornlocher, H.P., Kappe, S.H., Soares, M.P. and Mota, M.M. (2008) Heme oxygenase-1 is an anti-inflammatory host factor that promotes murine Plasmodium liver infection. *Cell Host Microbe* 3: 331-338 (Times cited: 10)(Journal IF: 7.436)

Pamplona, A., Ferreira, A., Balla, J., Jeney, V., Balla, G., Epiphanio, S., Chora, A., Rodrigues, C.D., Gregoire, I.P., Cunha-Rodrigues, M., Portugal, S., Soares, M.P. and Mota, M.M. (2007) Heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria. *Nat. Med.* 13: 703-710 (Times cited: 55)(Journal IF: 27.553)

Prudêncio, M., Rodriguez, A. and Mota, M.M. (2006) The silent path to thousands of merozoites: the Plasmodium liver stage. *Nat. Rev. Microbiol.* 4: 849-856 (Times cited: 37)(Journal IF: 14.310)

### Ongoing Research Projects

2009/2012 The function and dynamic of cholesterol during Plasmodium liver stage infection, European Commission. Fellow: G. Cabal. Coordinator: M. Mota (PIEF-2008-GA-235864)

2008/2011 The role of hepatic type cell polarity during Plasmodium, European Commission. Fellow: K. Hanson. Coordinator: M. Mota (PIEF-GA-2008-221854)

2008/2012 Intervention strategies against malaria, European Commission. Coordinator: NIMR, London (PITN-GA-2008-215281)

2008/2011 The role of host cell kinases in Plasmodium development inside hepatocytes during the liver stage of malaria infections, FCT. Coordinator: M. M. Mota (PTDC/BIA-MIC/72211/2006)

2008/2011 The role of SR-BI in malaria infection, FCT. Coordinator: M. Prudêncio (PTDC/BIA-BCM/71920/2006)

2008/2011 Cellular and molecular mechanisms of action of carbon monoxide in protection from severe malaria, FCT. Coordinator: A. Pamplona (PTDC/SAU-MII/64125/2006)

2005/2009 Functional genomics in malaria – which are the host cell molecules and pathways required for Plasmodium development inside hepatocytes?, Howard Hughes Medical Institute. Coordinator: M.M. Mota (HHMI#55005523, International Research Scholars Program. Infectious Diseases and Parasitology - 2005)

2004/2009 The role of host cell factors in the full development of the malaria parasite inside hepatocytes, European Science Foundation. Coordinator: M.M. Mota (EURYi - European Young Investigators Awards - 2004).

### Publications in Peer Review International Journals 2009

Albuquerque, S.S., Carret, C., Grosso, A.R., Tarun, A.S., Peng, X., Kappe, S.H., Prudêncio, M and Mota, M.M. (2009) Host cell transcriptional profiling during malaria liver stage infection reveals a coordinated and sequential set of biological events. *BMC Genomics* 10: 270 (Journal IF: 3.926)

- Liehl, P., França, A.R., Prudêncio, M., Latz, E., Zaidman-Rémy, A. and Mota, M.M. (2009) Phosphothioate oligodeoxynucleotides inhibit Plasmodium sporozoite gliding motility. *Cell Microbiol. (Epub November 12, 2009)*(Journal IF: 5.598)
- Pamplona, A., Hanscheid, T., Epiphanio, S., Mota, M.M. and Vigário, A.M. (2009) Cerebral malaria and the hemolysis/methemoglobin/heme hypothesis: shedding new light on an old disease. *Int. J. Biochem. Cell Biol.* 41: 711-716 (Journal IF: 4.178)
- Parvanova, I., Epiphanio, S., Fauq, A., Golde, T.E., Prudêncio, M. and Mota, M.M. (2009) A small molecule inhibitor of signal peptide peptidase inhibits Plasmodium development in the liver and decreases malaria severity. *PLoS One* 4: e5078 (Journal IF: NA)
- Ploemen, I.H., Prudêncio, M., Douradina, B.G., Ramesar, J., Fonager, J., van Gemert, G.J., Luty, A.J., Hermsen, C.C., Sauerwein, R.W., Baptista, F.G., Mota, M.M., Waters, A.P., Que, I., Lowik, C.W., Khan, S.M., Janse, C.J. and Franke-Fayard, B.M. (2009) Visualisation and quantitative analysis of the rodent malaria liver stage by real time imaging. *PLoS One* 4: e7881 (Journal IF: NA)
- Prudêncio, M. and Lehmann, M.J. (2009) Illuminating the host - how RNAi screens shed light on host-pathogen interactions. *Biotechnol. J.* 4: 826-837 (Journal IF: NA)
- Prudêncio, M., Derbyshire, E.T., Marques, C.A., Krishna, S., Mota, M.M. and Staines, H.M. (2009) *Plasmodium berghei*-infection induces volume-regulated anion channel-like activity in human hepatoma cells. *Cell Microbiol.* 11: 1492-1501 (Journal IF: 5.598)
- Vale, N., Prudêncio, M., Marques, C.A., Collins, M.S., Gut, J., Nogueira, F., Matos, J., Rosenthal, P.J., Cushion, M.T., do Rosário, V.E., Mota, M.M., Moreira, R. and Gomes, P. (2009) Imidazoquinones as antimalarial and antipneumocystis agents. *J. Med. Chem.* 52: 7800-7807 (Times cited: 1)(Journal IF: 4.898)

### Communications in International Conferences

- Hanson, K. Does hepatocyte polarity influence liver stage Plasmodium infection? ESF-EMBO Conference on Cell Polarity and Membrane Traffic, Sant Feliu de Guixols, Barcelona, Spain. May 23-28, 2009 (Poster presentation)
- Portugal, S. Malaria liver infection is decreased during blood-stage infection. Vivax Malaria Research III: 2009 and Beyond, Gamboa Rainforest Resort, Panama. May, 24-28, 2009 (Oral presentation)
- Portugal, S. Malaria liver infection is decreased during blood-stage infection. 5th MIM Pan-African Malaria Conference, Nairobi, Kenya. October, 31-November, 13, 2009 (Oral presentation)
- Prudêncio, M. Investigating the Liver Stage of Malaria Infection – Recent Findings and Technical Advances. A Collaborative Solution for Tropical Diseases – The Luso-American Response, Lisbon, Portugal. July 8-10, 2009 (Oral presentation)
- Santos, C., Actin dynamics in Plasmodium sp. infected hepatocytes, Research Advances in Malaria. Cell Biology of Malaria: Understanding Infection, targeting Interventions, Baltimore, Maryland, USA. May 28-29, 2009 (Poster presentation)

### Invited Lectures and Seminars

- Mota, M.M. The impact of blood stage infection on Plasmodium development inside hepatocytes, Gordon Research Conferences - 2009 Program, Malaria - Parasite Biology And Host-Parasite Interactions, Magdalen College, Oxford, United Kingdom. September 6-11, 2009
- Mota, M.M. Identification of host factors and lead compounds for development of novel agents targeting Plasmodium-infect hepatocytes - can they be used to target hypnozoite stages? American Society for Tropical Medicine and Hygiene Meeting, Washington, USA. November, 18-22, 2009

Prudêncio, M. The liver stage of malaria infection. Second Meeting of Plataforma Ibérica de Malária, GSK Diseases of the Developing World Research Centre, Tres Cantos, Madrid, Spain. December 17-18, 2009

Mota, MM. Malaria blood stage infection impairs *Plasmodium* development inside hepatocyte, Second Meeting of Plataforma Ibérica de Malária, GSK Diseases of the Developing World Research Centre, Tres Cantos, Madrid, Spain. December 17-18, 2009

Mota, M.M. Approaching Malaria from Various Angles, Harvard Medical School Portugal Program - First International symposium, Centro Cultural de Belém, Lisbon, Portugal. December 14-15, 2009

### **Organization of National and International Conferences**

Mota, M.M. co-organized the 5th Annual BioMalPar Conference on the Biology and Pathology of the Malaria Parasite, Heidelberg, Germany. May 18-21, 2009

### **Prizes, Honours and Awards**

Prémio Mulher Activa 2009 – Third Prize awarded to Maria Mota for the contribute given through her work in malaria research and as co-founder and Presidente of Associação Viver a Ciência (AvC) to the development of Portuguese society and Portuguese speaking communities.

### **Patents**

Method for the prevention of malaria infection of humans by hepatocyte growth factor antagonists, South Africa: Issued Patent No.: 2005/08178. Based on International PCT Publication No. WO2004/080420, African Reg. Ind. Prop. Org. - Application No. AP/P/2005/003411, Brazil - Application No. PI0408268-0, China - Publication No. CN1809382A, Europe – Publication No. 1601378, Hong Kong – Publication No. 1093903A, Japan - Application No. 2006-507105, United States - Publication No.: US 2004-0185050 A1.

### **Advanced Teaching**

Mota, M.M. Approaching Malaria from the Host side, Predoc's Scientific Symposium, Dresden International Graduate School for Biomedicine and Bioengineering, Wroclaw, Poland. April 25-27, 2009

Santos, C. "Principles of Fluorescence Microscopy Seminar BioImaging Nanocourse on Microscopy", IMM PhD Programme, IMM, Lisbon. October 27-28, 2009

### **Public Perception of Science**

Prémio Mulher Activa 2009 – Third Prize attributed to Maria Mota for the contribute given through her work in malaria research and as co-founder and Presidente of Associação Viver a Ciência (AvC) to the development of Portuguese society and Portuguese speaking communities

Visit to the lab of a group of 20 pre-school children from APIA - Associação de Apoio à Infância da Ajuda, December 2009

## Thesis Completed

### Master Thesis

Ana Pena (2009) “Carbon monoxide and brain sequestration of *Plasmodium berghei* ANKA in experimental cerebral malaria” Mestrado em Biologia Celular e Biotecnologia, Faculdade de Ciências da Universidade de Lisboa. Supervisors: A. Pamplona and R. Zilhão.

## Cellular Immunology Unit

### Description and Objectives

The Cellular Immunology Unit studies mechanisms able to induce and maintain immune tolerance. We study diseases, or animal models of human pathologies, where the immune system has an inappropriate action, such as in autoimmunity, transplantation or allergy. We aim to reprogramme the immune system, inducing regulatory mechanisms, such as the reduction of pathogenic cells and the expansion of regulatory T cells, leading to a reset of the tolerance state. We always strive to validate our experimental results in collaboration with clinical scientists.

### Head of Unit

Luis Graça, MD, PhD – Assistant Professor, FMUL

### Research Team

Ana Água-Doce	- PhD fellowship, FCT
Catarina Almeida	- Master student, IEFP
Ivonne Wollenberg	- PhD fellowship, FCT
Joana Duarte	- PhD fellowship, FCT
Marta Caridade, MSc	- PhD fellowship, FCT
Marta Monteiro, PhD	- Post-Doc fellowship, FCT
Vanessa Oliveira, PhD	- Post-Doc fellowship, FCT

### Research Areas

Immune tolerance  
Regulatory T cells  
Natural Killer T cells (NKT)  
Allergy  
Transplantation  
Autoimmunity  
Hemophilia

### Major Scientific Achievements

- a) CD4-blockade can induce long-term tolerance in a mouse model of chronic autoimmune arthritis preserving immune competence. Tolerance is associated with alteration of Th17/Treg ratio in the synovia.

We found treatment with non-depleting anti-CD4 monoclonal antibody (MAb) could prevent the development of arthritis in an animal model of rheumatoid arthritis (SKG mice). The treated mice had a significant reduction of pro-inflammatory cytokines in the serum (IL-6, TNF, MCP-1, and IFN- $\gamma$ ) and although previous studies have reported a protective role for IL-10 in SKG mice, we did not observe increased IL-10 in mice treated with anti-CD4. However within the synovial membranes the ratio of Foxp3/IL-17 expressing cells was inverted in mice treated with the MAb.

We have also shown that once tolerance is induced it confers long-term protection from arthritis even following challenge with curran 60 days following initial MAb treatment. Furthermore, when the MAb is administered after the onset of the disease the disease does not remit but its progression is halted. Finally, we have shown that mice treated with non-depleting anti-CD4, and thus protected from arthritis, remain competent to mount CD4-dependent immune responses to ovalbumin (OVA).

- b) Suboptimal T cell stimulation leads to Foxp3 Treg conversion independent of external TGF- $\beta$ . Low intensity T cell stimulation triggers TGF- $\beta$  production that supports Treg conversion.

It is now well established that Foxp3<sup>+</sup> Treg cells can be peripherally induced in a process TGF- $\beta$ -dependent. Several reports have shown tolerance can be induced in TCR-transgenic RAG<sup>-/-</sup> mice, where T cells acquire *de novo* expression of Foxp3. Two well characterized examples are the induction of transplantation tolerance with non-depleting anti-CD4 and the induction of oral tolerance to OVA. Since this process is TGF- $\beta$ -dependent, it is usually assumed *in vitro* T cell stimulation in the presence of exogenous TGF- $\beta$  reproduces the *in vivo* peripheral conversion of Treg cells. However, while the *in vitro* system leads to frequencies of Foxp3<sup>+</sup> cells in excess of 50% of the T cells, the *in vivo* conversion results in 1%-2% Foxp3<sup>+</sup> cells in the appropriate draining lymph nodes. We also considered that most reagents that have been implicated in the induction of Treg-dependent dominant tolerance target molecules involved in the “immune synapse” suggesting that a possible common denominator for tolerance induction *in vivo* would be to make immunogenic stimuli to be perceived by CD4 T cells in a “suboptimal” way.

We addressed the hypothesis above making use of different OVA-specific TCR-transgenic RAG-deficient mice (DO11.10 and OT-II). We have induced oral tolerance to OVA, or tolerance with non-depleting anti-CD4 to OVA-expressing skin grafts, and shown that draining lymph nodes contained 1% - 2% Foxp3<sup>+</sup> Treg cells. *In vitro*, Foxp3<sup>-</sup> TCR-transgenic cells stimulated by dendritic cells loaded with the appropriate peptide can efficiently convert into Foxp3<sup>+</sup> Treg cells (>50%) when exogenous TGF- $\beta$  is added to the cultures. We found that in the absence of exogenous TGF- $\beta$ , we could observe a small percentage of conversion 1% - 2% when we reduced the concentration of peptide to suboptimal levels, or added anti-CD4 to the culture medium. This conversion was still TGF- $\beta$ -dependent as it was abrogated by addition of neutralizing anti-TGF- $\beta$  MAb.

- c) Long-term tolerance to allergens can be induced with anti-CD4 antibodies and is independent of Foxp3<sup>+</sup> Treg cells. Tolerance can be induced in sensitized hosts, is antigen-specific, and can prevent allergic airways disease and anaphylaxis.

We have shown CD4 blockade at the time of allergen exposure induces antigen-specific tolerance in mice, sparing immune competence to unrelated antigens. Tolerance can be induced to a model antigen (OVA) or a clinically relevant allergen (house dust mite, HDM).

Tolerance induction is robust enough to be effective in pre-sensitized or allergen-specific TCR-transgenic animals. Tolerant mice are protected from airways eosinophilia, lung infiltration with Th2 cells, production of allergen-specific IgG1 and IgE, and airway hyperreactivity. Using TCR-transgenic T cells allowed us to identify the underlying mechanism leading to tolerance induction. We found this mechanism to be distinct from previously described tolerance inducing processes: it is independent of Th1 deviation, IL-10, or TGF- $\beta$ . We also confirmed tolerance as being Foxp3-independent as treatment with CD4-blockade was efficient in Foxp3-deficient T/Bmc mice (OVA-specific TCR transgenic, HA-specific BCR knock-in, and RAG<sup>-/-</sup>).

Adoptive cell transfer experiments, allowed us to follow the fate of CFSE labeled OVA-specific TCR-transgenic T cells while tolerance was being established. We found that although CD4-blockade would not lead to T cell deletion in the absence of antigenic stimulation, a large number of antigen-specific T cells triggered apoptosis when exposed to antigen under the cover of CD4-blockade. Therefore, the tolerogenic treatment does not target T cell proliferation, but rather CD4 blockade reduces the numbers of effector T cells by activation-induced cell death. Our findings describe a strategy for tolerance induction applicable to other immunogenic proteins besides allergens.

- d) Foxp3<sup>+</sup> Natural Killer T cells can be induced *in vitro* and *in vivo* when activated in a TGF- $\beta$ -rich environment. These NKTreg cells can suppress T cell proliferation and retain liver-seeking behavior.

Functional plasticity in response to cytokines has been considered a characteristic of conventional T lymphocytes. We have shown that iNKT-cell activation with TGF- $\beta$  induces *de novo* Foxp3 expression. Foxp3<sup>+</sup>iNKT cells displayed a phenotype similar to Treg cells,

suppressing through a contact-dependent, GITR-mediated mechanism. Foxp3 expression was stably maintained upon adoptive transfer, although Foxp3<sup>+</sup>iNKT cells migrated to anatomical sites distinct from Tregs: while transferred Tregs are recovered from secondary lymphoid tissue, NKTreg cells are mainly recovered from the liver.

Following the delivery of the NKT cell ligand  $\alpha$ -GalCer by gavage, we observed *in vivo* generation of Foxp3<sup>+</sup>iNKT cells in the TGF- $\beta$ -rich gut environment. On the contrary, the same procedure in animals with NKT cells unable to respond to TGF- $\beta$  (TGF- $\beta$ RIL.DN) did not lead to Foxp3 expression among NKT cells from mesenteric LNs.

In conclusion, iNKT cells respond to environmental cues by following distinct differentiation pathways that enable them to synergize with CD4 T-cells in determining the outcome of immune responses.

### Recent Most Relevant Publications

Oliveira, V., Agua-Doce, A., Duarte, J., Soares, M.P. and Graca, L. (2006) Regulatory T cell maintenance of dominant tolerance: Induction of tissue self-defense? *Transpl. Immunol.* 17: 7-10 (Times cited: 9)(Journal IF: 1.869)

Graça, L., Le Moine, A., Cobbold, S.P. and Waldmann, H. (2003) Dominant Transplantation Tolerance. *Curr. Opin. Immunol.* 15: 499-506 (Times cited: 53)(Journal IF: 10.455)

Graça, L.\*, Lin, C-Y.\*, Cobbold, S.P. and Waldmann, H. (2002) Dominant transplantation tolerance impairs CD8<sup>+</sup> T cell function but not expansion. *Nat. Immunol.* 3: 1208-1213 \* Joint first authors (Times cited: 91)(Journal IF: 25.113)

Graça, L., Thompson, S., Lin, C-Y., Adams, E., Cobbold, S.P. and Waldmann H. (2002) Both CD4<sup>+</sup>CD25<sup>+</sup> and CD4<sup>+</sup>CD25<sup>-</sup> regulatory cells mediate dominant transplantation tolerance. *J. Immunol.* 168: 5558-5565 (Times cited: 219)(Journal IF: 6.000)

Graça, L., Cobbold, S.P. and Waldmann, H. (2002) Identification of regulatory T cells in tolerated allografts. *J. Exp. Med.* 195: 1641-1646 (Times cited: 255)(Journal IF: 15.504)

### Ongoing Research Projects

2007/2009 Co-receptor and co-stimulation blockade for the induction of regulatory T cells in allergic airways disease, FCT. Coordinator: L. Graça (PTDC/SAU-MII/64279/2006)

2009/2011 Invariant Natural Killer T Cells in Allergic Asthma and Tolerogenic Therapy, FCT. Coordinator: L. Graça (PIC/IC/82895/2007)

### Publications in Peer Review International Journals 2009

Caetano-Lopes, J., Nery, A.M., Henriques, R., Canhão, H., Duarte, J., Amaral, P.M., Vale, M., Moura, R., Pereira, P.A., Weinmann, P., Abdulghani, S., Souto-Carneiro, M., Rego, P., Monteiro, J., Sakaguchi, S., Kontinen, Y., Graça, L., Queiroz, M.V., Vaz, M.F. and Fonseca, J.E. (2009) Chronic arthritis directly induces quantitative and qualitative bone disturbances leading to compromised biomechanical properties. *Clin. Exp. Rheumatol.* 27: 475-482 (Journal IF: 2.366)

Mancuso, M.E., Graça, L., Auerswald, G. and Santagostino, E. (2009) Haemophilia care in children--benefits of early prophylaxis for inhibitor prevention. *Haemophilia Suppl*1: 8-14 (Journal IF: 2.394)

### Communications in International Conferences

Agua-Doce, A. et al Induction of allergen-specific immune tolerance in sensitized mice with non-depleting anti-CD4 monoclonal antibodies. *Allergy & Asthma Symposium: Bridging Innate and Adaptive Immunity*, Bruges, Belgium. May 28-29, 2009 (Poster presentation)

Agua-Doce, A. et al. Enhancing tolerogenic antibodies to prevent Factor VIII inhibitors. Bayer Schering Pharma Hematology Conference: Bridging the gap between science & practice, Riga, Latvia. September 17-19, 2009 (Poster presentation) – *Best poster award*

Agua-Doce, A. et al. Induction of antigen-specific tolerance to aeroallergens: regulation without regulatory T cells. Keystone Symposium in Allergy and Astma, Keystone, Colorado, USA. January 21-23, 2009 (Poster presentation)

Almeida, C. et al. CD4 Blockade prevents iNKT-cell contribution to Allergic Airways Disease. European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Caridade, M. et al. The role of microenvironment in tolerance induction to foreign antigens. 4th ENII-MUGEN Immunology Summer School, Cappo Caccia, Sardinia, Italy. May 17-24, 2009 (Poster presentation)

Duarte, J. et al. Reprogramming the immune system with monoclonal antibodies in a mouse model of peanut-induced anaphylaxis. European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Monteiro, M. et al. Acquisition of Foxp3 Expression and Regulatory Function by Invariant Natural Killer T Cells. 5th International Symposium on CD1/NKT Cells, Kamakura, Japan. March 23-27, 2009 (Oral presentation)

Monteiro, M. et al. Acquisition of Foxp3 Expression and Regulatory Function by Invariant Natural Killer T Cells. Keystone Symposium on Regulatory T Cells, Keystone, Colorado, USA. March 1-6, 2009 (Oral presentation)

Oliveira, V. et al. Suboptimal conditions of TCR stimulation favor the induction of FoxP3+CD4+ T cells. Keystone Symposium on Regulatory T cells, Keystone, Colorado, USA. March 1-6, 2009 (Poster presentation)

Wollenberg, I. et al. Kinetic of germinal center CD4+ T cells during a primary immune response. Germinal Center Conference, Frankfurt, Germany. July 5-9, 2009 (Poster presentation) - *Travel Award*

Wollenberg, I. In vivo blockade of OX40L inhibits asthmatic lung inflammation. European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation) - *Travel Award*

### Communications in National Conferences

Agua-Doce, A. et al. CD4-blockade induces antigen-specific tolerance both to artificial and natural allergens. XXXV Annual Meeting of the Portuguese Society for Immunology, Lisbon, Portugal. September, 28-30 2009 (Poster presentation)

Agua-Doce, A. et al. CD4-blockade induces antigen-specific tolerance both to artificial and natural allergens. 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon, Portugal. October 12-13, 2009 (Poster presentation) - *Best poster award*

Almeida, C. et al. CD4 Blockade prevents iNKT-cell contribution to Allergic Airways Disease. XXXV Annual Meeting of the Portuguese Society for Immunology, Lisbon, Portugal. September 28-30, 2009 (Poster presentation)

### Invited Lectures and Seminars

Graça L. Immunotolerance induction – the immunological perspective. 53rd Congress of the German Society for Thrombosis and Haemostasis, Vienna, Austria. February 6, 2009.

Graça L. Indução terapêutica de tolerância em doenças autoimunes. Annual Meeting of NEDAI, Torres Novas. April 24, 2009.

Graça L. The link between immune dysregulation and inhibitor development. Bayer Schering



Pharma Haematology Conference 2009, Riga, Latvia. September 17, 2009.

Graça, L. Conventional Natural Killer T cells with unconventional lifestyles. Annual Congress of the Portuguese Society for Immunology, Lisbon, Portugal. September 30, 2009.

Graça L. Indução terapêutica de tolerância em doenças autoimunes. Reunião de LES do Hospital de Santa Maria, Lisboa. October 23, 2009.

Graça, L. Induction of antigen-specific tolerance to allergens by promoting activation-induced cell death. Mucosal Immunology Symposium, Portuguese Society for Immunology, Lisbon, Portugal. December 11, 2009.

### **Organisation of National and International Conferences**

Graca L. Organizer of Mucosal Immunology Symposium, from Sociedade Portuguesa de Imunologia, Instituto de Medicina Molecular, Lisbon. December 11, 2009.

### **Prizes, Honours and Awards**

Prof. Heimburger Award in Coagulation Diseases, CSL Behring, 2009 to Luis Graça.

Travel Award from the Germinal Centre Society to Ivonne Wollenberg to attend Germinal Center Conference, Frankfurt, Germany, 5-9 July 2009.

Bayer Schering Pharma Hematology Conference: Bridging the gap between science & practice Best Poster Award to Agua-Doce, A., Oliveira, V.G. and Graça, L. *Enhancing tolerogenic antibodies to prevent Factor VIII inhibitors.*

3<sup>rd</sup> IMM PhD Students Meeting Best Poster Award to Agua-Doce, A., Duarte, D., Oliveira, V.G. and Graça, L. *CD4-blockade induces antigen-specific tolerance both to artificial and natural allergens.*

### **Patents**

Água-Doce, A. and Graça, L. The use of adjuvant to facilitate the induction of tolerance to proteins. Universidade de Lisboa / Instituto de Medicina Molecular (13.11.2009).

Monteiro, M. and Graça, L. Foxp3+ regulatory natural killer T cells and a method for their generation. Universidade de Lisboa / Instituto de Medicina Molecular (13.11.2009)

### **Advanced Teaching**

Graça, L. Organizer “Immunology module”, Programa Gulbenkian de Formação Médica Avançada, held at IMM. January 2009.

### **Public Perception of Science**

Graça L. (2009) Alergia e Asma. In “Conhecer a Imunologia”. pp: 17-18. Livro editado pela Sociedade Portuguesa de Imunologia e distribuído com o jornal Público para celebrar o Dia da Imunologia.

## Thesis Completed

### Master Thesis

Catarina Almeida (2009) “Modulation of invariant Natural Killer T cells in immune mediated diseases” Mestrado em Bioquímica Médica, Faculdade de Ciências da Universidade de Lisboa.  
Supervisors: Marta Monteiro e Margarida Telhada.

## Molecular Immunology Unit

### Description and Objectives

We study the differentiation of T lymphocytes and their key roles in immunity to infection and cancer. Our projects focus on the development of these cells in the vertebrate thymus, and on their functions upon activation in the periphery. We are particularly interested in the anti-tumour and in pro- *versus* anti-inflammatory functions of specific subsets of T cells, currently focusing on  $\gamma\delta$  and on regulatory (Foxp3<sup>+</sup>)  $\alpha\beta$  T cells. We investigate differentiation and activation signals for T cells in the mouse system, which provides crucial *in vivo* models for infectious (such as malaria) and autoimmune diseases. We also study human peripheral blood T cells and, in particular, their recognition and elimination of lymphomas and leukemias. Overall, we envisage the identification of molecular mechanisms involved in the differentiation, activation and function of T cells, aiming towards the design of new treatments for cancer, on the one hand, and immune disorders, on the other.

### Head of Unit

Bruno Silva-Santos, PhD in Immunology, University College London; Assistant Professor, Faculdade de Medicina da Universidade de Lisboa.

### Principal Investigators

Ana Pamplona Santos, PhD in Biochemistry, Universidade Nova de Lisboa, Portugal.

### Research Team

Julie C. Ribot, PhD	- Post-Doc fellowship, EMBO
Anita Quintal Gomes, PhD	- Auxiliary Professor, ESTSL
Ana de Barros, MSc	- PhD fellowship, FCT
Daniel Vargas Correia, BSc	- PhD fellowship, FCT
Telma Lança, BSc	- PhD fellowship, FCT
Natacha Gonçalves-Sousa, BSc	- Research fellowship, FCT grant

### Research Areas

Thymic T cell development  
 T cell activation and functional differentiation  
 Tumour Immunology  
 Immunopathology of Severe Malaria

### Major Scientific Achievements

#### *Differentiation of murine $\gamma\delta$ T lymphocytes*

$\gamma\delta$  T lymphocytes make crucial contributions to cellular immune responses to pathogens through their production of the cytokines Interferon- $\gamma$  (IFN- $\gamma$ ) and Interleukin-17 (IL-17), which are also involved in tumor immunity (IFN- $\gamma$ ) and autoimmunity (IL-17 and IFN- $\gamma$ ). We hypothesized that these different outcomes could stem from distinct cellular subsets of  $\gamma\delta$  T lymphocytes present in the thymus and in secondary lymphoid organs (spleen, lymph nodes), which we set out to identify. Our results showed that most murine  $\gamma\delta$  lymphocytes express the Tumor Necrosis Factor Receptor (TNF-R) family-member CD27 and secrete IFN- $\gamma$ , whereas IL-17 production is restricted to CD27<sup>(-)</sup> cells. The distinguishing phenotypes of the mouse  $\gamma\delta$

subsets are essentially stable, even during infection (as that imposed by *Plasmodium* parasites). Critically, we demonstrated that these phenotypes are established during thymic development, where CD27 functions as a novel regulator of  $\gamma\delta$  cell differentiation, inducing the expression of lymphotoxin beta receptor (LT $\beta$ R) and genes associated with IFN- $\gamma$  production. Thus, CD27-regulated thymic events largely predetermine IFN- $\gamma$  versus IL-17 production by peripheral  $\gamma\delta$  cells. Moreover, when we tested multiple cytokine environments (rich in TGF- $\beta$ , IL-6, IL-21 or IL-23), we have not observed any “inter-conversion” between these phenotypes, suggesting that functional commitment of  $\gamma\delta$  cells is stable and indeed occurs in the thymus.

Furthermore, we were able to identify discrete intra-thymic progenitors (TCR $\gamma\delta^+$  CD27 $^+$  CD25 $^+$ ) shared by IFN- $\gamma$  and IL-17-producing  $\gamma\delta$  subsets. Since this common progenitor population expresses CD27, we investigated the effect of CD27 engagement with its ligand, CD70, a TNF-related transmembrane glycoprotein expressed abundantly on thymic epithelial cells. This resulted in a significant developmental bias toward CD27 $^+$  IFN- $\gamma^+$   $\gamma\delta$  thymocytes. Our data collectively established a novel model for the functional differentiation of pro-inflammatory  $\gamma\delta$  T cell subsets, where the balance between IFN- $\gamma$  and IL-17-producing  $\gamma\delta$  T cell subsets is established in the thymus and is critically determined by CD70:CD27 interactions.

#### *Tumour cell recognition by human $\gamma\delta$ T lymphocytes*

Human  $\gamma\delta$  T cells are potent killers of a variety of tumour cell lines. However, we and others have observed that fully-activated  $\gamma\delta$  T cells can only target a subset of all human tumour cell lines and primary biopsies tested. We have concentrated on identifying potential tumour antigens that underlie tumour cell targeting by  $\gamma\delta$  lymphocytes. Upon screening a panel of 20 lymphoma and leukaemia cell lines and 32 primary biopsies using a well established *in vitro* killing assay (with pre-activated  $\gamma\delta$  T cells), we performed cDNA microarray comparisons between “susceptible” (over 80% lysis in the assay) and “resistant” tumours (under 20% lysis) of identical origin (cytogenetic marks). We thus identified a panel of 11 differentially expressed genes encoding cell surface molecules that may be involved in  $\gamma\delta$ :tumour cell interactions and may constitute informative biomarkers for  $\gamma\delta$  T cell-mediated immunotherapy of leukemias and lymphomas.

Of the candidate genes identified, we have focused on ULBP-1, a member of the non-classical MHC class Ib protein family. Following from our data showing that ULBP-1 mRNA and protein expression on hematopoietic tumors strongly correlated with their susceptibility to  $\gamma\delta$  T cell-mediated killing, we have specifically knocked-out *ULBP-1* expression in susceptible tumours using RNA interference. Strikingly, we observed a significant reduction (60%) in  $\gamma\delta$  T cell-dependent leukaemia cell lysis, which was mirrored in essays where its receptor, NKG2D, was blocked through specific monoclonal antibodies. Moreover, ULBP-1 overexpression in resistant lymphoma cell lines was sufficient to make them susceptible to  $\gamma\delta$  T cell killing.

Provocatively, we have observed that primary many tumours down-regulate ULBP1 and are therefore resistant to  $\gamma\delta$  T cell targeting, which is suggestive of active “immuno-evasion” and “immuno-selection”. We are now attempting to revert this situation by inducing ULBP1 expression through therapeutic means.

#### **Recent Most Relevant Publications**

Gonçalves-Sousa, N., Ribot, J.C., deBarros, A., Correia, D.V., Caramalho, I. and Silva-Santos, B. (2010) Inhibition of murine gd lymphocyte expansion and effector function by regulatory ab T-cells is cell-contact dependent and sensitive to GITR modulation. *Eur. J. Immunol.* 40 : 61-70 (*Epub October 28, 2009*)(Journal IF: 4.865)

Ribot, J.C., deBarros, A., Pang, D.J., Neves, J.F., Peperzak, V., Girardi, M., Borst, J., Hayday, A.C., Pennington, D.J. and Silva-Santos, B. (2009) CD27 is a thymic determinant of the balance between IFN- $\gamma$ - and IL-17-producing  $\gamma\delta$  T cell subsets. *Nat. Immunol.* 10: 427-436 (Times cited: 7)(Journal IF: 26.218)

Correia, D.V., d'Orey, F., Cardoso, B.A., Lança, T., Grosso, A.R., deBarros, A., Martins, L.R., Barata, J.T. and Silva-Santos B. (2009) Highly active microbial phosphoantigen induces rapid yet sustained MEK/ Erk- and PI-3K/ Akt-mediated signal transduction in anti-tumor human  $\gamma\delta$  T cells. *PloS One*: e5657 (Times cited: 4)(Journal IF: NA)

Gomes, A.Q., Correia, D.V. and Silva-Santos, B. (2007) Non-classical MHC proteins as determinants of tumor immunosurveillance. *EMBO Rep.* 8: 1024-1030 (Times cited: 4)(Journal IF: 7.099)

Pennington, D.J., Silva-Santos, B., Escorcio-Correia, M., Silberzahn, T. and Hayday, A.C. (2006) Early events in the thymus affect the balance of effector and regulatory T cells. *Nature* 444: 1073-1077 (Times cited: 42)(Journal IF: 31.434)

### Ongoing Research Projects

2007/2011 Innate mechanisms of tumour immunosurveillance, EMBO. Coordinator: B. Silva-Santos (Installation Grant/ YIP project 1440)

2008/2010 Identification of ligands for  $\square\square$  T lymphocytes: a genomic and transcriptomic approach, FCT. Coordinator: B. Silva-Santos (PTDC/BIA-BCM/71663)

2008/2010. Mechanisms of tumour resistance to  $\square\square$  T cell-based cancer immunotherapy, Fundação Calouste Gulbenkian. Coordinator: B. Silva-Santos (SDH Oncologia 2008/99293)

### Publications in Peer Review International Journals 2009

Correia, D.V., d'Orey, F., Cardoso, B.A., Lança, T., Grosso, A.R., deBarros, A., Martins, L.R., Barata, J.T. and Silva-Santos, B. (2009) Highly active microbial phosphoantigen induces rapid yet sustained MEK/ Erk- and PI-3K/ Akt-mediated signal transduction in anti-tumor human  $\gamma\delta$  T cells. *PloS ONE*: e5657 (Times cited: 4)(Journal IF: NA)

Gonçalves-Sousa, N., Ribot, J.C., deBarros, A., Correia, D.V., Caramalho, I. and Silva-Santos, B. (2010) Inhibition of murine  $\gamma\delta$  lymphocyte expansion and effector function by regulatory ab T-cells is cell-contact dependent and sensitive to GITR modulation. *Eur. J. Immunol.* 40 : 61-70 (*Epub October 28, 2009*)(Journal IF: 4.865)

Ribot, J.C., deBarros, A., Pang, D.J., Neves, J.F., Peperzak, V., Girardi, M., Borst, J., Hayday, A. C., Pennington, D.J. and Silva-Santos, B. (2009) CD27 is a thymic determinant of the balance between IFN- $\gamma$ - and IL-17-producing  $\gamma\delta$  T cell subsets. *Nat. Immunol.* 10: 427-436 (Times cited: 7)(Journal IF: 26.218)

### Communications in International Conferences

Lança, T. et al. Lymphoma and leukemia cell targeting by human  $\gamma\delta$  T cells: the role of non-classical MHC proteins. 1<sup>st</sup> International Conference on Cancer immunotherapy and Immunomonitoring, Kiev, Ukraine. May 18-21, 2009 (Oral presentation)

deBarros, A. et al.  $\gamma\delta$  T cells receive critical activation and differentiation signals from the CD27 co-receptor. 2<sup>nd</sup> European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Oral presentation)

Ribot, J. et al. Thymic CD70-CD27 signals promote the differentiation of  $\alpha\beta$  and  $\gamma\delta$  T cell subsets. Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society and International Society for Interferon and Cytokine Research, Lisbon, Portugal. October 18-21, 2009 (Oral presentation)

Correia, D.V. et al. T cell receptor agonists and tumor biomarkers for  $\gamma\delta$  T cell-based immunotherapy of lymphomas and leukemias. Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society and International Society for Interferon and Cytokine Research, Lisbon, Portugal. October 18-21, 2009 (Oral presentation)

Ribot, J. et al. Developmental imprinting versus plasticity of  $\gamma\delta$  T-cell effector functions. 3<sup>rd</sup> World Immune Regulation Meeting, Davos, Switzerland. March 22-26, 2009 (Poster presentation)

Correia, D.V. et al. Activation of anti-tumor human  $\gamma\delta$  T cells by HMB-PP. 1<sup>st</sup> International Conference on Cancer immunotherapy and Immunomonitoring, Kiev, Ukraine. May 18-21, 2009 (Poster presentation)

deBarros, A. et al. Novel  $\gamma\delta$  T cell subsets with distinct functions in immunity to infection and tumours. Microanatomy of Immune Responses in Health and Disease Conference, University of Birmingham, UK. September 5-7, 2009 (Poster presentation)

Ribot, J. et al. Thymic CD70-CD27 signals promote the differentiation of  $\alpha\beta$  and  $\gamma\delta$  T cell subsets. 2<sup>nd</sup> European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Gomes, A.Q. et al. Identification of tumour-associated antigens underlying the anti-lymphoma activity of human  $\gamma\delta$  T cells. 2<sup>nd</sup> European Congress of Immunology, Berlin, Germany. September 13-16, 2009 (Poster presentation)

Gonçalves-Sousa, N. et al. Inhibition of murine  $\gamma\delta$  lymphocyte expansion and effector functions by regulatory  $\alpha\beta$  T-cells through GITR-mediated cell-cell interactions. Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society and International Society for Interferon and Cytokine Research, Lisbon, Portugal. October 18-21, 2009 (Poster presentation)

Lança, T. et al. The NKG2D ligand ULBP1 determines lymphoma and leukemia cell susceptibility to human  $\gamma\delta$  T cell cytotoxicity. Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society and International Society for Interferon and Cytokine Research, Lisbon, Portugal. October 18-21, 2009 (Poster presentation)

deBarros, A. et al. Novel  $\gamma\delta$  T cell subsets with distinct functions in immunity to infection and tumours. Tri-Society Annual Conference of the Society for Leukocyte Biology, International Cytokine Society and International Society for Interferon and Cytokine Research, Lisbon, Portugal. October 18-21, 2009 (Poster presentation)

### Communications in National Conferences

Ribot, J. et al. Thymic CD70-CD27 signals promote the differentiation of  $\alpha\beta$  and  $\gamma\delta$  T cell subsets. XXXV Annual Meeting of the Portuguese Society of Immunology, Lisbon. September 28-30, 2009 (Poster presentation)

Correia D.V. et al. Molecular signatures of tumors susceptible or resistant to human  $\gamma\delta$  T cell-mediated cytotoxicity. XXXV Annual Meeting of the Portuguese Society of Immunology, Lisbon. September 28-30, 2009 (Poster presentation)

Lança, T. et al. The NKG2D ligand ULBP1 determines lymphoma and leukemia cell susceptibility to human  $\gamma\delta$  T cell cytotoxicity. XXXV Annual Meeting of the Portuguese Society of Immunology, Lisbon. September 28-30, 2009 (Poster presentation)

deBarros, A. et al. CD27 expression segregates human  $\gamma\delta$  T cell subsets with distinct functions in immunity to tumors. XXXV Annual Meeting of the Portuguese Society of Immunology, Lisbon. September 28-30, 2009 (Poster presentation)

### Invited Lectures and Seminars

Silva-Santos, B.  $\gamma\delta$  T cell differentiation and activation: the role of the CD27 receptor. Institut Pasteur, Paris, France. October 26, 2009.

Silva-Santos, B. Critical receptors for  $\gamma\delta$  T cell differentiation and activation. Instituto Clinico Humanitas, Milan, Italy. October 5, 2009.

Silva-Santos, B. Critical receptors for  $\gamma\delta$  T cell differentiation and activation. XXXV Annual Meeting of the Portuguese Society of Immunology, Lisbon. September 28-30, 2009.

Silva-Santos, B. Thymic imprinting of functional T cell differentiation. EMBO Young Investigator Programme Annual Meeting, Istanbul, Turkey. May 13-15, 2009.

### Organization of National and International Conferences

Silva-Santos, B. Co-Organizer of the EMBO Young Investigator Programme PhD Annual Meeting, Heidelberg, Germany. September 13-19, 2009.

Silva-Santos, B. Co-Organizer of the Annual Meeting of the Portuguese Society of Immunology, Lisbon. September 28-30, 2009.

### Prizes, Honours and Awards

Pfizer / Society of Biomedical Sciences Award for Accomplishment in Clinical Research to B. Silva-Santos.

International Cytokine Society Young Investigator Award (2nd Prize) to B. Silva-Santos.

International Cytokine Society Post-Doctoral Award (1st Prize) to J. C. Ribot.

Best Paper of the Portuguese Society of Immunology to J. C. Ribot.

Outstanding Abstract and special Oral Presentation Award to T. Lança. 1st International Conference on Cancer Immunotherapy and Immunomonitoring, Kiev, Ukraine.

### Advanced Teaching

Silva-Santos, B. Cancer Immunotherapy. Programa de Formação Médica Avançada, Lisbon. January 22, 2009.

Silva-Santos, B. "Tumour Immunology" Biology of Cancer Nanocourse, IMM PhD Programme. Lisbon. April 30, 2009.

Silva-Santos, B. "T cell development" GABBA PhD Programme, Porto. June 4, 2009.

### Public Perception of Science

Silva-Santos, B. *Visualization and representation in Immunology*. Seminar at Workshop "Images that make Science", Faculdade de Ciências da Universidade de Lisboa. May 25, 2009.

Silva-Santos, B. *The Immune System against Cancer*. Chapter in booklet "Conhecer a Imunologia", Portuguese Society of Immunology. Published May 31, 2009.

deBarros, A. *Winning an Oscar in Immunology*. Article in the magazine "Science in School", European Molecular Biology Laboratory, Autumn 2009 (Issue 13).

Silva-Santos, B., Correia, D.V., Lança, T., deBarros, A., Pamplona, A, and Gonçalves-Sousa N. *Our Research in Immunology*. Seminars to visiting students from the Virgílio Ferreira High School (12<sup>th</sup> grade). November 26 and December 16, 2009.

## Nutrition and Metabolism Unit

### Description and Objectives

The Unit promotes: 1) Under and Post-Graduate Education, 2) Research in Clinical Nutrition and Metabolism, and delivers services to the Hospital (HSM) or Community.

1) A 4 year degree in Dietetics and Nutrition has been undergoing since 2004.

Following the of the Bologna process implementation, a whole Master Program in Dietetics and Nutrition was started in October 2008, whose board presided by Paula Ravasco includes Maria E. Camilo and Helena Cortez- Pinto. In 2009, students have been pursuing their research projects to attain the Master Degree. Besides, in 2009 a medical student chose to do her project, in Nutrition and Oncology, with Paula Ravasco to achieve the integrated Master degree in Medicine.

In 2007, Catarina Sousa Guerreiro and Ana Catarina Moreira, both with a Degree in Dietetics and a Master in Clinical Nutrition, became PhD students in Health Sciences (Nutrition) under the supervision of M.E. Camilo. The Theses are respectively entitled: “NUTRIÇÃO e GENES em DOENÇAS INTESTINAIS” and “Hemodiálise: Qualidade de Vida e Parâmetros nutricionais”.

2) Investigators pursue areas of research according to specific backgrounds and interests. Nutrition/life-style related risk factors and nutritional interaction with diseases prevail. Interconnections with basic science are searched: genetic polymorphisms of inflammatory mediators, cytokines, either as pathogenic mechanisms of lesion and/or nutritional deterioration, changes of the cellular nuclei and DNA degradation, and relation to apoptosis in metabolic (alcohol or fat-related) or chronic diseases (e.g. cancer), insulin resistance, etc.

### Head of Unit

Maria Ermelinda Camilo, MD, PhD – retired from Auxiliary Professor FMUL since June 2008, remains voluntarily as Head of the Unit.

### Principal Investigators

Isabel Monteiro Grillo, MD, PhD - Director of the Radiotherapy and of the Oncology Departments HSM, Associate Professor FMUL.

Helena Cortez-Pinto, MD, PhD - Consultant of Gastroenterology HSM, Auxiliary Professor FMUL (with *Agregação*)

Paula Ravasco, Degree in Nutrition, PhD in Health Sciences FMUL - Auxiliary Professor FMUL

### Research Team

Catarina Sousa Guerreiro (Master in Clinical Nutrition)	- PhD Student /Assistant FMUL
Mariana Verdelho Machado, MD	- Intern of Gastroenterology, HSM;
Regina Lourenço, PharmD	- Assistant FMUL, PharmD HSM;
Carolina Boléo-Tomé (Degree in Nutrition)	- Training Fellowship, IEFP (since March 16)
Mariana Chaves, Degree in Nutrition	- Training Fellowship, IMM (since March 16)

### Research Areas

Hepatology; steatohepatitis; obesity; insulin resistance.  
Oncology, radiotherapy



Nutrition and Outcomes  
Nutrition and Cancer

### Major Scientific Achievements

Having shaped the only Research Unit of Nutrition and Metabolism in Portugal, just with our own effort is a major achievement. A small multidisciplinary group, extended the knowledge and good practice in clinical nutrition, and reached a national/international level of cutting edge research of excellence, fully devised and conducted by us in two main areas.

1) Cortez-Pinto H focus on fat metabolism in the liver, from clinical to translational research, lately addressing the metabolic syndrome/insulin resistance; she is a prized speaker in major international meetings, recognized as an expert in steatohepatitis;

2) Ravasco P. focusing on Nutrition and Cancer evolved from clinical to translational research; she is a prized speaker in international meetings, an expert in the field and the first to perform nutritional clinical trials in cancer and value nutrition in patient's Quality of Life and its importance on outcomes. Among her awards, *Eminent Scientist of the Year 2004 & Millennium Golden International Award* in Nutrition and Clinical Oncology was based on unique academic and research contribution in Clinical Nutrition, attributed by The World Scientist Forum of the International Research Promotion Council.

We equally value our participation in activities to enhance the nutritional knowledge of the community, often involving young dieticians/nutritionists.

Recently, there are an increasing number of medical students who chose nutrition as the topic for the discipline of clinical investigation, as well as the topic to achieve the integrated Master degree in Medicine.

### Recent Most Relevant Publications

Guerreiro, C.S., Cravo, M.L., Brito, M., Vidal, P.M., Fidalgo, P.O. and Leitão, C.N. (2007) The D1822V APC polymorphism interacts with fat, calcium, and fiber intakes in modulating the risk of colorectal cancer in Portuguese persons. *Am. J. Clin. Nutr.* 85: 1592-1597 (Times cited: 4)(Journal IF: NA)

Machado, M., Marques-Vidal, P. and Cortez-Pinto, H. (2006) Hepatic histology in obese patients undergoing bariatric surgery. *J. Hepatol.* 45: 600-606 (Times cited 37)(Journal IF: 7.056)

Ravasco, P., Monteiro-Grillo, I., Marques-Vidal, P. and Camilo, M.E. (2005) Dietary counselling improves patient' outcomes: a prospective randomized controlled trial in colorectal cancer patients undergoing radiotherapy. *J. Clin. Oncol.* 23: 1431-1438 (Times cited: 66)(Journal IF: 17.157)

Ravasco, P., Monteiro-Grillo, I., Marques-Vidal, P. and Camilo, M.E. (2005) Impact of nutrition on outcome: A prospective randomized controlled trial in patients with head and neck cancer undergoing radiotherapy. *Head & Neck* 27: 659-668 (Times cited: 37)(Journal IF: 2.603)

Ribeiro, P., Cortez-Pinto, H., Solá, S., Castro, R., Ramalho, R., Baptista, A., Moura, M., Camilo, M.E. and Rodrigues, C. (2004) Hepatocyte Apoptosis, Expression of Death Receptors and Activation of NF- $\kappa$ B in the Liver of Non-alcoholic and Alcoholic Steatohepatitis Patients. *Am. J. Gastroenterol.* 99: 1708-17017 (Times cited: 88)(Journal IF: 6.444)

### Ongoing Research Projects

**Hepatology; alcoholic and non-alcoholic steatohepatitis**

**Nutrition and Endoscopy related procedures**

PI: Helena Cortez-Pinto

2009/2010 Efficacy, Safety and Tolerability of Levodopa-Carbidopa Intestinal Gel in Levodopa-

## 154 | 2009 IMM Activity Report

Responsive Parkinson's Subjects. Industry sponsored. Our group participation pertains to the placement of PEG/PEJ device. Coordinator: J. Ferreira

2009/2012 Insulin resistance in Wilson's disease – experimental study in a mice model knock-out ATP7B (-/-), that has features of Wilson's disease, evaluating insulin sensitivity, considering that it is probable the presence of insulin resistance in Wilson's disease, Sociedade Portuguesa de Gastroenterologia. Coordinator: H. Cortez-Pinto.

2008/2011 Nutraceuticals: a shield against chronic disease? -contribution for the rationale of a new therapeutic option. Coordinator: A. Sousa-Guerreiro (PTDC/SAU-OSM/66323/2006)

### **Nutricion and Cancer**

PI: Paula Ravasco

2009/2011 Nutrition in Cancer: relevance and guidelines for clinical practice. Coordinator: Paula Ravasco.

2008/2011 Body composition and metabolic pattern in cancer. Coordinator: Paula Ravasco.

2009 Body composition, physical activity and food intake pattern in cancer patients. Coordinator: I. Monteiro-Grillo.

2006/2010 Colorectal Cancer and Cytokines. Coordinator: P. Ravasco.

2003/2009 Cancer: metabolic dysfunction, nutrition and Quality of Life. Coordinator: P. Ravasco.

### **Oncology/ Radiotherapy**

PI: Isabel Monteiro-Grillo

2007/2009 O efeito de baixas doses de radiação ionizante. Coordinator: S. Constantino.

2009/2010 A qualidade de vida no doente com cancro da próstata em tratamentode radioterapia. Coordinator: I. Monteiro Grillo.

2009/2011 Desenvolvimento de novos métodos dosimétricos para aplicações médicas. Coordinator: I. Monteiro Grillo

2009/2010 Aceitação de um sistema de planeamento computadorizado. Coordinator: I. Monteiro Grillo.

## **Publications in Peer Review International Journals 2009**

Cansado, P., Ravasco, P. and Camilo, M. (2009) Hospital undernutrition in the elderly: a longitudinal study. *J. Nutr.Health Aging* 13: 159-164. (Journal IF: 2.321)

Cortez-Pinto, H. and Machado, M.V. (2009) Uncoupling proteins and non-alcoholic fatty liver disease. *J. Hepatol.* 50: 857–860 (Journal IF: 7.056)

Guerreiro, C.S., Ferreira, P., Tavares, L., Moura Santos, P., Neves, M., Brito, M. and Cravo, M. (2009) Fatty acids, IL-6 and TNF- $\alpha$  polymorphisms: An example of nutrigenetics in Crohn's disease. *Am. J. Gastroenterol.* 104: 2241-2249. (Journal IF: 6.444)

Machado, M., Ravasco, P., Martins, A., Almeida, M.R., Camilo, M.E. and Cortez-Pinto, H. (2009) Iron homeostasis and H63D mutations in alcoholics with or without liver disease. *World J. Gastroenterol.* 15: 106-111 (Journal IF: 2.081)

Machado, M.V. and Cortez-Pinto, H. (2009) Insulin resistance and steatosis in chronic hepatitis C. *Ann. Hepatol.* 8 (Suppl 1): S67-S75 (Journal IF: NA)

Machado, M.V., Martins, A., Almeida, R., Marques-Vidal, P., Gonçalves, M.S., Camilo, M.E. and Cortez-Pinto, H. (2009) Does the simultaneous tumor necrosis factor receptor 2 and Tumor Necrosis Factor promoter gene polymorphism represent a higher risk for alcoholic liver disease? *Eur. J. Gastroenterol. Hepatol.* 21: 201-205 (Journal IF: 2.080)

Marcelino, G., Oliveira, J.M., Ravasco, P. and Marques-Vidal, P. (2009) Weight concerns and

weight reduction practices of Portuguese adolescents. *Nutr. Hosp.* 24: 758 (Journal IF: 1.096)

Ravasco, P. (2009) Cancer and nutrition: key determinants of Quality of Life. *Eur. J. Cancer* 45 (Suppl 1): 409 (Journal IF: 4.475)

Ravasco, P., Aranha, M.M., Borralho, P.M., Moreira da Silva, I.B., Correia, L., Fernandes, A., Rodrigues, C.M.P. and Camilo, M.E. (2010) Colorectal carcinogenesis: interaction between nutrients, histology and NF-kB? *Clin. Nutr.* 29: 42-46 (*Epub July 1, 2009*)(Journal IF: 3.203)

Telles-Correia, D., Cortez-Pinto, H., Barbosa, A., Mega, I., Monteiro, E. (2009) Quality of life following liver transplantation: a comparative study between Familial Amyloid Neuropathy and liver disease patients. *BMC Gastroenterol.* 9: 54 (Journal IF: 2.136)

### Publications in Peer Review National Journals 2009

Coronha, A.L., Lourenço, C., Ferreira, M., Reis, N., Almeida, R., Cruz, C., Chaves, M., Monteiro Grillo, I., Camilo, M.E. and Ravasco, P. (2009) É possível integrar a Nutrição na prática clínica! A relevância do Estágio de Investigação Clínica. *Revista da Faculdade de Medicina de Lisboa série III vol. 14, nº3: 167-173.*

Cruz, C., Chaves, M., Ravasco, P., Monteiro Grillo, I. and Camilo, M. (2009) Validação do MUST em Oncologia: mobilização multidisciplinar. *Revista APNEP* 3: 2-8.

Fortunato, M., Feijó, S., Almeida, T., Mendonça, V., Aguiar, M., Jorge, M. and Monteiro Grillo, I. (2009) Braquiterapia Endoluminal HDR no Tratamento de Tumores Primários ou Recidivas na Árvore Traqueobrônquica. *Revista Portuguesa de Pneumologia Vol. XV nº2, March-April.*

Guerreiro, C.S., Ferreira, P., Tavares, L., Moura Santos, P., Neves, M., Brito, M. and Cravo, M. (2009) Doença de Crohn – A influência dos ácidos gordos e polimorfismos na actividade da doença. *APNEP* 3: 9-17.

Marcelino, A., Campanhe, T., Eusébio, V., Guerreiro, C.S. and Mendes, L. (2009) Estilos de vida e avaliação nutricional em dadores de sangue – caracterização de dadores de sangue. *APNEP* 3: 23-27.

### Books/Book Chapters

Machado, M.V. and Cortez-Pinto, H. (2009) Resistência à insulina e esteatose na hepatite crónica C *in* Terapêutica nas Hepatites. Sarmento e Castro (eds). Permanyer Portuguesa.

Monteiro Grillo, I., Tripero, J., Guinot, J.L. (2009) Aspectos Generales de la Braquiterapia en el câncer de mama *in* Medical Practice Group, España. Guia de Braquiterapia. Gunot, J.L., Lanzós, E. and Munoz, V. (eds)

Ravasco P. (2009) Nutrition in Cancer, *in* Handbook of Clinical Aspects in Gynecological Oncology (Official ESGO Reference Book) European Society of Gynaecological Oncology Council (eds). London: Campden Publishing Ltd.

### Communications in International Conferences

Bujor, L. and Monteiro Grillo, I. Enhanced toxicity with cetuximab and radiotherapy in patients with locally advanced head and neck cancer. 20-eme Congrès SFOR, Paris, France. October 21-23, 2009 (Poster presentation)

Chaves, M. et al. Body Composition pattern in cancer patients: cross-sectional characterisation. COSPI-3 Course (Combating Obesity: Strategies for Prevention and Intervention) 2009, Grunlsee, Austria. February 22 – March 7, 2009 (Oral presentation)

Cortez-Pinto, H. et al. Liver disease is the main burden of diseases attributable to alcohol drinking: results of a national study. 44th Annual Meeting of the European Association for the Study of the Liver, Copenhagen, Denmark. April 23, 2009. *Journal of Hepatology, Supplement*

2009 (Oral presentation)

Cruz, C. Pattern of nutritional status in cancer: cross-sectional characterisation. COSPI-3 Course (Combating Obesity: Strategies for Prevention and Intervention) 2009, Grunlsee, Austria. February 22 – March 7, 2009 (Oral presentation)

Eiras, M. et al. Patient safety at a radiotherapy department: how can we enhance safety? 10th Biennial ESTRO Conference on Physics and Radiation Technology for Clinical Radiotherapy, Maastricht, Netherlands. August, 2009 (Poster presentation)

Ferreira, C.N. et al. Infections at first decompensation of liver cirrhosis predict higher mortality at hospital admission and at long term. Digestive Disease Week, Chicago, Illinois, USA. May 30 – June 4, 2009. (Poster presentation)

Ferreira, C.N. et al. Renal insufficiency is relatively infrequent at first decompensation of liver cirrhosis and is associated with higher risk of nosocomial infections and poor prognosis. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, USA. October 30 – November 3, 2009. Hepatology (Supplement 1) 2009. (Poster presentation)

Ferreira, P. et al. The influence of eicosapentaenoic acid (EPA) on TNF- $\alpha$  production by peripheral blood mononuclear cells of Crohn's disease (CD) patients and cell kinetics accordingly to TNF- $\alpha$  857 c/t polymorphism. 4th Congress of ECCO, Hamburg, Germany. February 5-7, 2009. Journal of Crohn's & Colitis 2009; 3, S101. (Poster presentation)

Fidalgo, C. et al. Osteoporosis in Crohn's Disease: Who is to Blame? 4th Congress of ECCO, Hamburg, Germany. February 5-7, 2009. Journal of Crohn's & Colitis 2009; 3, S32. (Poster presentation)

Guerreiro, C.S. et al. Obesity : a report of portuguese situation. 31st ESPEN Congress, Vienna, Austria. August 29 – September 1, 2009. Clinical Nutrition 2009; 4: 107. (Poster presentation)

Guerreiro, C.S. Risk Factors For Metabolic Bone Disease In Patients With Crohn's Disease. 31<sup>st</sup> ESPEN Congress, Vienna, Austria. August 29 – September 1, 2009. Clinical Nutrition 2009; 4: 20. (Oral presentation) – *Best presentation award*

Jorge Lemos, M. et al. Adjuvant post-operative intravaginal high-dose rate Brachytherapy for stage IB and IC endometrial cancer. Gec-Estro, Porto, Portugal. May 13-16, 2009 (Oral presentation)

Machado, M. et al. In morbid obesity decreased adiponectin and increased leptin associates with higher risk of nonalcoholic steatohepatitis and higher necroinflammatory respectively. Special Conference: NAFLD/NASH and related metabolic disease, Bologna, Italy. September 24-26, 2009 (Poster presentation)

Machado, M.V. Do leptin and ghrelin levels correlate with the severity of nonalcoholic fatty liver in morbid obesity? United European Gastroenterology Week - UEGW (Gastro 2009), London, UK. November 21-25, 2009 (Poster presentation)

Machado, M.V. et al. Hepatic steatosis in HIV/ hepatitis C virus co-infected patients – a meta-analysis of the risk factors. 60th Annual Meeting of the American Association for the Study of Liver Diseases (AASLD), Boston, USA. October 30 – November 3, 2009. Hepatology (Supplement 1) 2009. (Poster presentation)

Machado, M.V. et al. Neither iron overload nor increased ferritin is present in morbid obesity patients with nonalcoholic fatty liver disease. United European Gastroenterology Week - UEGW (Gastro 2009), London, UK. November 21-25, 2009 (Poster presentation)

Machado, M.V. et al. Non-organ specific autoantibodies in morbid obesity: correlation with non alcoholic fatty liver disease severity and risk factors. United European Gastroenterology Week - UEGW (Gastro 2009), London, UK. November 21-25, 2009. (Poster presentation)

Machado, M.V. et al. One year follow up after the first episode of spontaneous bacterial peritonitis. United European Gastroenterology Week - UEGW (Gastro 2009), London, UK. November 21-25, 2009. (Poster presentation)

Machado, M.V., et al. Autoimmune hepatitis: retrospective evaluation of the last 10 years and

comparison of two diagnostic scores. United European Gastroenterology Week - UEGW (Gastro 2009), London, UK. November 21-25, 2009 (Poster presentation)

Mão de Ferro, S. et al. Increased circulating TNF  $\alpha$ , IL1 and IL6, in the absence of increased intestinal permeability in patients with compensated liver cirrhosis. 44th Annual Meeting of the European Association for the Study of the Liver. Copenhagen, Denmark. April 23, 2009. Journal of Hepatology, Supplement 2009. (Poster presentation)

Marques Vidal, P. et al. Body mass index and body fat independently and negatively influence fitness levels in Portuguese youth. Research Day "Genes and Diseases" Université de Lausanne – Faculté de Biologie et de Médecine, Lausanne, Switzerland. January 29, 2009. Métabolisme et Cardiovasculaire MCV-18 2008, 115. (Oral presentation)

Marques-Vidal, P. et al. Body mass index and body fat independently and negatively influence fitness levels in Portuguese youth. ESC (European Society of Cardiology) Congress, Barcelona, Spain. August 29 – September 2, 2009. European Heart Journal 2009; 30 (suppl), 662. (Poster presentation)

Mendes, D. et al. Nutritional status measurements: The best predictors of COPD severity. 31st ESPEN Congress, Vienna, Austria. August 29 – September 1, 2009. Clinical Nutrition 2009; 4: 134. (Poster presentation)

Mendonça, V. et al. Prostate cancer radiation dose escalation: early, late toxicity and biochemical outcome in 83 patients. GEC-ESTRO Annual Meeting (European Society for Radiotherapy and Oncology), Porto, Portugal. May, 2009 (Oral presentation)

Ravasco, P. et al. Insights on body composition in cancer patients. 31<sup>st</sup> Congress of the European Society for Clinical Nutrition and Metabolism (ESPEN), Viena, Áustria. August 29 – September 1, 2009. Clinical Nutrition 2009; 4 (suppl 2), 78-79. (Poster presentation)

Ravasco, P. et al. Insights on body composition in cancer patients. 5<sup>th</sup> Annual Chicago Supportive Oncology Conference, Chicago, Illinois, USA. October, 2009. The Journal of Supportive Oncology 2009; 15 (suppl1), 27. (Oral presentation)

Ravasco, P. et al. M. MUST in Oncology: a multidisciplinary tool. 5<sup>th</sup> Annual Chicago Supportive Oncology Conference, Chicago, Illinois, USA. October, 2009. The Journal of Supportive Oncology 2009; 15 (suppl1), 22. (Oral presentation)

Ravasco, P. et al. MUST in Oncology: a multidisciplinary tool. 31<sup>st</sup> Congress of the European Society for Clinical Nutrition and Metabolism (ESPEN), Viena, Áustria. August 29 – September 1, 2009. Clinical Nutrition 2009; 4 (suppl 2), 101. (Poster presentation)

Ravasco, P. et al. Nutritional status in cancer: diverse and challenging? 5<sup>th</sup> Annual Chicago Supportive Oncology Conference, Chicago, Illinois, USA. October, 2009. The Journal of Supportive Oncology 2009; 15 (suppl1), 26. (Oral presentation)

Ravasco, P., et al. Nutritional status in cancer: diverse and challenging? 31<sup>st</sup> Congress of the European Society for Clinical Nutrition and Metabolism (ESPEN), Viena, Áustria. August 29 – September 1, 2009. Clinical Nutrition 2009 4 (suppl 2), 72. (Poster presentation)

### Communications in National Conferences

Bujor, L. and Monteiro Grillo, I. Estudo de deleções 1p/19q em Oligodendroglioma Primários e Secundários. (Study of 1p/19 q deletations in primary and secondary Oligodendrogliomas). Portuguese Conference of Neurology Society, Lisbon. November 5-7, 2009 (Oral presentation)

Bujor, L. et al. Canal anal plasmocytoma – an uncommon intestinal bleeding cause. 5<sup>o</sup> Simpósio Nacional de Cancro, Lisbon. 2009 (Poster presentation)

Bujor, L. et al. Canal anal plasmocytoma- an uncommon presentation site. Reunião Anual da Sociedade Portuguesa de Hematologia, Tróia. October 29-30, 2009 (Poster presentation)

Bujor, L. et al. Disseminação Leptomeningea de um GBM secundário - caso clínico. 2<sup>o</sup> Congresso Nacional da Associação Portuguesa de Neuro-Oncologia, Porto. October 23-24, 2009

(Poster presentation)

Bujor, L. et al. Meningioma Cordóide subtipo raro e agressivo. 2º Congresso Nacional da Associação Portuguesa de Neuro-Oncologia, Porto. October 23-24, 2009 (Poster presentation)

Bujor, L. et al. Meylomatous meningitis. Reunião Anual da Sociedade Portuguesa de Hematologia, Tróia. October 29-30, 2009 (Poster presentation)

Bujor, L. et al. Toxicidade cutanea secundaria ao uso de Cetuximab e radioterapia concomitante-caso clinico. (Secondary skin toxicity in concomitant radiotherapy and cetuximab-clinical case). Portuguese National Congress of Head and Neck, Lisbon. 2009 (Poster presentation)

Bujor, L. et al. Toxicidade Hematológica em doente com Glioblastoma Multiforme e Insuficiência Renal Crónica. 2º Congresso Nacional da Associação Portuguesa de Neuro-Oncologia, Porto. October 23-24, 2009 (Poster presentation)

Bujor, L. Unusual relapse localizations of a human immunodeficiency virus (HIV) - associated lymphoma. Reunião Anual da Sociedade Portuguesa de Hematologia, Tróia. October 29-30, 2009 (Poster presentation)

Carrilho, G.L. et al. Excesso de peso: uma outra realidade em lares de idosos. XI Congresso anual da APNEP 2009, Lisboa. June 18-19, 2009. Revista da APNEP 2009; III(1):177. (Poster presentation)

Cebola, M. et al. Estado Nutricional da população geriátrica portuguesa: Dados de um estudo nacional "Nutriaction XXI" Congresso anual da APNEP 2009, Lisboa. June 18-19, 2009. Revista da APNEP 2009; III (1):180. (Poster presentation)

Chaves, M. et al. A diversidade do estado nutricional em oncologia! II Fórum de Projectos de Prevenção da Obesidade promovido pela Direcção Geral de Saúde e pela Plataforma contra a Obesidade, Lisboa. June 26-27, 2009 (Poster presentation)

Chaves, M. et al. A diversidade do estado nutricional em oncologia! XXIX Congresso Nacional de Gastreterologia e Endoscopia Digestiva, Porto, Portugal. June 18-20, 2009. Jornal Português de Gastreterologia 2009. 16, Nº4: 48. (Poster presentation)

Chaves, M. et al. Novas perspectivas sobre a composição corporal no doente oncológico. XI Congresso Anual da Associação Portuguesa de Nutrição Entérica e Parentérica (APNEP), Lisboa. June 18-19, 2009. Revista da APNEP 2009. Vol. III, Nº1: 153. (Oral presentation)

Cruz, C. et al. O estado nutricional em oncologia: a diversidade e o desafio. XI Congresso Anual da Associação Portuguesa de Nutrição Entérica e Parentérica (APNEP), Lisboa. June 18-19, 2009. Revista da APNEP 2009. Vol. III, Nº1: 154. (Oral presentation)

Cruz, C. Tumor gastrointestinal: avaliação multidisciplinar do risco nutricional. XXIX Congresso Nacional de Gastreterologia e Endoscopia Digestiva, Porto, Portugal. June 18-20, 2009. Jornal Português de Gastreterologia 2009. 16, Nº4: 22. (Oral presentation)

Machado, M.V. et al. Auto-anticorpos não específicos de órgão e obesidade mórbida: correlação com Fígado Gordo Não Alcoólico e seus factores de risco. XXIX Congresso Nacional de Gastreterologia e Endoscopia Digestiva, Porto. June 18-20, 2009. GE-Jornal Português de Gastreterologia, 70; 133 (Oral presentation)

Machado, M.V. et al. Doença hepática alcoólica e polimorfismos promotores de angiogenese. XXIX Congresso Nacional de Gastreterologia e Endoscopia Digestiva, Porto. June 18-20, 2009. GE-Jornal Português de Gastreterologia, 17; 31. (Oral presentation)

Machado, M.V. et al. Hepatite auto-imune: avaliação retrospectiva dos últimos 10 anos e comparação de 2 scores de diagnóstico. XXIX Congresso Nacional de Gastreterologia e Endoscopia Digestiva, Porto. June 18-20, 2009. GE-Jornal Português de Gastreterologia, 70; 135 (Poster presentation)

Oliveira, J. et al. Estado Nutricional como indicador de severidade da DPOC: qual o melhor parâmetro? XI Congresso anual da APNEP, Lisboa. June 18-19, 2009. Revista da APNEP 2009; III (1):149. Classified among the five best research works of the congress. (Oral presentation)

Pimentel-Nunes, P. et al. História natural do hepatocarcinoma na população portuguesa: coorte de 15 anos num hospital terciário. XXIX Congresso Nacional de Gastrenterologia e Endoscopia Digestiva, Porto. June 18-20, 2009. GE-Jornal Português de Gastrenterologia, 70; 13 (Poster presentation)

Ravasco, P. et al. O MUST em Oncologia: integração multidisciplinar. XI Congresso Anual da Associação Portuguesa de Nutrição Entérica e Parentérica (APNEP), Lisboa. June 18-19, 2009. Revista da APNEP 2009. Vol. III, Nº1: 155. (Oral presentation)

Velez, B. et al. Estado nutricional e padrão alimentar de profissionais de saúde de centros de saúde. Congresso Português de Dietética e Nutrição, Lisboa. September 25-26, 2009. Abstract book: Pag 16. (Poster presentation) - *Best Poster Award*

### Invited Lectures and Seminars

Chaves, M. Qual o estado nutricional do doente oncológico? – A diversidade do estado nutricional e composição corporal. XI Congresso Anual da APNEP (Associação Portuguesa de Nutrição Entérica e Parentérica), Lisboa, Portugal. June 19, 2009

Chaves, M.A. Nutrição como factor de risco ou protector da doença oncológica. Círculo de Conferências para Farmacêuticos, Delegação Norte da Associação Nacional de Farmácias, subordinada ao tema “Nutrição e Cancro”, Porto, Portugal. June 5, 2009

Cortez Pinto, H. and Ravasco, P. How different is the dietary pattern in non-alcoholic steatohepatitis patients? In the Clinical Nutrition Symposium for presentation of Original papers that have contributed most to the impact factor of the Clinical Nutrition 2008 Journal. 31<sup>st</sup> Congress of the European Society for Clinical Nutrition and Metabolism (ESPEN), Vienna, Austria. August 31, 2009

Cortez-Pinto, H. Actividade física e NASH. 12<sup>a</sup> Reunião Anual da Associação para o Estudo do Fígado, Estoril, Portugal. April 3-4, 2009

Cortez-Pinto, H. Doença hepática e obesidade grave. XII Simpósio Internacional da Terapêutica em Hepatite Viral, Salvador da Bahia, Brasil. July 23, 2009

Cortez-Pinto, H. Early Morning Workshop: Update on NASH. Co-moderation with Prof. Vlad Ratziu. 44th Annual Meeting of the European Association for the Study of the Liver, Copenhagen, Denmark. April 25, 2009

Cortez-Pinto, H. Fibrosis in NASH. Risk factors and progression. Special Conference: NAFLD/NASH and related metabolic disease, Bologna, Italy. 24-26 September 2009

Cortez-Pinto, H. Importância e Custos das doenças associadas ao álcool. II Congresso Português de Hepatologia, Torres Vedras, Portugal. March 12-14, 2009

Cortez-Pinto, H. Pathogenesis of fat accumulation in alcoholic and non-alcoholic fatty liver. Symposium: Alcoholic steatohepatitis: a lesson we can learn from non-alcoholic fatty liver. 12<sup>th</sup> Congress of European Society for Biomedical Research on Alcoholism (ESBRA), Helsinki, Finland. June 7, 2009

Cruz, C. A Alimentação na prevenção do Cancro / A Alimentação no doente Oncológico. Círculo de Conferências para Farmacêuticos, Delegação Norte da Associação Nacional de Farmácias, subordinada ao tema “Nutrição e Cancro”, Porto, Portugal. June 5, 2009

Cruz, C. O MUST em prática multidisciplinar. XI Congresso Anual da APNEP (Associação Portuguesa de Nutrição Entérica e Parentérica), Lisboa, Portugal. June 19, 2009.

Guerreiro, C.S. Aconselhamento nutrigenético - mito ou realidade? XI Congresso anual da APNEP, Lisboa, Portugal. June 18-19, 2009 (Abstract in Revista APNEP 2009 III (1): 49)

Guerreiro, C.S. Nutrigenética nas doenças intestinais. Congresso Português de Dietética e Nutrição, Lisboa, Portugal, September 25-26, 2009

Lourenço, R. and Ferreira, O. Produção em farmácia hospitalar. Velhos e novos desafios.

Preparação de medicamentos em Meio Hospitalar: Modelos de Boa Gestão e Impactos da Inovação Tecnológica – Programa do Medicamento Hospitalar. Lisbon, Portugal. January, 2009

Monteiro Grillo, I. Chairman: Cancro do Recto. Jornadas de Oncologia, Espinho, Portugal. July 1-3, 2009.

Monteiro Grillo, I. Chairperson Round Table: Segurança do doente: cultura de segurança nas organizações de saúde e gestão de equipas. Reunião Científica, Escola Nacional de Saúde Pública, Lisbon, Portugal. February 7, 2009

Monteiro Grillo, I. Implantación de la técnica tratamiento de próstata com alta tasa de dosis. 1ª Reunión de Usuários Braquiterapia- Avances en braquiterapia de Próstata en el Siglo XXI, Madrid, Spain. April 23-24, 2009

Ravasco P. Alimentação no Doente Oncológico - A importância e necessidade da terapêutica nutricional individualizada. Círculo de Conferências para Farmacêuticos promovido pela Delegação Norte da Associação Nacional de Farmácias, subordinada ao tema “Nutrição e Cancro”, Porto, Portugal. June 5, 2009

Ravasco P. Recomendações da World Cancer Research Fund – Recomendações mundiais para a prevenção do cancro. Círculo de Conferências para Farmacêuticos promovido pela Delegação Norte da Associação Nacional de Farmácias, subordinada ao tema “Nutrição e Cancro”, Porto. June 5, 2009

Ravasco, P. Cancer and Nutrition: Key determinants of Quality of Life. Annual Meeting ECCO15-ESMO34, European Cancer Organisation (ECCO) and European Society of Medical Oncology (ESMO), Berlin, Germany. September 21, 2009

Ravasco, P. Carcinoma do pâncreas. Round table: Suporte nutricional e doença pancreática. XI Congresso Anual da APNEP (Associação Portuguesa de Nutrição Entérica e Parentérica), Lisboa, Portugal. June 18, 2009. (Revista da APNEP 2009; vol. III, Nº1: 53)

Ravasco, P. Chairperson Round table: Developmental origins of breast and other cancers, 6th World Congress on Developmental and Origins of Health and Disease (DOHAD), Santiago, Chile. November 20, 2009

Ravasco, P. Chairperson Round table: Nutrição no doente crónico. Simpósio de Nutrição do Hospital da Luz, Lisboa, Portugal. October 16, 2009

Ravasco, P. Chairperson Round table: Qual o estado nutricional do doente Oncológico? XI Congresso Anual da APNEP (Associação Portuguesa de Nutrição Entérica e Parentérica), Lisboa, Portugal. June 19, 2009.

Ravasco, P. Dietary counselling, a simple but effective way to improve cancer outcomes. Symposium: Prevention, Nutrition and Cancer – The role of nutrition in the prevention and treatment of cancer, VU Medical Centre of Amsterdam, Amsterdam, Netherlands. September 25, 2009.

Ravasco, P. Dietary counselling: a simple but effective way to improve outcomes. Nutrition to improve patient outcomes - Symposium on Cancer and Nutrition, ESMO (European Society of Medical Oncology), Zurich, Switzerland. March 20, 2009

Ravasco, P. From dietary counselling to parenteral nutrition in the treatment of tumour patients. Nutrition and cancer: impact on outcome. Symposium on Meeting Annual International Multidisciplinary EDI 2009 of Clinical Nutrition, German Society of Nutritional Medicine, Berlin, Germany. February 27, 2009

Ravasco, P. Individualised nutrition: improved care and improved outcomes. Head and Neck Cancer Study Day, British Dietetic Association - Oncology Group, London, UK. November 9, 2009

Ravasco, P. Nutrição e Oncologia. Integrated in the cycle of Multidisciplinary Clinical Sessions of Hospital da Luz. April 15, 2009

Ravasco, P. Nutritional counselling, cancer outcome and quality of life. Annual Meeting on



Clinical Nutrition, University Hospital of Aarhus, Aarhus, Denmark. April 24, 2009

Ravasco, P. Prevenção na obesidade e cancro. Round table: Obesidade e Cancro. XI Congresso Anual da APNEP (Associação Portuguesa de Nutrição Entérica e Parentérica), Lisboa. Portugal, June 18, 2009 (Revista da APNEP 2009; vol. III, Nº1: 88)

### **Organisation of National and International Conferences**

Camilo, M.E. Organizing Committee of Congresso Português de Dietética e Nutrição, Lisboa, Portugal. September 25-26, 2009

Cortez-Pinto, H. Organizing Committee of “EASL Special Event on NAFLD/NASH”, Bologna, Italy. 24-26 September 2009

Cortez-Pinto, H. Organizing Committee of II Congress of Sociedade Portuguesa de Hepatologia, Torres Vedras, Portugal. March 12-14, 2009

Guerreiro, C.S. Organizing Committee of Congresso Português de Dietética e Nutrição, Lisboa, Portugal. September 25-26, 2009

Monteiro Grillo, I. Local organizer of ESTRO “Multidisciplinary Management of Breast Câncer”, Lisbon, Portugal. Abril 2-4, 2009

Ravasco, P. Member of the Scientific Committee of the XI Congresso Anual da APNEP (Associação Portuguesa de Nutrição Entérica e Parentérica), Lisboa, Portugal. June 18-19, 2009

### **Prizes, Honours and Awards**

2º Prémio Nutrição Clínica Fresenius Kabi awarded to Guerreiro, C.S., Ferreira, P., Tavares, L., Moura Santos, P., Neves, M., Brito, M., Cravo, M. “Doença de Crohn – A influência dos ácidos gordos e polimorfismos na actividade da doença”

ESPEN 2009 Travel Fellowship to Guerreiro, C.S., Ferreira, P., Santos, P.M., Tavares, L., Fidalgo, C., Brito, M., Cravo, M. “Risk Factors For Metabolic Bone Disease in Patients With Crohn’s Disease”.

Best poster award to Velez, B., Lopes, AR., Guerreiro, CS., Gil, F., Sousa, J., Mendes, L. Estado nutricional e padrão alimentar de profissionais de saúde de centros de saúde. Congresso Português de Dietética e Nutrição, Lisboa, Portugal. September 25-26, 2009

Classified among the five best research works of the congress. Oliveira, J., Pinto, A., Almeida, I., Lopes, J., Mendes, D., Guerreiro, C.S., Barbara, C., Mendes, L. Estado Nutricional como indicador de severidade da DPOC: qual o melhor parâmetro? XI Congresso Anual da Associação Portuguesa de Nutrição Entérica e Parentérica (APNEP), Lisbon, Portugal, June 18-19, 2009

COSPI Presentation Award for 1 of the 2 best presentations at the Erasmus Intensive Programme COSPI-3. Chaves, M., Cruz, C., Ravasco, P., Monteiro Grillo, I., Camilo, M. Body Composition pattern in cancer patients: cross-sectional characterisation. COSPI-3 Course (Combating Obesity: Strategies for Prevention and Intervention) 2009, Grunlsee, Austria. February 22 – March 7, 2009

COSPI Presentation Award for 1 of the 2 best presentations at the Erasmus Intensive Programme COSPI-3. Cruz, C., Chaves, M., Ravasco, P., Monteiro Grillo, I., Camilo, M. Pattern of nutritional status in cancer: cross-sectional characterisation. COSPI-3 Course (Combating Obesity: Strategies for Prevention and Intervention) 2009, Grunlsee, Austria. February 22 – March 7, 2009

### **Advanced Teaching**

Camilo, M.E. COSPI=Combating Obesity: Strategies for Prevention and Intervention, ERASMUS programme (Network Participant)

Ravasco, P. DIETS= Dietitians Improving Education Training Standards across Europe , ERASMUS programme (Network Participant)

Ravasco, P. Member of the working group DELPHI – international panel of 17 specialists in caquexia.

Ravasco, P. President of the Master Program in Dietetics and Nutrition 2008-2010; Board: Paula Ravasco, Maria Ermelinda Camilo and Helena Cortez Pinto.

Ravasco, P. President of the newly proposed Master Program in Nutrition (organized in 2009 due to start in January 2010) Board: P. Ravasco, M.E. Camilo and H. Cortez-Pinto.

Ravasco, P. Supervisor of Carolina Boléo-Tomé and Mariana Chaves research projects.

Ravasco, P. Supervisor of 5 students' research projects for the Masters Thesis in Nutrition and Dietetics.

Ravasco, P. Supervisor of research projects for Integrated Masters Thesis in nutrition of 5 medical students. Project Tutors: Mariana Chaves and Carolina Boléo-Tomé.

Ravasco, P. Supervisora do estágio de investigação na área da Nutrição Clínica de dois alunos de 4º e 5º anos do curso de Medicina da *Comenius University Martin* (Martin) da Eslováquia (Jana Ferencova e Stanislav Krajnak), no âmbito do projecto de cooperação internacional “*Standing Committee on Research*” (SCORE) da “*International Federation of Medical Students' Associations*” (IFMSA) entre a Faculdade de Medicina da Universidade de Lisboa e outras faculdades de Medicina a nível internacional. Project Tutors: Mariana Chaves and Carolina Boléo-Tomé.

Ravasco, P. Suplementos nutricionais no desporto in the Pharmacology module. 7<sup>th</sup> Post Graduate Course in Medicina Desportiva , Faculdade de Medicina da Universidade de Lisboa. April 18, 2009.

Cravo, M., Lourenço, R. Curso Nutrição Clínica – Entérica e Parentérica. XI Congresso anual da APNEP. Lisboa, Portugal. June 18-19, 2009.

Cortez-Pinto, H. Is NAFLD a significant co-factor in other liver diseases? NAFLD/NASH, incluído no Curso Pós-Graduado Special Conference: NAFLD/NASH and related metabolic disease, Bologna, Italy. September 24-26, 2009.

Cortez-Pinto, H. Fígado gordo. Epidemiologia conhecida. Referenciação à Gastreterologia – Reunião organizada para Médicos de Medicina Familiar, Vila Nova de Gaia, Portugal. February 7, 2009.

Cortez-Pinto, H. Complicações hepáticas da obesidade. Curso Pós-Graduado: Obesidade e Gastreterologia, XXIX Congresso Nacional de Gastreterologia e Endoscopia Digestiva, Porto, Portugal. June 18-20,2009.

Cortez-Pinto, H. Supervisor of research projects for Masters Thesis in Medicine of 4 post-graduate students, and one in Human Molecular Biology – Faculdade de Ciências de Lisboa.

Monteiro Grillo, I. Co-orientadora na Tese de Mestrado “Impacto do meio Sócio-económico-demográfico, na qualidade de vida e reacções emocionais (ansiedade e depressão) da mulher mastectomizada”.

Monteiro Grillo, I. Co-orientadora na Tese de Mestrado “Hipodermoclise uma Forma de Dignificar a Vida na Prestação de Cuidados de Saúde no Domicilio”

Monteiro Grillo, I. Orientadora na Tese de Mestrado “Cuidados Paliativos: Dificuldades dos Enfermeiros do HSM”

Monteiro Grillo, I. Orientadora na Tese de Doutoramento de Margarida Maria de Matos Rodrigues e Silva Eiras “Gestão da segurança do doente na prestação de cuidados de saúde”.

Lourenço, R., Correia, M. Fármacos e fluidoterapia: preparação, conservação e administração. Curso de Transporte de Doentes Críticos em Pediatria. Lisbon, Portugal. January 2009.

**Public Perception of Science**

Ravasco, P., Boléo-Tomé, C., Chaves M. Sessão de esclarecimento para um grupo de alunos do 12º ano de escolaridade do Escola Secundária Miguel Torga em Monte Abraão, no contexto da Área de Projecto subordinada ao tema – “Uma vida melhor para doentes oncológicos”, Faculdade de Medicina da Universidade de Lisboa. Dezembro 4, 2009.

## **Immunobiology Unit**

### **Description and Objectives**

Immunological studies are crucial for public health providing knowledge for vaccine development and for the treatment of immune disorders, transplantation rejection, infectious diseases and cancer. Understanding the mechanisms underlying differentiation of immune cells and the control of immune responses involves analyses ranging from lymphoid organogenesis to lymphocyte differentiation and function.

Immune protection involves the generation of immunological memory, through which an individual acquires the capacity to respond better to the initial antigen on re-exposure. Immune and neuronal memory are characterized by obvious differences, but also striking parallels. Indeed, mounting evidence indicates that some of the most potent neurotrophic factors may act on or are produced by immune cells.

Our research projects are centred on the cellular and molecular mechanisms governing lymphoid organogenesis and on the role of neurotrophic factors in lymphocyte development and function.

### **Head of Unit**

Henrique Veiga-Fernandes, DVM, PhD

### **Research Team**

Afonso Almeida, PhD	- Post-Doc fellowship, FCT
Diogo Pereira	- PhD fellowship, FCT
Mariana Campos	- Master student (left in November 2009)
Francisca Almeida	- Master student
Inês Barbosa	- Technician
Lara dos Santos	- Technician
Gonçalo Ramos	- Trainee

### **Research Areas**

Lymphoid organogenesis  
Haematopoiesis  
Lymphocyte function  
Lymphocyte development

### **Major Scientific Achievements**

The Immunobiology unit initiated its activity in October 2008. This unit ensured international funding, being awarded an ERC starting grant and an EMBO installation grant. During 2009 we recruited a research team and we secured additional funding for our projects.

Natural Killer cell commitment

During the past year, in collaboration with Hugh Brady at Imperial College in London, we made an important contribution for the understanding of Natural Killer (NK) cell lineage commitment and development. We found that the basic leucine zipper transcription factor E4BP4 is essential for generation of the NK cell lineage. E4BP4 acted in a cell-intrinsic manner downstream of the interleukin 15 receptor and through the transcription factor Id2. These data were published in Nature Immunology (2009), and due to their impact a “news & views” on this work was published in the same issue of this journal.

Enteric organogenesis

We investigated which mechanisms empower cells from different embryonic origin, ie, nervous and haematopoietic cells, to use RET signalling but with different enteric colonisation outcomes. We found that LTin haematopoietic cells respond efficiently to all known RET ligands, which is in great contrast to nervous cells that respond exclusively to the ligand GDNF. Moreover, LTin cells use RET signalling in trans, while nervous cells are restricted to RET responses in cis. This unique property relies on LTin single cell expression signature. Conversely to nervous cells, LTin cells expressed Ret but did not co-express Ret co-receptors. To further support this hypothesis, we reprogrammed LTin cells to co-express Gfra1 in cis and showed that in this context LTin cells respond preferentially to GDNF, mimicking nervous cells responses. These findings demonstrate that during enteric organogenesis, by using different patterns of Ret and Gfra co-expression at a single cell level, cells from different origins can use the same key molecule but with different biological outcomes. A manuscript with these data is currently in preparation.

#### Embryonic haematopoiesis

We accessed the function of the tyrosine kinase RET during foetal haematopoiesis. We found that Ret and its co-receptors Gfra1 and Gfra2 were expressed in embryonic Haematopoietic Stem Cells (HSCs). Using mice homozygous for a null mutation of Ret we found that the HSC compartment of Ret deficient embryos had a 2.2 fold reduction when compared to their WT littermate controls. Accordingly, ablation of this gene had a major impact in total foetal liver cellularity. Strikingly, Ret null HSCs also had a reduction of quiescent cells, which correlated with a reduction in short-term and long-term reconstitution potential.

These finding strongly implicate RET signalling involvement in early foetal haematopoiesis, suggesting that GFLs might be used to modulate haematopoietic differentiation. We are currently dissecting the downstream events of this signalling axis in HSCs.

#### Generation of transgenic models for de-regulated Ret expression in lymphocytes

Over expression and expression of aberrant forms of RET are characteristic of oncogenic diseases including leukaemia. In order to further elucidate the role of Ret in lymphocytes, we started to develop tools allowing de-regulation of the normal expression patterns of RET in the haematopoietic system.

Thus, in the laboratory we have generated different transgenic constructs taking advantage of the human CD2 (hCD2) regulatory elements (promoter and Locus Control Region (LCR) of the hCD2). The latter constitute a powerful tool to drive expression of transgenes in lymphocytes. The hCD2 promoter and LCR allow tissue-specific, position independent and copy number dependent expression of transgenes, avoiding position effect variegation frequently observed in transgenesis. We have generated a hCD2-Ret construct that will enforce RET over-expression in lymphocytes, hCD2-RetMEN2A and hCD2-RetMEN2B that determine the expression of constitutively activated forms of RET. These constructs are currently being injected by Mauro Tolaini at NIMR, in London. Screening for founder will be performed in the next few months. A possible outcome from these models is the development of leukaemia mouse model, since Ret was shown to be differentially expressed in leukemic cells.

### Recent Most Relevant Publications

Gascoyne, D.M., Long, E., Veiga-Fernandes, H., de Boer, J., Williams, O., Seddon, B., Coles, M., Kioussis, D. and Brady, H.J. (2009) The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol.* 10: 1118-1124 (Times cited: 1)(Journal IF: 26.218)

Veiga-Fernandes, H., Coles, M.C., Foster, K.E., Patel, A., Williams, A., Natarajan, D., Barlow, A., Pachnis, V. and Kioussis, D. (2007) Tyrosine kinase receptor Ret is a key regulator in Peyer's Patch organogenesis. *Nature* 446: 547-551 (Times cited: 15)(Journal IF: 31.434)

Peixoto, A., Evaristo, C., Munitic, I., Monteiro, M., Charbit, A., Rocha, B. and Veiga-Fernandes, H. (2007) CD8 single-cell co-expression reveals three different effector types present at distinct phases of the immune response. *J. Exp. Med.* 204: 1193-1205 (Times cited: 14)(Journal IF: 15.504)

Veiga-Fernandes, H. and Rocha, B. (2004) High expression of active CDK6 in the cytoplasm of CD8 memory cells favors rapid division. *Nat. Immunol.* 5: 31-37 (Times cited: 45)(Journal IF: 25.113)

Veiga-Fernandes, H., Walter, U., Bourgeois, C., McLean, A. and Rocha, B. (2000) Response of naive and memory CD8+ T cells to antigen stimulation in vivo. *Nat. Immunol.* 1: 47-53 (Times cited: 254)(Journal IF: 25.113)

### **Ongoing Research Projects**

2008/2011 Role of neurotrophic factors in the immune system, EMBO. Coordinator: H. Veiga-Fernandes (EMBO installation grant, 1648)

2008/2013 Role of the proto-oncogene Ret in lymphocyte function, ERC. Coordinator: H. Veiga-Fernandes (ERC starting grant, 207057)

### **Publications in Peer Review International Journals 2009**

Gascoyne, D.M., Long, E., Veiga-Fernandes, H., de Boer, J., Williams, O., Seddon, B., Coles, M., Kioussis, D. and Brady, H.J. (2009) The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol.* 10: 1118-1124 (Times cited: 1)(Journal IF: 26.218)

### **Communications in International Conferences**

Fonseca-Pereira, D. et al. Role of the proto-oncogene Ret in foetal haematopoiesis". International Life Sciences Master students research conference. Maastricht, Netherlands. June, 2009 (Oral presentation)

Veiga-Fernandes, H. Common regulatory mechanisms in enteric lymphoid and neuronal organogenesis. 4th European Molecular Imaging Meeting, Barcelona, Spain. May, 2009 (Oral presentation)

Veiga-Fernandes, H. Common regulatory mechanisms in enteric lymphoid and neuronal organogenesis. Microanatomy of Immune Responses in Health and Disease. Birmingham, UK. September, 2009 (Oral presentation)

### **Communications in National Conferences**

Fonseca-Pereira, D., et al. Role of the proto-oncogene Ret during haematopoiesis". XXXV Reunião anual da Sociedade Portuguesa de Imunologia, Lisboa. September 28-29, 2009 (Oral presentation)

Fonseca-Pereira, D. et al. Role of the proto-oncogene Ret during haematopoiesis". 3rd IMM PhD Student Meeting, Lisboa. October 12-13, 2009 (Poster presentation)

### **Invited Lectures and Seminars**

Veiga-Fernandes, H. Shedding light on enteric lymphoid organogenesis. Instituto Gulbenkian de Ciência, Lisbon, Portugal. March 2009.

Veiga-Fernandes, H. Tools and lymphoid organogenesis. Jubilee of Prof. Maria de Sousa, Cascais, Portugal. November 2009.

Veiga-Fernandes, H. Lymphoid organogenesis and beyond. Instituto de Medicina Molecular, Lisboa Portugal. November 2009.

Veiga-Fernandes, H. Mechanisms in enteric lymphoid organogenesis. Centro de Malaria e outras

Doenças Tropicais, Lisboa, Portugal. November 2009.

### **Prizes, Honours and Awards**

Prize “Figures of the future in Science” by Correio da Manhã (2009) award to Henrique Veiga-Fernandes, Lisbon, Portugal.

### **Advanced Teaching**

Veiga-Fernandes H. Lymphoid organogenesis. GABBA PhD programme, Universidade do Porto, Portugal. June 2009.

Almeida, A. Lymphocyte homeostasis. GABBA PhD programme, Universidade do Porto, Portugal. June 2009.

### **Thesis Completed**

#### **Master Thesis**

Mariana Rodrigues Campos (2009) “Role of the proto-oncogene Ret during haematopoietic differentiation” Master thesis. Faculdade de Ciências da Universidade de Lisboa. Supervisor: Henrique Veiga-Fernandes.

## Molecular Parasitology Unit

### Description and Objectives

Malaria parasites (*Plasmodium*) cause severe disease in humans. The parasites develop and multiply asexually as haploid forms in red blood cells in the human host and move through a mosquito vector in order to infect new individuals. Transmission from the human host (or a mammalian model such as the mouse) to the mosquito, and *vice versa*, represent not only a severe bottleneck in the parasites' life cycle but depends on the development of specialized cell types. Male and female forms differentiate in the blood and once they arrive in the mosquito midgut - having been taken up during a blood meal of a female mosquito - take part in fertilization. The resulting diploid zygote develops into a motile ookinete that escapes the midgut pushing through the epithelium to continue its life cycle; sporozoites finally reside in mosquito salivary glands ready to infect a new individual. We have recently shown that post-transcriptional gene silencing is essential for the development of the zygote; much of the RNA is stockpiled in female precursor cells (gametocytes) where it relies on conserved protein factors to maintain this P-body related mRNP. Similarly, a related mechanism may play a role in the sporozoite. Our aim is to understand the protein factors that are involved in the translational regulation of mRNA and how transcription, RNA steady state levels and protein synthesis are controlled at these crucial moments. Strategies that interfere with the *Plasmodium* transmission may help reduce the spread of malaria.

### Head of Unit

Gunnar R. Mair, PhD in Molecular Parasitology, The Queen's University of Belfast, N. Ireland, U.K.

### Research Team

Celine Carret, PhD	- Post-Doc fellowship, FCT
Ana Rita Gomes	- MSc Student
Alexandre Costa	- Training fellowship (left in March)
Ana Maria Guerreiro, MSc	- Technician
Catarina Wanzeller Teofilo	- Training fellowship (left in September)
Marcelo Pereira	- Research student

### Research Areas

Malaria  
Gene expression control  
RNA biology  
Transmission

### Major Scientific Achievements

Malaria is a vector borne disease that according to a 2002 WHO report is responsible for 3-500 millions of clinical cases each year and is one of the major causes of disease in tropical and subtropical areas. Transmission of the parasite *Plasmodium* sp. relies on the uptake of sexual precursor cells or gametocytes into the mosquito vector and the subsequent generation of infective sporozoites that are transferred into a new individual during the bite of an infected mosquito. The *P. falciparum* genome harbours approximately 5400 genes. Comparative genomics on a number of other *Plasmodium* species (including the rodent malaria species *P. berghei* and *P. yoelii*) show highly syntenic chromosome blocks between the various species and similar number of predicted genes.  $\frac{2}{3}$  of open reading frames are still annotated as hypothetical



with unknown functions and no homologs in other organisms. Thus, an understanding of basic molecular and cell biological mechanisms is required, that once understood, will have the potential to translate into novel intervention strategies.

Throughout the *Plasmodium* life cycle the transcriptome and the resulting proteome changes dynamically with specific protein expression witnessed in all life cycle stages. How this is controlled giving rise to this diversity and specificity with few clearly recognizable transcription factors is largely unknown.

We have shown that post-transcriptional mechanisms play an important role during sexual development of the parasite that may also operate in salivary gland sporozoites prior to host infection. Gametocytes are cell cycle arrested and circulate in the vertebrate host, waiting to be taken up by a female mosquito during a blood meal. In the female gametocyte exist large mRNA pools that are translationally quiescent only to be activated following fertilization. We have identified the major protein factors involved in maintaining and stabilising translationally silenced transcripts. In the absence of core protein factors post-fertilization development is aborted, most likely due to the destabilization of 100s of transcripts in the female gametocyte. Among these destabilized mRNAs are parasite surface antigens and recently identified DNA binding proteins homologous to the AP2/ERF family of plant transcription factors, strongly suggesting that these proteins play an important role during transcription in the newly formed zygote and developing ookinete, possibly extending into oocyst and sporozoite stages. This event may be analogous to maternal-to-zygote transition in multicellular organisms when transcription is initiated in the newly formed zygote,.

More recently – and in collaboration with the Malaria Unit – we have been able to show that a similar mechanism may operate during sporozoite to liver stage transformation. There, RNA binding proteins appear to maintain the differentiated state of salivary gland sporozoites while awaiting transfer to a new mammalian host by inhibiting the translation of certain mRNAs.

### Recent Most Relevant Publications

Baum, J., Papenfuss, A.T., Mair, G.R., Janse, C.J., Waters, A.P., Cowman, A.F., Crabb, B.S. and de Konig-Ward, T. (2009) Molecular genetics and comparative genomics suggests RNAi is not functional in malaria parasites. *Nucleic Acid Res.* 37: 3788-3798 (Times cited: 1)(Journal IF: 6.968)

Yuda, M., Iwanaga, S., Shigenobu, S., Mair, G.R., Janse, C.J., Waters, A.P., Kato, T. and Kaneko, I. (2009) Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. *Mol. Microbiol.* 71: 1402-1414 (Times cited: 3)(Journal IF: 5.462)

Braks, J.A., Mair, G.R., Franke-Fayard, B., Janse, C.J. and Waters, A.P. (2008) A conserved U-rich RNA region implicated in regulation of translation in Plasmodium female gametocytes. *Nucleic Acids Res.* 36: 1176-1186 (Times cited: 5)(Journal IF: 6.968)

Mair, G.R., Braks, J.A., Garver, L.S., Wiegant, J.C., Hall, N., Dirks, R.W., Khan, S.M., Dimopoulos, G., Janse, C.J., and Waters, A.P. (2006) Regulation of sexual development of Plasmodium by translational repression. *Science* 313: 667-669 (Times cited: 67)(Journal IF: 26.372)

Khan, S.M., Franke-Fayard, B., Mair, G.R., Lasonder, E., Janse, C.J., Mann, M. and Waters, A.P. (2005) Proteome analysis of separated male and female gametocytes reveals novel sex-specific Plasmodium biology. *Cell* 121: 675-687 (Times cited: 82)(Journal IF 29.887)

### Publications in Peer Review International Journals 2009

Baum, J., Papenfuss, A.T., Mair, G.R., Janse, C.J., Waters, A.P., Cowman, A.F., Crabb, B.S. and de Konig-Ward T. (2009) Molecular genetics and comparative genomics suggests RNAi is not functional in malaria parasites. *Nucleic Acid Res.* 37: 3788-3798 (Journal IF 6.968)

McVeigh, P., Mair, G.R., Atkinson, L., Ladurner, P., Zamanian, M., Novozhilova, E., Marks, N.J.,

## 170 | 2009 IMM Activity Report

Day, T.A. and Maule A.G. (2009) Discovery of multiple neuropeptide families in the phylum Platyhelminthes. *Int. J. Parasitol.* 39: 1243-1252 (Journal IF 3.392)

Yuda, M., Iwanaga, S., Shigenobu, S., Mair, G.R., Janse, C.J., Waters, A.P., Kato, T. and Kaneko, I. (2009) Identification of a transcription factor in the mosquito-invasive stage of malaria parasites. *Mol. Microbiol.* 71: 1402-1414 (Journal IF 5.462)

Lavazec, C., Moreira, C.K., Mair, G.R., Waters, A.P., Janse, C.J. and Templeton TJ. (2009) Analysis of mutant *Plasmodium berghei* parasites lacking expression of multiple PbCCp genes. *Mol. Biochem. Parasitol.* 163: 1-7 (Journal IF: 2.896)

### **Prizes, Honours and Awards**

Best Oral Communication in the 12<sup>th</sup> GAPIC Program 2009 award to A.R. Gomes.

### **Advanced Teaching**

Gunnar Mair 2009 Invited Lecture: Course "Regulação e Diferenciação" of the Genetics and Molecular Biology Master, Faculdade de Ciências da Universidade de Lisboa.

## Mycobacteria-Host Interactions Unit

### Description and Objectives

The ability of Koch's bacilli to create tuberculosis or a latent infection relay, in part, on the powerful mechanisms it has evolved to parasitize host macrophages and dendritic cells. Our major aim for the current period was to better understand the molecular mechanisms by which 1) a non-pathogenic mycobacterium is killed by a macrophage and 2) how a pathogenic one survives. Given that all these processes are happening at the level of individual phagosomes in cells, we argue that the signalling networks occurring in the phagosomal membrane, which provides the key boundary between the pathogen and the rest of the cell, represents the key to understanding the two main objectives. Direct links between actin assembly and the inflammatory response leading to killing of non-pathogenic and pathogenic mycobacteria have been found. Towards objective 1 mycobacterial surface virulence factors and pro and anti-inflammatory ligands are being tested for their effects on phagosomal signalling networks. Towards objective 2 a broad cell biological strategy is being used that links signalling network analysis to membrane trafficking events and to the process of inflammation and anti-inflammation. Closely linked to these questions is the ongoing goal 3) -to be able to understand, and rationally manipulate the signalling networks in the phagosomal membrane controlling key functions in order to boost the innate killing propensity of macrophages.

### Head of Unit

Elsa Anes, PharmD, PhD) - Associate Professor, FFUL

### Research Team

Luísa Jordao, PhD	- Post-Doc fellowship, FCT (left in September)
Bibhuti Bhusan Mishra, Msc	- PhD fellowship, FCT (left in September)
Paulo Bettencourt, Msc	- PhD fellowship, FCT
Nuno Baltazar Carmo, Msc	- PhD fellowship, FCT
Marta Filipa Simões	- Master student (left in May)
David Pires	- Master student
Marta Barroso	- Master student

### Research Areas

Innate immunity  
Intracellular trafficking  
Host-pathogen interactions  
Tuberculosis

### Major Scientific Achievements

#### *Links Between Phagosomal Actin Assembly and Inflammatory Response in vitro*

Plasma membranes can transiently assemble actin filaments in response to receptor activation. Isolated macrophage latex bead phagosomes (LBP) also polymerize actin, a process stimulated by many phosphorylated lipids, such as sphingosine-1-phosphate (S1P) or phosphoinositide-4-phosphate. We now show that these lipids induce trans-membrane transport of ADP into the phagosome lumen where it is converted by adenylase kinase to ATP. This ATP is hypothesized to bind the phagosomal purinergic receptor P2X7 that regulates actin assembly on the LBP cytoplasmic surface. On plasma membranes of macrophages the addition of serum or S1P induces an immediate extra-cellular release of ADP that is converted to ATP by soluble adenylate kinase released outside. This ATP binds the P2X7 receptor that signals to the underlying

machinery that assembles actin- with only 5 seconds between S1P binding and actin assembly. In contrast, the membrane assembly of actin that occurs during phagocytosis operates independently of extra-cellular nucleotides. In addition we show that five lipids-- phosphatidylinositol-4-phosphate, phosphatidylinositol-(4,5)-bisphosphate, sphingosine-1-phosphate (S1P), ceramide-1-phosphate and phosphatidic acid stimulate both actin assembly and transport of ADP across the phagosomal membrane into the lumen. Once there, this ADP is converted to ATP by adenylate kinase activity. High luminal ATP concentrations correlated well with phagosome actin assembly under different conditions. We propose that luminal ATP accumulates in response to selected lipids and activates the P2X7R that signals across the phagosomal membrane to trigger actin assembly on the cytoplasmic membrane surface. (Kuehnel et al., 2009. J Cell Science).

During the last year we focused on the effects of lipids in killing mycobacteria via NF-KB. We have shown that several lipids can modulate the macrophage innate immune response against mycobacteria and enhance their killing. Since NF-kappaB is required for mycobacterial killing, we tested the ability of lipids to activate NF-kappaB in uninfected macrophages and those infected with mycobacteria. In uninfected cells, sphingomyelin (SM), phosphatidylinositol-4-phosphate (PIP) and arachidonic acid (AA) enhanced NF-kappaB activation and the cell surface expression of CD69, a macrophage activation marker regulated by NF-kappaB. Sphingosine (Sph), sphingosine-1-phosphate (S1P), diacylglycerol (DAG), eicosapentanoic acid (EPA) and phosphatidyl choline (PC) failed to activate either NF-kappaB or CD69. Ceramide (Cer) activated CD69 expression without activating NF-kappaB. In *Mycobacterium smegmatis*-infected cells, SM, PIP and AA transiently activated NF-kappaB in a manner that was enhanced. In contrast *Mycobacterium avium* mostly repressed NF-kappaB activation and only SM and AA could induce its partial activation. While lipids that activate NF-kappaB in uninfected cells tend to kill mycobacteria in macrophages Sph and S1P failed to activate NF-kappaB under most conditions but nevertheless enhanced killing of *M. smegmatis*, *M. avium* and *M. tuberculosis* H37Rv. Our results argue that both NF-kappaB-dependent and -independent mechanisms are involved in macrophage killing of mycobacteria and that both mechanisms can be enhanced by selected lipids. (Gutierrez et al., 2009 Cell Microbiol).

#### Other macrophage pro-inflammatory mechanisms during tuberculosis: The P2X7 receptor and the inflammasome

The mechanism(s) of *Mycobacterium tuberculosis* induced IL-1 $\beta$  secretion at the site of infection is poorly understood. We performed an shRNA-based screen to probe the role of known NOD-like receptors (NLRs) and major CARD-domain proteins (CARDs) in casp-1 activation and IL-1 $\beta$  secretion. We found that multiple NLRs/CARDs are involved in the response to *Mtb* in macrophages albeit, NALP3 was the inflammasome with the strongest contribution. Using a panel of different *Mtb* ligands, we showed that ESAT-6 activated casp-1 in a NALP3/ASC dependent fashion. Ag85 alone was not able to activate casp-1 but in combination with ESAT-6, it reached the cytosol and lead to a stronger casp-1 activation. These results establish the NALP3 as the main inflammasome involved in IL-1 $\beta$  secretion in response to *Mtb* and ESAT-6 as the dominant PAMP triggering casp-1 activation (Mishra et al submitted).

#### Role of macrophage and dendritic cells cathepsins and their inhibitors on Mycobacteria phagosome and on intracellular killing

Dendritic cells also play a central role in tuberculosis pathogenesis. Proteolytic enzymes named cysteine cathepsins are known to play a crucial role in antigen processing being an important part of the complex immune response. Further understanding of the proteolytic mechanisms involved and intracellular distribution of these proteases and their inhibitors (cystatins) will provide a valuable approach to modulate the immune response to tuberculosis. We found endo and exo peptidases with differentially killing activities on mycobacteria within activated macrophages and mature Dcs.

#### Effects of Mycobacterial porins on intracellular resistance survival within macrophages

Non-pathogenic mycobacteria such as *Mycobacterium smegmatis* reside in macrophages within phagosomes that fuse with late endocytic/lysosomal compartments. This sequential fusion process is required for the killing of non-pathogenic mycobacteria by macrophages. Porins are

proteins that allow the influx of hydrophilic molecules across the mycobacterial outer membrane. Deletion of the porins MspA, MspC and MspD significantly increased survival of *M. smegmatis* in J774 macrophages. However, the mechanism underlying this observation is unknown. Internalization of wild-type *M. smegmatis* (SMR5) and the porin triple mutant (ML16) by macrophages was identical indicating that the viability of the porin mutant in vivo was enhanced. This was not due to effects on phagosome trafficking since fusion of phagosomes containing the mutant with late endocytic compartments was unaffected. Moreover, in ML16-infected macrophages, the generation of nitric oxide (NO) was similar to the wild type-infected cells. However, ML16 was significantly more resistant to the effects of NO in vitro compared to SMR5. Our data provide evidence that porins render mycobacteria vulnerable to killing by reactive nitrogen intermediates within phagosomes probably by facilitating uptake of NO across the mycobacterial outer membrane. (for this project we received a PhD student from Brasil: Fabrino et al. 2009 *Microbes Infect*).

#### Development of New Strategies to treat tuberculosis

In collaboration with the iMed- FFUL we combined efforts to develop new pro-drugs, against TB, encapsulated in liposomes containing pro-inflammatory lipids having killing ability as assessed by the present study. Thus included ceramide or arachidonic acid. So far the new liposomal drugs possess bactericidal effects in macrophages infected with usually resistance strains to current antibiotic therapies. (Simões et al., 2009, Pires *et al.* in prep)

#### **Recent Most Relevant Publications**

Kuehnel, M., Rybin, V., Anand, P., Anes, E. and Griffiths, G. (2009) Lipids regulate P2X7 receptor-dependent actin assembly by phago- somes via ADP translocation and ATP synthesis in the phagosome lumen. *J. Cell Sci.* 122: 499- 504 (Times cited: 5)(Journal IF: 6.4247)

Gutierrez, M.G\*, Mishra, B.B.\*, Jordão, M.L., Elliot, E., Anes, E. and Griffiths, G. (2008) NF-kappa B activation controls phago-lysosome fusion-mediated killing of mycobacteria by macrophages. *J. Immunol.* 18: 2651-2663 \*Joint first authors (Times cited: 6)(Journal IF: 6.000)

Jordao, L., Bleck, CKE., Mayorga, L., Griffiths, G. and Anes, E. (2008) On the killing of mycobacteria by macrophages. *Cell Microbiol.* 10: 529-548 (Times cited: 10)(Journal IF: 5.598)

Anes, E., Peyron, P., Staali, L., Jordão, L., Gutierrez, M.G., Kress, H., Hagedorn, M., Maridonneau-Parini, I., Skinner, M.A., Wildeman, A.G., Kalamidas, S.A., Kuehnel, M. and Griffiths, G. (2006) Dynamic life and death interactions between *Mycobacterium smegmatis* and J774 macrophages. *Cell Microbiol.* 8: 939-960 (Times cited: 21)(Journal IF: 5.598)

Anes, E., Kuehnel, M.P., Bos, E., Moniz-Pereira, J., Habermann, A. and Griffiths, G. (2003) Selected lipids activate phagosome actin assembly and maturation resulting in killing of pathogenic mycobacteria. *Nat. Cell. Biol.* 5: 793-802 (Times cited: 115)(Journal IF: 17.774)

#### **Ongoing Research Projects**

2007/2009 Identification and manipulation of phagosomal signaling networks to activate killing mechanisms of macrophages infected with mycobacteria, FCT. Coordinator: E. Anes (PPCDT/BIA-BCM/55327/2004)

2009/2011 Interference of *Mycobacterium tuberculosis* with the endocytic/ antigen presentation pathways on macrophages and dendritic cells from healthy and immuno-compromised donors, FCT. Coordinator: E. Anes (PIC/IC/82859/2007)

2008/2009 Interaction of tuberculosis bacteria with dendritic cells, Bilateral collaboration Slovenia-Portugal, with the group of Tina Zavašnik Bergant, Instituto Jožef Stefan, Liubliana, FCT. Coordinator: E. Anes (SLO-PT 08/09)

### Publications in Peer Review International Journals 2009

Fabrino, D.L., Bleck, C.K.E., Anes, E., Hasilik, A., Melo, R.C.N., Niederweis, M., Griffiths, G. and Gutierrez, M.G. (2009) Role of porins in the susceptibility to nitric oxide-mediated killing of mycobacteria. *Microbes Infect.* 11: 868-875 (Times cited: 2)(Journal IF 3.7)

Simões, M.F., Valente, E., Rodriguez-Gomez, M.J., Anes, E\* and Constantino, L.\* (2009) Lipophilic POA amide and ester prodrugs. Stability, activation and activity against *M. tuberculosis*. *Eur. J. Pharm. Sci.* 37: 257-263 \* joint corresponding authors. (Journal IF 3.1)

Kuehnel, M., Rybin, V., Anand, P., Anes, E. and Griffiths, G. (2009) Lipids regulate P2X7 receptor-dependent actin assembly by phagosomes via ADP translocation and ATP synthesis in the phagosome lumen. *J. Cell Science* 122: 499-504 (Times cited: 5)(Journal IF 6.4)

Gutierrez, M.G., Gonzalez, A.P., Anes, E. and Griffiths, G. (2009) Role of lipids in killing mycobacteria by macrophages: evidence for NF-KB dependent and independent killing induced by different lipids. *Cell Microbiol.* 11: 406-420 (Times cited: 2)(Journal IF 5.6)

### Communications in International Conferences

Jordao, L. et al. Mycobacteria interaction with macrophages and dendritic cells: Is there a role for cathepsins?, Keystone Symposia, Dendritic cells, Banff, Alberta, Canada. March 29 - April 30 (Poster presentation)

Mishra, B.B. et al. *M. tuberculosis* activates the inflammasome leading to processing and release of IL-1 $\beta$  from macrophages. Keystone Symposia, Immune Pattern Recognition, Banff, Alberta, Canada. March 29 -April 30 (Poster presentation)

Bettencourt, P. MicroRNAs are involved in early steps of mycobacteria infection on macrophages. Host Genetics Control of Infectious Diseases, Institut Pasteur, Paris, France. October 7-9, 2009 (Poster presentation)

### Communications in National Conferences

Bettencourt, P. and Anes, E. Are microRNAs involved in Mycobacterial infections? URJA Workshop Mycobacterium Biology and Pathogenesis, Faculdade de Farmácia da Universidade de Lisboa, Lisboa. April 8, 2009 (Oral presentation)

Carmo, N.B. and Anes, E. Searching for host factors involved in Mycobacterium tuberculosis infection. URJA Workshop Mycobacterium Biology and Pathogenesis, Faculdade de Farmácia da Universidade de Lisboa, Lisboa. April 8, 2009 (Oral presentation)

Simões, M.F. and Anes, E. New PZA derivatives active against mycobacteria PZA resistance species. URJA Workshop Mycobacterium Biology and Pathogenesis, Faculdade de Farmácia da Universidade de Lisboa, Lisboa. April 8, 2009 (Oral presentation)

### Organisation of National and International Conferences

Anes, E. organizer of the URJA/IMM Workshop Mycobacterium Biology and Pathogenesis, Faculdade de Farmácia da Universidade de Lisboa, Lisboa, Portugal. April 8, 2009

Anes, E. organizer of URJA/IMM Symposium Host-pathogen interactions: strategies for therapeutic development, Faculdade de Farmácia da Universidade de Lisboa, Lisboa. Portugal. November 16, 2009

### Advanced Teaching

Anes, E. "Innate immunity mechanisms for bacterial infections", Módulo Microbiologia e Saúde, Mestrado de Microbiologia Aplicada da Universidade de Lisboa.

Anes, E. Coordinator of Biologia Celular, Integrated master in Pharmaceutical Sciences, Faculdade de Farmácia da Universidade de Lisboa.

Anes, E. “Immunity to tuberculosis”, Imunologia, Integrated master in Pharmaceutical Sciences, Faculdade de Farmácia da Universidade de Lisboa.

### **Thesis Completed**

#### **PhD Thesis**

Bibhuti Bhusan Mishra (2009) Identification and manipulation of phagosomal signaling networks to activate killing mechanisms of macrophages infected with mycobacteria. Faculdade de Farmácia da Universidade de Lisboa. Supervisor: E. Anes

#### **Master Thesis**

Marta Filipa Simões (2009) Desenvolvimento de pró-fármacos derivados da pirazinamida para tratamento de micobacterioses e estudo do efeito combinado com anti-inflamatórios não esteróides. Mestrado em Microbiologia Clínica, Universidade de Lisboa. Supervisor: E. Anes

#### **Diploma Thesis**

Pires D. 2009. Receptores citosólicos e o inflamassoma no contexto de infeções bacterianas. . Licenciatura em Microbiologia da Universidade de Lisboa. Supervisor: E. Anes

## **Retroviruses Unit**

### **Description and Objectives**

The main goal of this unit is to study the molecular interactions of HIV with the cell and use its replication strategies for biotechnological applications. We aim to understand the mechanisms underlying the function of cellular restriction factors and co-factors in HIV-1 and HIV-2 infection. Specifically, we are concentrated in the viral protein Vif and investigate how it can protect viral infectivity of HIV by neutralizing APOBEC family proteins. This goal is integrated in a broader study to comprehend the host-virus interaction at the cellular and molecular level. Here, the importance of cellular proteins and miRNA to help or restrict HIV-1 infection is crucial to develop concepts of viral infectivity or latency. By using shRNA libraries and zinc-finger libraries we look into elucidate the major pathways of virus interaction with the cell.

In a different angle of research and using our understanding of HIV biology we aim to identify biochemical targets for viral neutralization and develop molecular strategies to knock-out HIV-1 infection. We are deeply interested to impede viral entry into cells and intervene in viral latency. The strategies we explore are based on the platform of synthetic zinc-fingers and engineering antibody-derived protein scaffolds that use unique variable regions. Our goal is to develop more effective and adapted strategies that can explore the weaknesses of the virus.

The final aim of our unit wants to explore HIV-1 replication strategies for biotechnology purposes to generate novel therapeutic proteins and better gene therapy strategies by programming cell targeting of engineered lentiviral particles.

### **Head of Unit**

Joao Gonçalves, PhD – Associate Professor, Faculdade de Farmácia da Universidade de Lisboa

### **Principal Investigators**

Inês Soeiro, PhD

### **Research Team**

Mariana Santa-Marta, PhD	- Ciência 2009 fellowship
Paula Brito, PhD	- Post-Doc fellowship, FCT
Sylvie Rato	- PhD fellowship, FCT
Lidia Ferreira	- PhD fellowship, FCT
Andreia Couto	- PhD fellowship, FCT
Soraia Oliveira	- PhD fellowship, FCT
Acilino Vieira	- PhD fellowship, FCT
Cátia Cantante	- PhD fellowship, FCT
Iris Couto	- PhD fellowship, FCT (left in June)
Frederico Aires da Silva	- PhD fellowship, FCT (left in March)
Luis Ferreira	- Training Fellowship
André Fonseca	- Training Fellowship
Catarina Santos	- Training Fellowship
Ana Catarina Santos	- Master Student

### **Research Areas**

Regulation of APOBEC3G as a tool for antiviral therapy  
Genetic screening of HIV-1 restriction factors  
miRNA expression and regulation of viral replication



## Antibodies derived by viral mutagenesis and antibody engineering

**Major Scientific Achievements**

- A. We have provided evidence that the mechanism of HIV-1 Vif induced degradation of APOBEC3G is via ubiquitination by the new SOCS box of Vif protein. In addition, our studies have shown that besides degradation, Vif interaction with APOBEC3G can inhibit its deamination activity. Moreover, we have demonstrated that this direct inhibition of deaminases by Vif can be also shown for AID. In an effort to translate our work to HIV-2, we have demonstrated that APOBEC3G does not have an antiviral effect over HIV-2.
- B. We identified new 14 proteins essential for HIV-1 replication in T lymphocytes that do not affect cell viability. These proteins are described to be involved in MAPK, JNK and ERK pathways, vesicular traffic and DNA repair. Moreover, we show that most proteins do not affect viral integration but rather affect viral transcription/translation. This study brings new insights for the complex interplay of HIV-1/host cell and opens new possibilities for antiviral strategies.
- C. We have designed new small domain intrabodies that result from camelization of anti-Vif scFv and mapped its inhibitory region. In an effort to expand the technology of intrabody knock-out of intracellular antigens we have developed new scFv that can inhibit the function of KSHV LANA protein and consequently eliminate the majority of viral episomes from B-cell lymphoma.
- D. Our applied research aims to engineer new artificial zinc-fingers against HIV-1 promoter and resulted in the identification of two new sites on HIV-1 LTR capable to inhibit its expression and replication when targeted by the zinc-fingers.
- E. Our vision to deliver these molecular strategies to cells resulted in the construction of a lentiviral vector that expresses a chimeric Sindbis envelope at its surface displaying a scFv capable to recognize the CCR5 cell receptor.
- F. We have designed new small domain antibodies capable to construct new biosensors based on a piezoelectric surface. This work demonstrates the usefulness of piezoelectric biosensors in biorecognition analysis and evidences the advantages on using simultaneous impedance analysis to bring analytical significance to measured data, and thus to improve piezoelectric sensors sensitivity and applicability. These results broaden the intervention and applications of recombinant antibodies in the field of diagnosis.

**Recent Most Relevant Publications**

Rato, S., Maia, S., Brito, P.M., Resende, L., Pereira, C.F., Moita, C., Freitas, R.P., Moniz-Pereira, J., Hacothen, N., Moita, L.F. and Goncalves, J. (2010) Novel HIV-1 knockdown targets identified by an enriched kinases/phosphatases shRNA library using a long-term iterative screen in Jurkat T-cells. *PlosONE Accepted for publication* (Journal IF: NA)

Cadima-Couto, I., Freitas-Vieira, A., Nowarski, R., Britan-Rosich, E., Kotler, M. and Goncalves, J. (2009) Ubiquitin-fusion as a strategy to modulate protein half-life: A3G antiviral activity revisited. *Virology* 393: 286-294 (Times cited: 1)(Journal IF: 4.2)

Aires da Silva, F., Corte-Real, S. and Goncalves, J. (2008) Recombinant antibodies as therapeutic agents: pathways for modeling new biodrugs. *BioDrugs* 22: 301-314 (Times cited: 6)(Journal IF: 2.253)

Ferreira, G.N., Encarnação, J.M., Rosa, L., Rodrigues, R., Breyner, R., Barrento, S., Pedro, L., Aires da Silva, F. and Goncalves J. (2007) Recombinant single-chain variable fragment and single domain antibody piezoimmunosensors for detection of HIV1 virion infectivity factor. *Biosens. Bioelectron.* 23: 384-392 (Times cited: 8)(Journal IF: 5.243)

Costa, M.J., Pedro, L., Alves de Matos, A.P., Aires-Barros, M.R., Belo, J.A., Goncalves, J. and

Ferreira, G.N. (2007) Molecular construction of bionanoparticles: Chimeric Simian Immunodeficiency - Human Immunodeficiency nanoparticles with minimal viral protein content. *Biotechnol. Appl. Biochem.* 48: 35-43 (Times cited: 10)(Journal IF: 3.2)

### Ongoing Research Projects

2008/2010 Regulation of intracellular anti-viral defense factor APOBEC3G as therapeutic strategy to control HIV-1 infection, FCT. (PTDC/SAU-FCF/74613/2006)

2008/2010 Screening the role of kinases/phosphatases in early stages of HIV-1 infection using shRNA-encoded lentiviral libraries., FCT. (PTDC/SAU-MII/65346/2006)

2008/2010 Genome-wide screening with libraries of synthetic zinc-finger proteins capable to modulate host gene expression: Application in HIV-1 infection, FCT. (PTDC/SAU-GMG/71599/2006)

2008/2010 A Novel Lentiviral Target-Specific Strategy for Molecular and Gene Therapy, FCT. (PTDC/BIA-BCM/64275/2006)

2008/2010 Cellular and Molecular Response of HIV-2 and HIV-1 infection to integration modulation, Merck Sharp & Dohme.

### Publications in Peer Review International Journals 2009

Cadima-Couto, I., Freitas-Vieira, A., Nowarski, R., Britan-Rosich, E., Kotler, M. and Goncalves J. (2009) Ubiquitin-fusion as a strategy to modulate protein half-life: A3G antiviral activity revisited. *Virology* 393: 286-294 (Times cited: 1)(Journal IF: 4.2)

Fonseca, L., Aires-da-Silva, F., Santa-Marta, M., Barata, J., Barbas, C.F. 3rd and Goncalves, J. (2009) Programming specificity of lentiviral cell targeting using anti-FITC single-chain antibody. *Human Gene Therapy* 20: 1417–1545 (Journal IF: 5.2)

Corte-Real, S., Fonseca, L. , Barbas, C.F. 3rd, Chang, Y., Moore, P. and Goncalves, J. (2009) Intrabody-based mapping of latency-associated nuclear antigen from Kaposi's sarcoma-associated herpesvirus show conserved epitopes for viral latency inhibition. *Open J. Hematol.* (Times cited: 5)(Journal IF: NA)

### Communications in International Conferences

Soeiro, I. and Gonçalves, J. Regulation of HIV-1 Vif function by phosphorylation. Cold Spring Harbor Laboratory, NY, USA. May 23-27, 2009 (Poster presentation)

Rato S. et al. Functional shRNA screen reveals kinases and phosphatases essential for HIV-1 replication. 2009 CSHL Meeting on Retroviruses, Cold Spring Harbor Laboratory, New York, USA, May 18-23, 2009

Fonseca, L. et al. Programming specificity of lentiviral cell targeting using anti-FITC single-chain antibody. ISGT 2009, Hannover, Germany. November, 2009 (Poster presentation)

Couto, A. and Gonçalves, J. Human scFv derived from naïve library that target gp41 are strong candidates to inhibit HIV-1 Fusion Process. FEBS 2009, Prague, Czech Republic. September, 2009 (Poster presentation)

Rato, S.\*, Eberhardy, S., Goncalves, J., Barbas, C. Artificial zinc-fingers and siRNA targeting the primer binding site differentially inhibit reverse transcription and viral expression of HIV-1. 32th annual meeting on Retroviruses Cold Spring Harbor. Pp 254.

Couto, A. and Gonçalves, J. Human scFv derived from naïve library that target gp41 are strong candidates to inhibit HIV-1 fusion process. 34<sup>th</sup> FEBS Congress. 2009.

### Communications in National Conferences

Rato, S. et al. Identification of new cellular proteins essential for HIV-1 replication: the importance of kinases and phosphatases in HIV-1 life cycle. Microbiotec09, Vilamoura, Portugal. November 28-30, 2009

Couto, A. and Gonçalves, J. An HIV-1 gp120 loop grafted in a single domain VL antibody is sufficient for CD4 binding. Microbiotec 2009. Vilamoura, Portugal, November 28-30, 2009

Santos, C. et al. Design of synthetic single domain V<sub>L</sub> antibodies that inhibit HIV-1 infection through binding to gp41 ectodomain. Microbiotec 2009. Vilamoura, Portugal, November 28-30,

Design of synthetic single domain VL antibodies that inhibit HIV-1 infection through binding to gp41 ectodomain. MicroBiotec09. Vilamoura, Portugal, November 28-30, 2009 (Poster presentation)

Oliveira, S.R. and Gonçalves, J. New combinatorial strategy to multimerize single-domain antibodies to increase therapeutic potency. Microbitech09, Vilamoura. Portugal. November 28-30, 2009.

Rato S. et al. New targets for HIV-1 infection. National congress of infectious diseases and clinic microbiology. AIDS and Parasitology, Vilamoura, Portugal, October 8-11, 2009.

### Patents

2007 - Engineered Rabbit Antibody Variable DFomains and uses thereof. Application serial n° 60/916,226 (owner: TechnoPhage).

2008 - Albumin-Binding Domain-Fusions and Use Thereof to Improve Protein Pharmacokinetics. Application serial n° 61104513 (owner: TechnoPhage).

## Parasite Molecular Genetics Unit

### Description and Objectives

To escape the grip of the human immune system, *Trypanosoma brucei*, the parasite that causes African sleeping sickness, performs an infamous disappearing act. Every time the host's immune system gets close to eliminating the infection, a small number of trypanosomes avoids detection by shedding their surface 'coat' and putting on a new one, distinct from those the immune system already recognizes. The coat covers the entire surface of the parasite and consists of a dense layer of a Variant Surface Glycoprotein (VSG). This process of changing coats is known as antigenic variation.

There are hundreds of VSG genes throughout the trypanosome's genome, but, at any given time, only one is actively transcribed from a specialized subtelomeric locus known as the expression site. During antigenic variation, some parasites switch transcription to a new expression site, causing the surface coats to change their molecular identity. Such transcriptional changes do not involve alterations in the DNA sequence but are inherited nevertheless, which indicates that VSG transcription is under epigenetic control. Recently, it was shown that enzymes that remodel or modify the structure of chromatin at expression sites are important for antigenic variation. Moreover we now know that the active expression site is organized in a more open chromatin structure than of silent sites.

Our goal is to understand the role chromatin plays in antigenic variation in *T. brucei*. Specifically, we intend to identify the mechanisms and molecules that define open and closed chromatin structures at expression sites. To that end, we will use modern molecular and genetic strategies including RNA interference, chromatin immunoprecipitation, high-throughput deep sequencing and bioinformatics. Understanding how antigenic variation works should allow us to develop drugs that will disrupt VSG regulation, which in turn, would make parasites, at long last, more vulnerable to the host immune defences.

### Head of Unit

Luísa Figueiredo, PhD from the University of Porto and Institut Pasteur, France

### Recent Most Relevant Publications

Figueiredo, L.M. and Cross, G.A.M. (2010) Nucleosomes are depleted at the VSG expression site transcribed by RNA polymerase I in African trypanosomes. *Eukaryot. Cell* 9: 148-154 (Journal IF: 3.830)

Figueiredo, L.M., Cross, G.A.M., Janzen, C.J. (2009) Epigenetics in African trypanosomes. *Nat. Rev. Microbiol.* 7: 504-513 (Journal IF: 14.31)

Yang, X., Figueiredo, L.M., Espinal, A., Okubo, E. and Li, B. (2009) RAP1 Is essential for silencing telomeric variant surface glycoprotein genes in *Trypanosoma brucei*. *Cell* 137: 99-109 (Journal IF: 31.253)

Figueiredo, L.M., Janzen, C.J. and Cross, G.A. (2008) A Histone methyltransferase modulates antigenic variation in African Trypanosomes. *PLoS Biology* 6: e161 (Journal IF: 12.683)

Figueiredo, L.M., Freitas-Junior, L.H. and Scherf, A. (2002) A central role for *Plasmodium falciparum* subtelomeric regions in spatial positioning and telomere length regulation. *EMBO J.* 21: 815-824 (Journal IF: 8.295)

**Research Proposal**  
(Unit established in December)

THE STRUCTURE AND ROLE OF CHROMATIN IN ANTIGENIC VARIATION

Antigenic variation is a mechanism employed by certain pathogens in order to escape to the host immune system. In *Trypanosoma brucei*, antigenic variation relies on the tight transcriptional regulation of a multigene family of Variant Surface Glycoproteins (VSG). Only one VSG gene is transcribed at a time by RNA polymerase I (pol I), and this occurs from one of the ~15 specialized subtelomeric loci called bloodstream expression sites (BES). BES transcription is controlled epigenetically, but so far very few chromatin factors have been identified. My work performed in the laboratory of George Cross has demonstrated that two epigenetic factors, the histone methyltransferase DOT1B and the telomeric protein RAP1, are necessary for VSG silencing and for fast switching. I have also shown that the chromatin of the actively transcribed BES is essentially devoid of regularly spaced nucleosomes, in contrast to inactive BES, suggesting important structural differences between the chromatin of active and silent BESs. My proposed research will build upon this work, in an effort to understand the role of chromatin in the transcriptional regulation of VSG genes. My working hypothesis is that the “open” and “closed” chromatin, at the active and silent BESs respectively, result from the presence of different structural components, the action of chromatin modifying or remodelling enzymes and/or histone chaperones. To identify such proteins and their mechanisms of action, I will use complementary forward and reverse genetic approaches. Understanding the players and molecular mechanisms involved in the establishment and maintenance of specific epigenetic states at BESs will not only facilitate our understanding of antigenic variation, but may lead to the identification of new therapeutic targets.

RESEARCH AIMS:

1) *Test role of Histone H3 variant in BES silencing and switching*

Recent genome-wide studies revealed that Histone H3 variant (H3V) is enriched at telomeres and subtelomeric regions, and also at the end of polycistronic transcription units (Nicolai Siegel, personal communication). This new observation suggests that H3V might be involved in fine-tuning VSG telomeric silencing, which would have not been detected by Lowell and Cross 1. To test this hypothesis, I will use a similar approach to the one I developed to characterize the role of DOT1B in VSG switching 2. H3V will be knocked-out in a reporter strain containing two tagged BESs, which will allow me to measure transcript up-regulation by real-time PCR analysis, to measure switch rates and to follow VSG expression in individual cells by FACS analysis. If H3V plays a role in VSG silencing, I intend to test whether this effect is exacerbated in other mutant backgrounds, such as the DOT1B null mutant or others identified in aim 3). Building a map of genetic interactions will be useful in understanding the multiple pathways involved in VSG gene regulation.

2) *Identify factors that open/close BES chromatin using a candidate-gene approach*

I intend to use RNA interference (RNAi) to knockdown the expression of genes identified by genome analysis that are predicted to encode chromatin proteins or chromatin modifying enzymes (E.g. Histone H1, ISWI, JBP2, Asf1, Nap1, HAT1-3, etc.) and use a formaldehyde-dependent enrichment procedure (FAIRE) as a read-out of chromatin condensation 3. For those genes that are capable of disrupting the “open” or “closed” epigenetic states of BES, their effects on antigenic variation will be investigated as described in aim 1). Their precise role in chromatin condensation will be studied in cell-free systems such as super-coiling assay. Finally, these proteins will be epitope-tagged to determine their nuclear and chromosomal localizations by immunofluorescence microscopy and by the sequencing of chromatin immunoprecipitated DNA (ChIP-seq), respectively.

*1Luisa M Figueiredo: Research Proposal*

3) *Identify Pol I transcription activators using an RNAi genetic screen*

During a BES switch, a previously silent BES becomes activated. Like in higher eukaryotes, this transcriptional activation probably involves the removal of silencing proteins and binding of an

activator complex, which not only recruit the RNA polymerase, but also remodel the chromatin

4. Here, I aim to identify genes that are necessary to establish pol I transcription using an unbiased genetic approach. For that I intend to do an RNAi screen in the insect stage, where transfection efficiency is higher. Since BES promoters are not efficiently used in this life-cycle stage, my reporter strain will consist of an ectopic rDNA promoter that will drive the expression of a fusion protein HSV Thymidine Kinase-Puromycin and eGFP. A tet operator will be inserted just downstream of the promoter to allow conditional expression. A second cassette will be used to distinguish between transcription activators that exclusively affect transcription by pol I and not pol II (Figure). In contrast to a previous RNAi screen 5, my genome-wide RNAi library will be made on a tightly regulated hairpin-based construct. Knock-down of a pol I specific

activator will turn cells resistant to Gancyclovir and Blasticidin, but sensitive to Puromycin. FACS will be used to confirm the drug-resistance phenotypes and to quantitatively assess reporter expression levels in individual cells. After drug selection, the pool of transgenic lines will be subcloned and the targeted gene will be identified by PCR.

Epigenetic regulation of VSG expression is probably mediated by a complex network of factors and intersecting mechanisms. This research proposal is a logical follow-up of my previous work and aims at uncovering some of the molecular players of this network. My approaches range from rapid and straightforward (aim 1) to more long-term and risky (aim 3). The molecules identified in this study will open the doors for future studies on antigenic variation not only because they can be used as baits to identify interacting partners, but also because they will give us critical insight into the underlying molecular mechanisms, which can be used to formulate new hypothesis and thus help the field move forward.

1. Lowell, J. E. & Cross, G. A. A variant histone H3 is enriched at telomeres in *Trypanosoma brucei*. *J Cell Sci* 117, 5937-5947 (2004).
2. Figueiredo, L. M., Janzen, C. J. & Cross, G. A. A histone methyltransferase modulates antigenic variation in African trypanosomes. *PLoS Biol* 6, e161 (2008).
3. Giresi, P. G., Kim, J., McDaniell, R. M., Iyer, V. R. & Lieb, J. D. FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) isolates active regulatory elements from human chromatin. *Genome Res* 17, 877-885 (2007).
4. McStay, B. & Grummt, I. The Epigenetics of rRNA Genes: From Molecular to Chromosome Biology. *Annu Rev Cell Dev Biol* (2008).
5. Morris, J. C., Wang, Z., Drew, M. E. & Englund, P. T. Glycolysis modulates trypanosome glycoprotein expression as revealed by an RNAi library. *EMBO J* 21, 4429-4438 (2002).

## Neurosciences

**Programme Coordinator:** Ana Sebastião and Mamede de Carvalho

**Heads of Unit:**

Joaquim Alexandre Ribeiro, MD, PhD

Mamede de Carvalho, MD, PhD

Isabel Rocha, PharmD, PhD

José Ferro, MD, PhD

Tiago Fleming Outeiro, PhD

**Principal Investigators:**

Joaquim Alexandre Ribeiro, Ana Sebastião, Alexandre Mendonça, Luísa Lopes, Maria José Diógenes

Mamede de Carvalho, Michael Swash, Anabela Pinto, Conceição Azevedo Coutinho

Isabel Rocha

José Ferro, Alexandre Casto Caldas, Alexandre de Mendonça, Isabel Pavão Martins, José Pimentel, Cristina Sampaio, Teresa Paiva, Sofia A. Oliveira

Tiago Fleming Outeiro, Teresa Pais





## Neurosciences Unit

### Description and Objectives

This unit aims the elucidation of the cellular and molecular mechanisms involved in the tuning and fine-tuning of neuronal communication including receptor activation and transducing systems involved and membrane transporters. The unit is composed by four independent groups. Each group has its own research team and ongoing financed research projects. Therefore, in this report groups will be identified independently. Since there is a tight collaboration between the groups, which happens at the level of the students, post-docs and leaders, most of the papers, the communications and other output initiatives are co-authored and co-organized. Thus, to avoid repetitions, in the second part of this report, which deals with the 2009 output of the unit, the activities of the groups are pooled together. Also, due to the tight interaction between the groups, and to avoid repetitions, the major scientific achievements are pooled together.

### Head of Unit

Joaquim Alexandre Ribeiro, MD, PhD – Full Professor, FMUL

### Principal Investigators

Ana Maria Ferreira de Sousa Sebastião – PhD., Associate Professor, FMUL

Alexandre Valério de Mendonça, PhD., Principal Investigator, FMUL

Luisa V. Lopes PhD, Staff Scientist, IMM

Maria José Diógenes, Auxiliar Professor, FMUL

### Major Scientific Achievements

Following the broad objective of the Unit – to understand how neurons are finely tuned, or in other words, how neuronal activity is metamodulated by adenosine – major scientific achievements during 2009 include the following findings:

- 1) Adenosine A2A receptors allow synaptic plasticity, and this has been observed at both hippocampal synapses and the cortico-striatal pathway. These actions are most probably connected to the facilitatory influence exerted by A2A receptors upon the actions of neurotrophic factors. These molecules, besides influencing synaptic activity also influence neuronal maturation and neuronal death. We have been focusing on Brain Derived Neurotrophic Factor (BDNF) and Glial Derived Neurotrophic factor (GDNF) due to their involvement in neurodegenerative pathologies, such as Alzheimer's Disease, Huntington's Disease and Parkinson's Disease.
- 2) Adenosine A2A receptors facilitate the reuptake of the inhibitory transmitter, GABA, at nerve terminals, highlighting the possibility that A2A receptors shape the GABAergic control of excitability, therefore with potential implications for epilepsy.
- 3) While carefully looking at the influences of adenosine A2A receptors on synaptic transmission in very young (peri-weaning) animals, we could identify for the first time that at these ages tonic activation of A2A receptors predominates over A1 receptor activation, challenging an old paradigm that inhibition by adenosine predominates over excitation.

In conclusion, the major scientific achievements along the year reinforce concepts that have been developed and highlighted by the Unit members over the last years, that adenosine, via regulating the action of other agents that protect and reinforce synapses, has a major role in the process of 'synchronizing' or 'desynchronizing' directly or indirectly neuronal synaptic activity.

## NEUROMODULATION AND PLASTICITY GROUP

**PI:** Joaquim Alexandre Ribeiro, MD, PhD, Full Professor, FMUL

### Research Team

Alexandra Marçal, PhD	- Investigator, FCUL
Diana Cunha Reis, PhD	- Post-Doc fellowship, FCT
António Pinto Duarte, MSc ,	- PhD fellowship, FCT
Catarina Gomes, MSc.	- PhD fellowship, FCT
Paula I. Pousinha, MSc.	- PhD fellowship, FCT
Ana Rita Gagean de Sousa Guerra Costenla	- PhD fellowship, FCT
Frederico Simões do Couto, MD	- Assistant, FMUL
Nuno Canas, MD, MSc	- Assistant, FMUL
António F. Francisco, MD, MSc	- Assistant, FMUL
Nádia Rodrigues, BSc	- Msc Student
Ewa Tocarczyk	- MSc Neurobiology, Univ. Krakow, Poland
Joao Baião	- Technician, FMUL

### Research areas

Mechanisms of Neurosecretion.

Genesis of Neuromodulators

Neuro-Modulation and Metamodulation: a need to communicate between synapses

Experimental models of functional synaptic plasticity: implications for neurodegenerative diseases.

### Research objectives

The research performed by this group aims the elucidation of fine tune regulation of the chemical synapses by neuromodulators (e.g. purines, neuropeptides). Electrophysiological (extracellular and intracellular recordings) and neurochemical techniques are used to recognize the way neurotransmitters are controlled at the synaptic level and the implications of this knowledge in plasticity and in brain dysfunctions.

### Most Relevant Publications

Illes, P. and Ribeiro, J.A. (2004) Molecular physiology of P2 receptors in the central nervous system. *Eur. J. Pharmacol.* 483: 5-17 (Times cited: 72)(Journal IF: 2.787)

Ribeiro, J.A., Sebastião, A.M. and De Mendonça, A. (2002) Adenosine receptors in the nervous system: pathophysiological implications. *Prog. Neurobiol.* 68, 377-392. (Times cited: 160)(Journal IF: 9.130)

Ribeiro, J.A. and Sebastião, A.M. (1987) On the role, inactivation and origin of endogenous adenosine at the frog neuromuscular junction. *J. Physiol. Lond.* 384: 571-585 (Times cited: 106)(Journal IF: 4.649)

Ribeiro, J.A. and Sebastião, A.M. (1986) Adenosine receptors and calcium: basis for proposing a third (A3) adenosine receptor. *Prog. Neurobiol.* 26: 179-209 (Times cited: 258)(Journal IF: 9.130)

McQueen, D.S. and Ribeiro, J.A. (1981) Effect of adenosine on carotid body chemoreceptor activity in the cat. *Br. J. Pharmacol.* 74: 129-136 (Times cited: 105)(Journal IF: 4.902)

Ribeiro, J.A., Sa-Almeida, A.M. and Namorado, J.M. (1979). Adenosine and adenosine triphosphate decrease  $^{45}\text{Ca}$  uptake by synaptosomes stimulated by potassium. *Biochem. Pharmacol.* 28: 1297-1300 (Times cited: 100)(Journal IF: 4.838)

Ribeiro, J.A. and Walker, J. (1975) The effects of adenosine triphosphate and adenosine

diphosphate on transmission at the rat and frog neuromuscular junctions. *Br. J. Pharmacol.* 54: 213-218 (Times Cited: 144)(Journal IF: 4.902)

### Ongoing Research Projects

2008/2011 Membrane receptors and mediators involved in plastic changes induced by neuronal insults: implications for neuroprotection, FCT. Coordinator: J.A. Ribeiro (PTDC/SAU-FCF/68607/2006)

2005/2009 Interaction between endocannabinoids and adenosine in the hippocampus, FCT. Coordinator: J.A. Ribeiro (POCTI/SAU-FCF/57973/2004)

2005/2009 Role of TrkB receptors in ageing, Fundação Calouste Gulbenkian. Coordinator: J.A. Ribeiro (JG/ N°. 0494)

2007/2010 – COST B30 European Science Foundation Action on Neural Regeneration and Plasticity (NEREPLAS).

2006/2009. Blockade of adenosine A2A receptors prevents protein phosphorylation in the striatum induced by cortical stimulation and interactions with galanin receptors. Coordinators: S. Ferre (NIH, Bethesda, USA), J.A. Ribeiro (IMM) and R. Franco (Univ. Barcelona).

### NEUROPROTECTION GROUP

**PI:** Ana Maria Ferreira de Sousa Sebastião, PhD, Associate Professor (with *Agregação*), FMUL

#### Research Team

Claudia Valente, PhD	- Post-Doc fellowship, FCT
Catarina Fernandes, BSc	- PhD fellowship, FCT
Natália Assaife-Lopes, PharmD	- PhD fellowship, FCT
Vasco Sousa, BSc	- PhD Fellowship, FCT
Raquel Dias, BSc	- PhD Fellowship, FCT
Sandra Vaz, BSc	- PhD Fellowship, FCT
Sofia Cristóvão-Ferreira, BSc	- PhD Fellowship, FCT
Diogo Rombo, , BSc	- PhD Fellowship, FCT
Rita Aroeira, BSc	- MSc Student
Raquel Tomé, BSc	- MSc Student
Ana Filipa Ribeiro, BSc	- MScStudent
Vassiliki Kyrargyti	- Visiting PhD Student (February - May 2009)
Ricardo Reis	- Invited Professor (July 2009)
Susanne Gothan	- Ghest Student, University of Kholm
Elvira Duarte	- Technician, FMUL
João Baião	- Technician, FMUL

#### Research areas

Tripartite synapse mechanisms  
 Cross-talk between neuroprotective agents  
 Synaptic mechanisms of neuroprotection

#### Research objectives

The main objective of the Neuroprotection group is to investigate mechanisms involved in neuronal protection. When neuronal cells suffer an insult they release substances, some of them being involved in regulation of their activity and neuroprotection. One of these substances is adenosine that besides acting itself as a neuroprotective agent, interacts with other substances (e.g. neurotrophins such as BDNF, GDNF), also relevant for cell survival. Our aim is to evaluate the

interplay between different neuroprotective substances and, therefore, to contribute for the understanding of how neurones can be helped to survive and recover from insults.

### Most relevant publications

Fontinha, B.M., Delgado-García, J.M., Madroñal, N, Ribeiro, J.A., Sebastião, A.M., Gruart, A. (2009) Adenosine A2A receptor modulation of hippocampal CA3-CA1 synapse plasticity during associative learning in behaving mice *Neuropsychopharmacology* 34: 1865-1874 (Journal IF: 6.835)

Fernandes, C.C., Pinto-Duarte, A., Ribeiro, J.A. and Sebastião, A.M. (2008) Postsynaptic action of brain-derived neurotrophic factor attenuates alpha7 nicotinic acetylcholine receptor-mediated responses in hippocampal interneurons. *J. Neurosci.* 28: 5611-5618 (Times cited: 5)(Journal IF: 7.452)

Sebastião, A.M., de Mendonça, A., Moreira, T. and Ribeiro, J.A. (2001) Activation of synaptic NMDA receptors by action potential-dependent release of transmitter during hypoxia impairs recovery of synaptic transmission on reoxygenation. *J. Neurosci.* 21: 8564-8571 (Times cited: 31) (Journal IF: 7.452)

Sebastião, A.M. and Ribeiro, J.A. (2000) Fine tuning neuromodulation by adenosine. *Trends Pharmacol. Sci.* 21: 341-346 (Times cited: 125)(Journal IF: 9.340)

Sebastião, A.M. and Ribeiro, J.A. (1996) A2 receptor mediated excitatory actions of adenosine in the nervous system. *Prog. Neurobiol.* 48: 167-189 (Times cited: 180) (Journal IF: 9.130)

### Ongoing Research Projects

2007/2010 Trophic actions of neurotrophic factors: dependency of co-activation of A2A adenosine receptors, FCT. Project coordinator: A.M. Sebastião (PTDC/SAU-NEU/64126/2006)

2007/2010 COST B30 Action (EU): Neural Regeneration and Plasticity (NEREPLAS): Chair of the whole action: Delgado-Garcia, JM (Sevilla, Spain). A.M. Sebastião is the Chair of Neurobiology Group:

2008/2010 - Plasticidade Dopaminérgica na Retina e suas implicações funcionais, Programa CAPES (Brasil)-GRICES (Portugal). Coordenador: F. Garcia de Mello; PI at IMM: A.M. Sebastião; PI at CNC, Coimbra: João Malva.

2009/2010 - Role of neuronal NF- $\kappa$ B in synaptic transmission in the adult murine hippocampus. Collaborative project with Lesley Probert, Pasteur Institute, Hellenic Pasteur Institute, Athens, Greece.

2008/2010 – Mechanisms of neuroprotection by citokines: interplay with adenosine. Collaborative project with Knut Biber (Groningen), involving research to be done in parallel at Groningen University and at IMM in complementary techniques.

2008/2010 – Modulation by adenosine of the BDNF synaptic influences. Collaborative project with Detlev Boisson (Oregon), Moses Chao (New York, USA) and with Regeneron Pharmaceuticals (USA).

2007/2010 - BDNF acute Regulation of Nicotinic alpha-7 receptor clusters on hippocampal GABAergic neurons. Collaborative project with Darwin Berg (Univ California, San Diego).

2007/2010 – Interplay between A1 receptors and CB1 receptors at the behavioural level. Collaborative project with Prof. Judith Pratt and Dr. Ros Brett (Glasgow).

**RECEPTOR BIOLOGY AND COGNITION GROUP****PI:** Luisa Vaqueiro Lopes, PhD, IMM Staff Scientist**Research Team**

Vânia Batalha, BSc	- PhD student
Joana Vital, BSc	- Master student
Jorge Valadas, BSc	- Master student
João Baião	- Technician, FMUL

**Research areas**

Ageing, chronic stress and cognition  
 Animal models of disease  
 Neuromodulation by adenosine A<sub>2A</sub> receptors

**Research objectives**

Our main interest is to understand how brain structures involved in memory are affected in situations of cognitive decline. We are particularly interested in changes occurring with ageing and whether these changes are hastened or exacerbated by several pathological conditions, namely depression and anxiety, epilepsy, neurodegenerative diseases. By the use of experimental models mimicking these situations, we investigate the impact on cognition both at neuronal and behavioral levels. We are mainly focused into exploring the role of adenosine A<sub>2A</sub> receptors as potential cognitive modulators both in vitro and in vivo.

**Most relevant publications**

Lopes, L.V., Marvin-Guy, L.F., Fuerholz, A., Affolter, M., Ramadan, Z., Kussmann, M., Fay, L.B. and Bergonzelli, G.B. (2008) Maternal deprivation affects the neuromuscular protein profile of the rat colon in response to an acute stressor later in life. *J. Proteomics* 71: 80-88 (Times Cited: 2) (Journal IF: NA)

Chen, J.F., Sonsalla, P.K., Pedata, F., Melani, A., Domenici, M.R., Popoli, P., Geiger, J., Lopes, L.V. and de Mendonça, A. (2007) Adenosine A<sub>2A</sub> receptors and brain injury: broad spectrum of neuroprotection, multifaceted actions and "fine tuning" modulation. *Prog. Neurobiol.* 83: 310-331 (Times cited: 25)(IF: 9.130)

Rebola, N., Coelho, J.E., Costenla, A.R., Lopes, L.V., Parada, A., Oliveira, C.R., Soares-da-Silva, P., de Mendonça A. and Cunha, R.A. (2003) Decrease of adenosine A<sub>1</sub> receptor density and of adenosine neuromodulation in the hippocampus of kindled rats. *Eur. J. Neurosci.* 18: 820-828 (Times cited: 24)(IF: 3.385)

Lopes, L.V., Cunha, R.A., Kull, B., Fredholm, B.B. and Ribeiro, J.A. (2002) Adenosine A<sub>2A</sub> receptor facilitation of hippocampal synaptic transmission is dependent on tonic A<sub>1</sub> receptor inhibition. *Neuroscience* 112: 319-329 (Times cited: 44)(IF: 3.556)

Lopes, L.V., Cunha R.A. and Ribeiro J.A. (1999) Increase in the number, G-protein coupling and efficiency of facilitatory adenosine A<sub>2A</sub> receptors in the limbic cortex, but not striatum, of aged rats. *J. Neurochem.* 73: 1733-1738 (Times cited: 24)(IF: 4.500)

Lopes, L.V., Cunha, R.A. and Ribeiro J.A. (1999) Crosstalk between A<sub>1</sub> and A<sub>2A</sub> adenosine receptors in the hippocampus and cortex of young adult and old rats. *J. Neurophysiol.* 82: 3196-3203 (Times cited: 47)(IF: 3.648)

### Ongoing Research Projects

2009/-- Effect of neurotrophic factors on postnatal gut motility, NESTEC. Coordinator: L.V. Lopes (IMM/474/2008)

2003/-- Effect of stress on the maturation of the enteric nervous system, Collaborative project with M. Faure. Coordinator: L.V. Lopes

### Regulation of Neuronal Death group

**PI:** Maria José Diógenes, PhD, Auxiliary Professor, FMUL

### Research Team (established in 2009)

André J Santos, MSc	- PhD Student
Daniela Calçada, BSc	- MSc Student
Kelsey Rogers	- Invited PhD Student, Univ Wisconsin, USA

### Research areas

Apoptosis and Autophagy  
Aging consequences on synaptic transmission and plasticity  
Synaptic actions of neurotrophins

### Most relevant publications

Fontinha, B.M., Diógenes, M.J., Ribeiro, J.A. and Sebastião A.M. (2008) Enhancement of long-term potentiation by BDNF requires adenosine A<sub>2A</sub> receptor activation by endogenous adenosine. *Neuropharmacology* 54: 924-933 (Times cited: 8)(Journal IF: 3.344)

Diógenes, M.J., Assaife-Lopes, N., Pinto-Duarte, A, Ribeiro, J.A. and Sebastião, A.M. (2007) Influence of age on BDNF modulation of hippocampal synaptic transmission: interplay with adenosine A<sub>2A</sub> receptors. *Hippocampus* 17: 577-585 (Times cited: 12)(Journal IF: 5.230)

Diógenes, M.J., Fernandes, C.C., Sebastião, A.M. and Ribeiro, J.A. (2004) Activation of adenosine A<sub>2A</sub> receptor facilitates BDNF modulation of synaptic transmission in hippocampal slices. *J. Neurosci.* 24: 2905-2913 (Times cited: 37)(Journal IF: 7.452)

### Ongoing Research Projects

2008/-- Deciphering the role of A $\beta$  toxicity in the loss of endogenous neuroprotection. Coordinator: M.J. Diógenes

---

### Publications in Peer Review International Journals 2009

Costenla, A.R., Cunha, R.A. and de Mendonça, A. (2010) Caffeine, adenosine receptors and synaptic plasticity. *J. Alzheim. Dis. In press* (Journal IF: 5.101)

Cristóvão-Ferreira, S., Vaz, S.H., Ribeiro, J.A. and Sebastião, A.M. (2009) Adenosine A<sub>2A</sub> receptors, through PKA activation, restrain PKC inhibition of GAT-1-mediated GABA uptake by nerve terminals. *J. Neurochem.* 109: 336-347 (Journal IF: 4.500)

Fontinha, B.M., Delgado-García, J.M., Madroñal, N., Ribeiro, J.A., Sebastião, A.M. and Gruart, A. (2009) Adenosine A<sub>2A</sub> receptor modulation of hippocampal CA3-CA1 synapse plasticity during associative learning in behaving mice *Neuropsychopharmacology* 34: 1865-1874 (Journal IF: 6.835)

Gomes, C.A., Simões, P.F., Canas, P.M., Quiroz, C., Sebastião, A.M., Ferré, S., Cunha, R.A. and Ribeiro, J.A. (2009) GDNF control of the glutamatergic cortico-striatal pathway requires tonic activation of adenosine A<sub>2A</sub> Receptors. *J. Neurochem.* 108: 1208-1219 (Journal IF: 4.500)

Pousinha, P.A., Sebastião, A.M and Ribeiro, J.A. (2010). Predominance of adenosine excitatory over inhibitory effects on transmission at the neuromuscular junction of infant rats. *J. Pharmacol. Exp. Ther.* 332: 153-163 (*Epub September 29, 2009*) (Journal IF: 4.309)

Ribeiro, J.A. and Sebastião A.M. (2010). Modulation and MetaModulation of Synapses by Adenosine. *Acta Physiol. In press* (Journal IF: 2.455)

Ribeiro, J.A. and Sebastião, A.M. (2010) Caffeine and Adenosine. *J. Alzheim. Dis. In press* (Journal IF: 5.101)

Sebastião, A.M. and Ribeiro, J.A. (2009) Triggering neurotrophic factor actions through adenosine A<sub>2A</sub> receptor activation: implications for neuroprotection. *Br. J. Pharmacol.* 158: 15-22 (Journal IF: 4.902)

Sebastião, A.M. and Ribeiro, J.A. (2009) Tuning and Fine-tuning synapses with adenosine, *Curr. Neuropharmacol.* 7: 180-194 (Journal IF: 1.151)

Serpa, A., Ribeiro, J.A. and Sebastião, A.M. (2009) Cannabinoid CB<sub>1</sub> and Adenosine A<sub>1</sub> receptors independently inhibit hippocampal synaptic transmission. *Eur. J. Pharmacol.* 623: 41-46 (Journal IF: 2.787)

#### Publications in Peer Review National Journals 2009

Serpa, A., Ribeiro, J.A. and Sebastião, A.M. (2009) Adenosine A<sub>1</sub> and cannabinoid CB<sub>1</sub> receptors independently inhibit hippocampal synaptic transmission. *Rev. Fac Med Lisboa*, 14, 59-64.

Ribeiro, J.A. (2008). Como a Ubaína uniu o Grupo do Professor Fernando Peres Gomes no Instituto Gulbenkian de Ciencia: Um tributo à Professora Tice Anastácio de Macedo. Pp 75-86. Coimbra (published in 2009).

#### Books / Book Chapters

Sebastião, A.M. and Ribeiro, J.A. (2009) Adenosine Receptors and the Central Nervous System *in Handbook Exp. Pharmacol.* 193: 471-534.

Pedata, F., Pugliese, A.M., Sebastião, A.M. and Ribeiro J.A. (2010) Adenosine A<sub>3</sub> receptor signaling in the central nervous system *in Handbook Exp. Pharmacol.* 194: 165-188 (accepted in 2009)

Outeiro, T.F. and Lopes, L.V. (2009) Synaptic dysfunction in Parkinson's disease: from protein misfolding to functional alterations *in Protein folding for the synapse*. Wytenbach and V. O'Connor (eds). Springer.

#### Communications in International Conferences

Assaife-Lopes, N. et al. TrkB receptor function and translocation to lipid rafts: Involvement of adenosine A<sub>2A</sub> receptors. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Batalha, V.L., et al. Effect of neonatal stress on adenosine receptor levels in the rat hippocampus. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Correia, A.M. et al. Net Modulation of adenosine A<sub>1</sub>/A<sub>2A</sub> receptors of the neuromuscular transmission upon aging. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Cristóvão-Ferreira, S. et al. Adenosine modulates astrocytic GABA uptake in a biphasic way.

39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Cunha-Reis, D. et al. On the cellular and molecular pathways involved in VIP inhibition of LTP in the CA1 area of the hippocampus. Proceedings of the 9<sup>th</sup> International Symposium on VIP, PACAP and related peptides, Kagoshima, Japan. October 5-8, 2009 (Oral presentation)

Dias, R.A. et al. Enhancement of AMPA currents and GluR1 membrane expression through PKA- coupled adenosine A<sub>2A</sub> receptors. Meeting Purines: 80 years and very much alive, Stockholm, Sweden. September 2009 (Poster presentation)

Diógenes, M.J. et al. Effect of BDNF upon LTP in aging: influence of adenosine A<sub>2A</sub>. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Pousinha, P.A. et al. Modulation of the rat neuromuscular transmission by endogenous adenosine upon aging. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Sebastião, A.M. et al. Endogenous activation of adenosine A<sub>2A</sub> receptors plays a pivotal role in associative learning and hippocampal plasticity in behaving mice. Meeting Purines: 80 years and very much alive, Stockholm, Sweden. September 2009 (Poster presentation)

Sousa, V.C. et al. Chronic caffeine administration increases 9-THC -induced deficits on short-term memory. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Valente, C.A. et al. Expression pattern of glycine receptor in rat hippocampus: changes from embryonic to postnatal stages. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Vaz, S.H. et al. Brain-derived neurotrophic factor (BDNF) enhances GABA uptake in cortical astrocytes by translocation of GAT-1 to the plasma membrane. 39<sup>th</sup> Annual Meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

### Communications in National Conferences

Aroeira, R.I. et al. Age-related changes of the glycine receptor in rat hippocampus. 40<sup>th</sup> Meeting of the Portuguese Society of Pharmacology, Porto, Portugal. December 2-4, 2009 (Oral presentation)

Aroeira, R.I. et al. Spatial-temporal characterization of the Glycine Receptor in Rat Hippocampus. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster presentation)

Batalha, V.L. Adenosine receptor levels in the rat hippocampus: stress-induced changes. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster presentation)

Batalha, V.L. et al. Effect of neonatal stress in the rat hippocampus, 40<sup>th</sup> Meeting of the Portuguese Society of Pharmacology, Porto, Portugal. December 2-4, 2009 (Oral presentation)

Cunha-Reis, D. et al. VIP inhibition of LTP in the CA1 area of the hippocampus: cellular and molecular pathways. 40<sup>th</sup> Meeting of the Portuguese Society of Pharmacology, Porto, Portugal. December 2-4, 2009 (Oral presentation)

Dias, R.A. et al. Activation of G<sub>s</sub>- coupled adenosine A<sub>2A</sub> receptors enhances AMPA currents and GluR1 membrane expression. 3rd IMM PhD Student Meeting, Instituto de Medicina Molecular, Lisbon, Portugal. October 12-13, 2009 (Oral presentation)

Diógenes, M.J. et al. Exogenous and endogenous BDNF effects upon LTP in aging. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster presentation)

Jerónimo, A.S. et al. Amyloid-beta induces changes in TrkB receptors densities in rat neuronal



cultures: Influence on apoptosis. 40<sup>th</sup> Meeting of the Portuguese Society of Pharmacology, Porto, Portugal. December 2-4, 2009 (Oral presentation)

Jerónimo, A.S. et al. BDNF effect upon apoptosis induced by amyloid-beta peptide: changes in TrkB receptors expression. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster Presentation)

Neves-Tomé, R. et al. Trophic actions of BDNF are independent of those of the adenosine A<sub>2A</sub> receptor. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster presentation)

Rombo, D. et al. Adenosine A<sub>1</sub> receptors negatively modulate GABA<sub>A</sub> mediated postsynaptic currents in rat hippocampal CA1 pyramidal cells. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster presentation)

Rombo, D. et al. Inhibition of GABAergic currents in rat hippocampal neurons by adenosine A1 receptor activation. 40<sup>th</sup> Meeting of the Portuguese Society of Pharmacology, Porto, Portugal. December 2-4, 2009 (Oral presentation)

Vital, J. et al. Maternal separation compromises hippocampal-dependent memory in aged rats. 11<sup>th</sup> Meeting Portuguese Society for Neurosciences, Braga, Portugal. June 4-6, 2009 (Poster presentation)

### Invited Lectures and Seminars

Assaife-Lopes, N. Role of lipid rafts in TrkB-BDNF signaling: Implications for neuroprotection, Neurology Meeting Sessions, Hospital de Santa Maria, Lisboa, Portugal. July 23, 2009.

Cristóvão-Ferreira, S. GABA transporters modulation by adenosine, Lisbon Area Neuroscience Meeting, Instituto de Medicina Molecular, Lisboa, Portugal. February 18, 2009.

Diógenes, M.J. Cross-talk between adenosine A<sub>2A</sub> receptors and Trk receptors for BDNF. Lisbon Area Neuroscience Meeting, Instituto de Medicina Molecular, Lisboa, Portugal. May 20, 2009.

Ribeiro J.A. Modulation and Metamodulation of Synapses by Adenosine, Purines – 80 years and very much alive. Nobel Forum, Karolinska Institut, Stockholm, Sweden.

Ribeiro, J.A. VIP modulation of GABAergic transmission and plasticity in the rat hippocampus, Bilbao, Spain

Sebastião, A.M. Adenosine, neurotrophic factors and neurodegenerative diseases: new pieces for a puzzle. International Workshop on Influence of Degeneration and Repair in the CNS and Periphery, Budapest, Hungary

Sebastião, A.M. Modulation of nicotinic receptors and synaptic plasticity at the hippocampus by neurotrophins and adenosine, University la Sapienza, Rome, Italy.

Sebastião, A.M. Modulation of nicotinic  $\alpha 7$  receptors by BDNF: implications for plasticity, Cyprus.

Vaz, S. Regulation of GABA transporters: Brain-derived neurotrophic factor effect on GABA uptake, Lisbon Area Neuroscience Meeting, Instituto Gulbenkian de Ciência, Oeiras, Portugal. December 16, 2009.

Sebastião, A.M. Modulation of nicotinic responses and plasticity at the hippocampus. IMM Seminar

Assaife-Lopes, N. Lipid rafts and Neurodegenerative diseases, Clinical Neurology Meeting.

Tomé AR, 'Effect of BDNF on synaptic transmission in transgenic mice that express altered levels of Adenosine Kinase' and 'Trophic actions of BDNF: dependency of Adenosine A<sub>2A</sub> receptors co-activation'. Neurosciences Seminar, IMM, January 2009

Valente, C. 'Glycinergic transmission in the hippocampus'. Neurosciences Seminar, IMM,

## 194 | 2009 IMM Activity Report

January 2009

Cristovão-Ferreira, A.S. 'Adenosine A<sub>2A</sub> receptors enhance GABA transport into nerve terminals by restraining PKC inhibition of GAT-1'. Neurosciences Seminar, IMM, January 2009

Vale, F.M. 'Corticosteroids pharmacodynamics explains late effects and may be critical to optimize therapeutic outcomes'. Neurosciences Seminar, IMM, February 2009

Tokarczyk, E. 'The Lisbon Story about stereotaxy, melanopsin and OPT (olivary pretectal nucleus)'. Neurosciences Seminar, IMM, March 2009

Assaife-Lopes, N. 'Role of lipid rafts in the cross-talk between TrkB and A<sub>2A</sub> receptors'. Neurosciences Seminar, IMM, April 2009

Kyrargyti, V. 'The role of NF-κB in synaptic plasticity'. Neurosciences Seminar, IMM, April 2009

Ribeiro, J.A. 'Introduction to Neuroethics'. Neurosciences Seminar, IMM, May 2009

Vaz, S. 'Brain-derived neurotrophic factor (BDNF) enhances GABA uptake in cortical astrocytes by translocation of GAT-1 to the plasma membrane'. Neurosciences Seminar, IMM, May 2009

Ribeiro, J.A. 'Adenosine receptors as targets for Caffeine'. Neurosciences Seminar, IMM, June 2009

Chen, J.-F. 'What knock animals tell us about the effects of caffeine'. Neurosciences Seminar, IMM, June 2009

Rogers, K. 'Investigation of the role of TrkB receptors in modulating autophagy in primary cortical neurons'. Neurosciences Seminar, IMM, June 2009

Rombo, D. 'Adenosine A<sub>1</sub> receptors negatively modulate GABA<sub>A</sub> receptor mediated postsynaptic currents in rat hippocampal CA1 pyramidal cells'. Neurosciences Seminar, IMM, June 2009

Tomé, R. 'Adenosine Levels in transgenic mice that express altered levels of Adenosine Kinase'. Neurosciences Seminar, IMM, July 2009

Reis, R. 'Diferenciação funcional de neuroesferas de retina de ratinhos'. Neurosciences Seminar, IMM, July 2009

Aroeira, R. 'Spatial-temporal characterization of the glycine receptor in rat hippocampus'. Neurosciences Seminar, IMM, July 2009

Jerónimo Santos, A. 'Influence of adenosine A<sub>2A</sub> receptors on neuroprotective effect of BDNF upon neuronal death induced by amyloid-beta peptide'. Neurosciences Seminar, IMM, July 2009

Batalha, V. 'Adenosine A<sub>2A</sub> receptors in the Rat Hippocampus: stress related changes'. Neurosciences Seminar, IMM, September 2009

Lopes, L.V. 'Adenosine A<sub>2A</sub> receptors and chronic stress – implications for hippocampal function'. Neurosciences Seminar, IMM, September 2009

Sousa, V. 'Modulation of cannabinoid signaling by adenosine A<sub>1</sub> receptors and chronic caffeine administration: a behavioural and neurochemical approach'. Neurosciences Seminar, IMM, September 2009

Cunha Reis, D. 'Endogenous control of LTD and depotentiation in the hippocampus by VIP'. Neurosciences Seminar, IMM, December 2009

Gomes, C. 'Neurotrophic factors modulation by adenosine receptors and dopamine release in the rat striatum'. Neurosciences Seminar, IMM, December 2009

### Organization of National and International Conferences

Ribeiro, J.A. Member of Scientific Committee of PURINES 2010 meeting (Terragona).

Sebastião, A.M. Member of Scientific Committee of PURINES 2010 meeting (Terragona).

Sebastião, A.M. Head of the Working group on Neurobiology and organizer of a session in COST B30 on Neuronal Plasticity, Bilbao.

### **Advanced Teaching**

Sebastião, A.M. Coordinator and lecturer of the Master/PhD course in Neurosciences, FMUL. Most staff members and students at the Neurosciences Unit have been involved in Lectures, Seminars and Lab. Demonstrations at this Course

Sebastião, A.M. Member of the Coordinating Committee of the Master/PhD course in Cognitive Sciences, Universidade de Lisboa.

Ribeiro, J.A. Lecture on The Toxic effects of Psychopharmacological Agents, Master course in Legal Medicine and Forensic Sciences

Ribeiro JA. Lecture on Neuroethics, Master Course in Bioethics

Lopes, L.V. Lecturer and co-coordinator of the course “Neurochemistry”, Master course in Biochemistry, Faculdade de Ciências da Universidade de Lisboa

Cunha-Reis, D. Lecturer and co-coordinator of the course “Neurochemistry” of the Mastercourse in Biochemistry, Faculdade de Ciências da Universidade de Lisboa

Sebastião, A.M. Lecturer and co-coordinator of the course “Neurochemistry” of the Mastercourse in Biochemistry, Faculdade de Ciências da Universidade de Lisboa

Diógenes, M. J. Lecture on Synaptic Plasticity, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

Sebastião, A.M. Lecture on Electrophysiological Techniques, Instituto Superior Técnico, Universidade Técnica de Lisboa.

Sebastião, A.M. Lecture on Electrophysiological Techniques, Faculdade de Farmácia da Universidade de Lisboa

Bi-monthly Journal Club by PhD and Msc students, EEM, FMUL.

### **Public Perception of Science**

Institute of Molecular Medicine open day – The Institute was open on March 20th to visits from high schools. The students participated on «hands on activities» coordinated by investigators from the Neurosciences Unit.

Tournament “Play-Decide” organized by Pavilhão do Conhecimento, Lisbon and chaired by neuroscientists.

Seminars and round-tables related to brain issues, held in schools and other institutions, by neuroscience researchers. Lectures, workshops and interaction between scientist and high school students were promoted.

#### Interaction with Media

Sebastião AM - Phone interview (January 2009) to the newspaper 'Metro' (free distribution, daily) to comment on the essay published by Young in Nature, 457, Jan 8th 'Love: Neuroscience reveals all'

Sebastião AM - Phone interview (January 2009) to the magazine 'Nova Gente' (weekly distribution) to comment on brain differences in left handed persons (prompted by election of President Obama)

Sebastião AM - TV interview (February 2009, 9:00 a.m. news) to comment on plasticity at single neuron level, prompted by the paper by Wallace et al published in Nature Neuroscience, 12, 2009

#### Lectures and sessions to a broad audience

Diógenes, M.J. “O envelhecimento neuronal” - Palestra no Club Setubalense, April 2009.

Sebastião, A.M. ‘How addictive drugs act in the brain’ - special session to teachers of Biology (S. Bruno basic school, Caxias).

Visits to Basic and Secondary Schools during Brain Awareness Week

Members of the Neurosciences Unit went to the following Basic and High Schools, who applied to have a session on the Brain to their students. Total Number of participants: 1690 people. Activity coordination: Luísa V. Lopes and Paula A. Pousinha

Grade	School	Localization	Investigator
EB	Jacques Magalhães	Alverca	Raquel Dias
EB	S. Bruno	Caxias	Ana Sebastião
EB	Colégio José Álvaro Vidal	Alverca	Sofia Ferreira
ES	M. <sup>a</sup> Amália Vaz de Carvalho	Lisboa	Paula Pousinha
ES	Colégio N. S. Fátima	Leiria	Paula Pousinha
ES	Prof. Herculano Carvalho	Lisboa	Diana Cunha-Reis
ES	Bocage	Setúbal	M. <sup>a</sup> José Diógenes
ES	Ferreira Dias	Aguilva-Cacem	Catarina Gomes
ES	Rainha D. Leonor	Lisboa	Luísa Lopes
ES	Anselmo de Andrade	Almada	Paula Pousinha
ES	Colégio Manuel Bernardes	Lisboa	Cláudia Valente
ES	Colégio S. Miguel	Fátima	Frederico Couto

**Participation of the Unit at the IMM open day “hands on activities”: «O que é que te passa pela cabeça?».** Activity coordination: Luísa V. Lopes and Paula A. Pousinha

The Institute of Molecular Medicine was open on 20th march to visits from high schools.

Investigators of the Unit Involved	Participating Schools
UNF Luísa V. Lopes Paula A. Pousinha M. <sup>a</sup> José Diógenes Ana Rita Costenla Diogo Rambo Rita Aroeira Vânia Batalha André Santos Dina Silva (Califórnia memory test) Sofia Ferreira, Raquel Dias (Brain Quiz)	ES Maria mália vaz de Carvalho ES Ferreira Dias ES Cascais ES Rainha D. Leonor ES Domingos Sequeira.

The activity was sponsored by ZEISS (Eng.<sup>a</sup> Laura Machado). Total number of participants: 160 students and 10 teachers.

**Thesis Completed****PhD Thesis**

Gomes C.A. Neurotrophic Factors Modulation by Adenosine Receptors and Dopamine Release in the Rat Striatum. Submitted to Faculty of Medicine University of Lisbon, July 2009. Supervision: J A Ribeiro.

**Master Thesis**

Aroeira, R.I. (2009) Caracterização espaço-temporal do receptor da glicina no hipocampo de rato. Submitted to Faculdade de Ciências da Universidade de Lisboa. Supervision: C. Valente e P. Lima.

Rombo, D. (2009) Adenosine Modulation of Inhibitory Postsynaptic Currents Mediated by GABA A receptors in Rat Hippocampal Neurons. Submitted to Faculdade de Ciências da Universidade de Lisboa. Supervision: A M Sebastião M Ramos.

Santos, A.J. (2009) Influência dos Receptores da Adenosina no Efeito Neuroprotector do BDNF na Morte Neuronal Induzida. Submitted to Faculdade de Ciências da Universidade de Lisboa. Supervision: M J Diógenes e P Lima.

Batalha, V. (2009) Adenosine A2A Receptors in the Rat Hippocampus: Stress Related Changes. Submitted to Faculdade de Ciências da Universidade de Lisboa. Supervision: L.V. Lopes e P Lima.

Rodrigues, N. (2009) Vias de Transdução Envolvidas no Efeito do VIP na LTP na área CA1 do Hipocampo. Submitted to Faculdade de Ciências e Tecnologia da Universidade de Lisboa. Supervision: D. Cunha-Reis and C. Duarte.

## Neuromuscular Unit

### Description and Objectives

The main role of the Neuromuscular Unit is the investigation of Neuromuscular Disorders. This encompasses clinical phenotypes, clinical neurophysiology, neuropathology, rehabilitation, trials, and studies on molecular biomarkers. Although, we favor clinical investigation, we collaborate with others institutions and with other groups inside IMM towards basic investigation. We are much involved in searching biomarkers of respiratory insufficiency in motor neuron disease-amyotrophic lateral sclerosis. Moreover, we search motor cortex excitability and motor unit recruitment. We are particularly interested in exploring the interplay between clinical conditions and neurophysiology in order to develop new areas, including nervous system plasticity.

In two main areas we are internationally very competitive. In motor neuron disease-amyotrophic lateral sclerosis -amyotrophic lateral sclerosis, specifically on respiratory care and rehabilitation and neurophysiology; and in familial amyloid polyneuropathy, in particular on markers of early involvement investigation and longitudinal studies after liver transplantation.

### Head of the Unit

Mamede de Carvalho, MD, PhD – Assistant Professor, FMUL

### Research Team

Michael Swash, MD, PhD	- Invited Full Professor, FMUL
Anabela Pinto, MD, PhD	- Assistant Professor, FMUL
Conceição Azevedo Coutinho, MD, PhD	- Assistant Professor, FMUL
Teresinha Evangelista, MD	- Assistant, FMUL
Isabel Conceição, MD	- Investigator
Cândida Barroso, MD	- Investigator
Benjamim Ohana, MD	- Investigator
Luz Rocha, Speech Therapist	- Investigator
Susana Pinto, MD	- PhD student
Pedro Pereira, Technician	- PhD student
José Pedro Almeida, MD	- PhD student
Luz Rocha, Speech Therapist	- Investigator
José Castro, Technician	- Master Student

### Research areas

Neuromuscular Diseases  
Amyotrophic Lateral Sclerosis  
Familial Amyloidosis  
Clinical Neurophysiology  
Skin biopsy

### Major Scientific Achievements

In motor neuron disease-amyotrophic lateral sclerosis: we described a new neurophysiological measurement to quantify disease progression and lower motor neuron loss (the neurophysiological index), which is now applied in clinical trials; we showed that phrenic nerve motor responses are reliable and sensitive to detect hypoventilation in ALS patients; we presented evidence that patients with normal diaphragm can have abnormal respiratory function in sleep, in particular a periodical desaturation, not related to REM, which predicts a poor

outcome; we described that paraspinal muscles are early affected and that large motor neurons are not more prone to degeneration than small ones; we demonstrated that different conventional respiratory tests target to measure inspiration depend on the function of one muscle more than other, variable between specific tests.

In familial amyloid polyneuropathy: we observed that pain and organ involvement is more frequent in old-onset patients, as opposed to young-onset who present with autonomic dysfunction in general; we showed that skin sympathetic skin response is an early marker of small fiber dysfunction in patients; we described that skin biopsy is sensitive in this group of patients, we reported that sympathetic denervation is an early marker of autonomic lesion of the heart.

### Recent Most Relevant Publications

Pinto, S., Turkman, A., Pinto, A., Swash, M. and de Carvalho, M. (2009) Predictive value of respiratory tests for respiratory insufficiency in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 120: 941-946 (Journal IF: 2.972)

Fonseca, A.C., Martins, A.C., Silvestre, A.R., Serrano, M.R., Calado, A., Vilarinho, L. and Evangelista, T. (2009). Mitochondrial changes in patients with polymyositis. *Eur. J. Neurol.* 16 Suppl. 3: 217 (Journal IF: 2.732)

de Carvalho, M., Pinto, S. and Swash, M. (2008) Paraspinal and limb motor neuron involvement within homologous spinal segments in ALS. *Clin. Neurophysiol.* 119: 1607-1613 (Times cited: 4)(Journal IF: 2.971)

de Carvalho, M., Dengler, R., Eisen, A., England, J.D., Kaji, R., Kimura, R., Mills, K., Mitsumoto, H., Nodera, H., Shefner, J. and Swash, M. (2008) Electrodiagnostic criteria for diagnosis of ALS: consensus of an International Symposium sponsored by IFCN. *Clin. Neurophysiol.* 119: 497-503 (Times cited: 22)(Journal IF: 2.972)

Conceição, I., Castro, J.F., Scotto, M. and de Carvalho M (2008) Neurophysiological markers in familial amyloid polyneuropathy: early changes. *Clin. Neurophysiol.* 119: 1082-1087 Times cited: 2)(Journal IF: 2.972)

### Ongoing Research Projects

1994/present ESTEEM - European Project for Investigation in Electromyography. (no current funding).

2008/2018 Transthyretin-associated amyloidoses outcomes survey (THAOS). A Global, Multi-Center, Longitudinal, Observational Survey of Patients with Documented Transthyretin (TTR) Mutations or Wild-Type TTR Amyloidosis, FoldRx Pharmaceuticals, Inc.

2008/2010 An Open-Label Extension of Study Fx-005. Evaluating Long-Term Safety and Clinical Outcomes of Fx-1006A in Patients with Transthyretin Amyloid Polyneuropathy, FoldRx Pharmaceuticals, Inc. (Clinical Protocol Number Fx-006)

2010/2011 Open-Label Safety Evaluation of Fx-1006A in Patients with Transthyretin Amyloidosis, FoldRx Pharmaceuticals, Inc.

2008/2010 Early Markers in FAP. To determine early disease markers in asymptomatic and early symptomatic Val30Met carriers, FoldRx Pharmaceuticals, Inc.

2009/2011 Complement polymorphism analyses in FAP type I populations. Collaborative project with Instituto de Biologia Molecular e Celular do Porto and Center for Neurology and Genetics – Chipre.

2008/2009 - Alterações mitocondriais em doentes com miosite idiopática. Coordinator: T. Evangelista

2008/2012 Prevalence and severity of fatty liver in morbid obesity: correlation and mechanisms

related to the role of ectopic fat in insulin resistance, FCT. Coordinator: H. Cortez-Pinto. (PTDC/SAU- OSM/100878/2008)

2009/2011 Caracterização Clínica e Diagnóstica de Doentes com Patologia Neuromuscular – Estudo Epidemiológico em Portugal (ENDOMUS), Sociedade Portuguesa de Estudos de Doenças Neuromusculares. Coordinator: T. Evangelista

2009-2011 Respiratory Rehabilitation in Amyotrophic Lateral Sclerosis: Clinical and Biochemical Impact, FCT. Coordinator: M. de Carvalho. (PICIIC/82765/2007)

2009/2011- MUNIX – Multicentric International study to evaluate a new method for motor unit number estimation.

2009-2012 – Awaji criteria validation, International collaborative project. Coordinator: M. de Carvalho.

### Publications in Peer Review International Journals (2009)

Almeida, V., Mestre, T. and de Carvalho, M. Pseudobulbar syndrome in two patients with Human Immunodeficiency virus infection. *Amyotr. Lat. Scler. In press* (Journal IF: 1.823)

Cudkowicz, M.E. and Swash, M. Cerebrospinal fluid markers in amyotrophic lateral sclerosis: has the time come? *Neurology In press* (Journal IF: 7.043)

de Carvalho M, Swash M (2009). An interesting case: a woman, her butcher and a Japanese name. *Pract. Neurol.* 9: 284-288 (Journal IF: NA)

de Carvalho, M. (2009) Natural history of young-adult amyotrophic lateral sclerosis. *Neurology* 73: 649 (Journal IF: 7.043)

de Carvalho, M. and Swash, M. (2009) Stratifying disease stages with different progression rates determined by electrophysiological tests in patients with amyotrophic lateral sclerosis. *Muscle Nerve* 40: 318 (Journal IF: 2.574)

de Carvalho, M. and Swash, M. (2009) What is really new in progressive muscle atrophy? *Arch. Neurol.* 66: 1448-1429 (Journal IF: 5.874)

de Carvalho, M. and Swash, M. Fasciculation-cramp syndrome preceding anterior horn cell disease: an intermediate syndrome? *J. Neurol. Neurosurg. Psychiatry In press* (Journal IF: 4.622)

de Carvalho, M. and Swash, M. Sensitivity of electrophysiological tests for upper and lower motor neuron dysfunction in ALS: a six-month longitudinal study. *Muscle and Nerve In press* (Journal IF: 2.574)

de Carvalho, M., Costa, J., Pinto, S. and Pinto, A. (2009) Percutaneous nocturnal oximetry in amyotrophic lateral sclerosis: periodical desaturation. *Amyotr. Lat. Scler.* 10: 154-161 (Journal IF: 1.823)

de Carvalho, M., Pinto, S. and Swash, M. (2009) Motor unit changes in thoracic paraspinal muscles in ALS. *Muscle Nerve* 39: 83–86. (Journal IF: 2.574)

de Carvalho, M., Pinto, S., Swash, M. (2009) Association between paraspinal muscles and diaphragm denervation. *Amyotroph. Lateral Scler.* 15: 1-4 (Journal IF: 1.823)

de Carvalho, M., Swash, M. (2009) Awaji diagnostic algorithm increases sensitivity of El Escorial criteria for ALS diagnosis. *Amyotr. Lat. Scler.* 10: 53-57 (Journal IF: 1.823)

Evangelista, T., Ferro, J.M., Pereira, P. and de Carvalho, M. (2009) A case of asymptomatic cytoplasmic body revealed by simvastatin. *Neuromusc. Disord.* 19: 66-68 (Journal IF: 2.932)

Fonseca, A.C., Martins, A.C., Silvestre, A.R., Serrano, M.R., Calado, A., Vilarinho, L. and Evangelista, T. (2009). Mitochondrial changes in patients with polymyositis. *Eur. J. Neurol.* 16 Suppl. 3: 217 (Journal IF: 2.732)

Foreid, H., Barroso, C., Evangelista, T., Campos, A. and Pimentel, J. Intracerebral amyloidoma:



case report and review of the literature. *Clin. Neuropath. In press* (Journal IF: 1.200)

Jungbluth, H., Lillis, S., Zhou, H., Abbs, S., Swash, M. and Muntoni, F. (2009) Late onset axial myopathy with cores associated with a novel dominant mutation in skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscul. Disord.* 19: 344-347 (Journal IF: 2.932)

Pimentel, J., Barroso, C., Miguéns, J., Firmo, C. and Antunes, J.L. (2009) Papillary glioneuronal tumor – prognostic value of the extension of surgical resection. *Clin. Neuropath.* 28: 287-294 (Journal IF: 1.200)

Pinto, S. and de Carvalho, M. Is a 4-hour use of non-invasive ventilation enough to define compliance? *Amyotr. Lat. Scler. In press* (Journal IF: 1.823)

Pinto, S., Geraldès, R., Vaz, N., Pinto, A. and de Carvalho, M. (2009) Changes of the phrenic nerve motor response in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 120: 2082-2085 (Journal IF: 2.972)

Pinto, S., Turkman, A., Pinto, A., Swash, M. and de Carvalho, M. (2009) Predictive value of respiratory tests for respiratory insufficiency in amyotrophic lateral sclerosis. *Clin. Neurophysiol.* 120: 941-946 (Journal IF: 2.972)

Pugdahl, K., Fuglsang-Frederiksen, A., Johnsen, B., Tankisi, H., de Carvalho, M., Fawcett, P., Labarre-Vila, A., Liguori, R., Nix, W. and Schofield, I. Variation in the neurophysiological examination of Amyotrophic Lateral Sclerosis in Europe. *Amyotr. Lat. Scler. In press* (Journal IF: 1.823)

Ringel, S. and Swash, M. (2009) Money and Medicine. *Neurology.* 72: 766-768 (Journal IF: 7.043)

Swash, M. (2009) Clinical questions need perceptive answers. *Pract. Neurol.* 9: 117 (Journal IF: NA)

Swash, M. (2009) Peer review and “openness”. *J. R. Soc. Med.* 102: 506-507 (Journal IF: 1.020)

Swash, M. (2010) Apraxia contributes to the motor deficit in Parkinson’s Disease and Multiple System Atrophy. *Eur J Neurol. (Epub December 30, 2009)*(Journal IF: 2.732)

#### Publications in Peer Review National Journals 2009

Pinto, S. and de Carvalho, M. (2009) Patologia neuromuscular e distúrbios do sono. *Sinapse* 9: 22-31.

#### Communications in International Conferences

Almeida, J.P. et al. Cost-effectiveness of home telemonitoring non-invasive ventilation in ALS patients. 20th International Symposium on ALS/MND, Berlin, Germany. December 2009 (Poster presentation)

Almeida, V. and de Carvalho, M. Lesion of the deep palmar branch of the ulnar nerve: causes and outcome. 13th Congress of the European Federation of Neurological Societies, Florence, Italy. September 2009 (Poster presentation).

Almeida, V. et al. Treatable flail-leg syndrome. 20th International Symposium on ALS/MND, Berlin, Germany. December 2009 (Poster presentation)

Azevedo Coutinho, M.C. et al. Contribution of myocardial I123 metaiodobenzylguanidine scintigraphy for selecting patients with familial amyloidotic polyneuropathy for liver transplantation. ESC Congress 2009, Barcelona, Spain. August 2009 (Oral presentation)

Braga, A.C. et al. Regional Differences in the Management of ALS in Portugal. 20th International Symposium on ALS/MND, Berlin, Germany. December 2009 (Poster presentation)

Coelho, T. et al. A landmark clinical trial of a novel small molecule transthyretin stabilizer, Fx-1006A, in patients with TTR amyloid polyneuropathy: A Phase II/III, randomized, double-blind,

## 202 | 2009 IMM Activity Report

placebo-controlled study. Peripheral Nerve Society, Würzburg, Germany. July 2009 (Oral presentation)

Coelho, T. et al. A landmark clinical trial of a novel small molecule transthyretin stabilizer, Fx-1006A, in patients with TTR amyloid polyneuropathy: Tafamidis (Fx-1006A): A first-in-class disease-modifying therapy for Transthyretin Amyloid Polyneuropathy. American Neurological Association (ANA) 2009, Baltimore, USA. October 2009 (Oral presentation)

Conceição, I. et al Multimodal neurophysiologic assessment of small fiber involvement in Val30Met carriers. Xth Quantitative EMG Conference. Venice, Italy, May,2009 (Poster presentation).

de Carvalho, M. et al. Does LMN loss in ALS derive from primary UMN degeneration? 20th International Symposium on ALS/MND, Berlin, Germany. December 2009 (Poster presentation)

de Carvalho, M. et al. Results of a randomized controlled phase II trial of memantine for ALS. 20th International Symposium on ALS/MND, Berlin, Germany. December 2009 (Poster presentation)

Fonseca, A.C. et al. Mitochondrial changes in patients with polymyositis. 13th Congress of the European Federation of Neurological Societies, Florence, Italy. September 2009 (Oral presentation)

Geraldes, R. et al. Preliminary evaluation of myasthenic crisis clinical risk factors. Euromyasthenia, Paris, France. December 2009 (Oral presentation)

Kirsten, P. et al. Is Continuing medical audit worthwhile? Xth Quantitative EMG Conference. Venice, Italy. May 2009 (Poster presentation)

Palma, A. et al. Proteomics of plasma from Portuguese patients with familial Amyotrophic Lateral Sclerosis. 9th International Conference AD/PD 2009, Prague, Czech Republic. March 2009 (Poster presentation)

Pinto, A. et al. Wireless solutions for the control of home ventilation in patients with ALS - functional and survival outcome prediction. 12th International Conference on Home Mechanical Ventilation, Barcelona, Spain. March 2009 (Oral presentation)

Pinto, S. and de Carvalho, M. Is the involvement of diaphragm symmetric in Amyotrophic Lateral Sclerosis? 7th European ALS Congress. Research Workshop and Young investigators Meeting, Turin, Italy. May 2009 (Oral presentation)

### Communications in National Conferences

Almeida, V. and de Carvalho, M. Esclerose Lateral Primária – uma série de casos. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Almeida, V. and de Carvalho, M. Lesão do ramo profundo do nervo cubital –precisamos de cirurgião? Neuro 2009, Algarve. May 14-16, 2009 (Poster presentation)

Almeida, V. et al. Coexistência de duas neuropatias desmielinizantes. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Almeida, V. et al. Disferriopatia manifestando-se como hiperCKemia idiopática. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Almeida, V. et al. Dois casos tratáveis de “Flail-Lef syndrome”. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Azevedo Coutinho, M.C. et al. Associação do envolvimento cardíaco ao compromisso neurológico em doentes com Polineuropatia Amiloidótica Familiar. XXX Congresso Portugues

de Cardiologia, Vilamoura, Portugal. April 19-22, 2009 (Poster presentation)

Azevedo Coutinho, M.C. et al. Cintigrafia miocárdica com metaiodobenzilguanidina marcada com I123 na detecção de envolvimento cardíaco em portadores assintomáticos de polineuropatia amiloidótica familiar. XXX Congresso Portugues de Cardiologia, Vilamoura, Portugal. April 19-22, 2009 (Poster presentation)

Azevedo, A.L. et al. Miotonia electromiográfica em doente com Síndrome de Cushing. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Castro, J. et al. Estimulação magnética transcraniana e paralisia psicogénica. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Faria, C. et al. Alterações genéticas associadas com baixa sobrevida livre de doença em doentes com tumores oligodendrogliais. Neuro 2009, Algarve. May 14-16, 2009 (Oral presentation)

Figueiredo, R. et al. Miosite distal associada a Espondiloartropatia indiferenciada e Lúpus Eritematoso Sistémico. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Figueiredo, R. et al. Mononeuropatia múltipla secundária SAAF como síndrome paraneoplásico. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Fonseca, A.C. et al. Alterações mitocondriais em doentes com polimiosite. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Foreid, H. et al. Paralisia periódica hipocaliémica tirotoxicosa simulando clinica e electrofisiologicamente uma síndrome de Guillain-Barré. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Poster presentation)

Geraldes, R. et al. Avaliação preliminar de marcadores clínicos de crise miasténica. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Lobo, P. et al. Polineuropatia amiloidótica adquirida após transplante hepático sequencial. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Lobo, P. et al. Abscesso do tronco cerebral: terapêutica médica, Neuro 2009, Algarve. May 14-16, 2009 (Poster presentation)

Lobo, P. et al. Poliradiculopatia Pós-raquianestesia com Bupivacaína. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares, Sesimbra. April 2009 (Oral presentation)

Lobo, P.P. et al. Polineuropatia Amiloidótica Adquirida após Transplante Hepático Sequencial. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Lobo, P.P. et al. Ventilação não-invasiva não melhora o declínio da função respiratória nos doentes com Esclerose Lateral Amiotrófica. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Neto, L. et al. Hemangioblastoma do hipocampo, Neuro 2009, Algarve. May 14-16, 2009 (Poster presentation)

Pinto, S. and de Carvalho, M. Valor da resposta do nervo frénico como factor predictivo da sobrevida nos doentes com Esclerose Lateral Amiotrófica. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa, Portugal. November 5-8, 2009 (Oral presentation)

Pinto, S. et al. O valor predictivo da variabilidade nocturna da frequência cardíaca na sobrevida dos doentes com Esclerose Lateral Amiotrófica. Neuro 2009, Algarve. May 14-16, 2009 (Poster presentation)

Rodrigues, T. et al. Estudo de deleção 1p/19q em oligodendrogliomas anaplásicos primários e secundários. Neuro 2009, Algarve. May 14-16, 2009 (Oral presentation)

Silva, V. et al. Relação genotipo-fenotipo nos oligodendrogliomas. Neuro 2009, Algarve. May 14-16, 2009 (Oral presentation)

Sousa, M. et al. Impacto ecográfico e electromiográfico de uma infiltração de corticosteróides na bainha do flexor comum dos dedos da mão na sintomatologia associada ao Síndrome do Túnel Cárpico – Resultados preliminares do Projecto Carpo. XVII Jornadas Internacionais do Instituto Português de Reumatologia, Lisboa. Dezembro 2009 (Poster presentation)

Sousa, M. et al. Síndrome do Túnel Cárpico: avaliação clínica, ecográfica e electromiográfica prospectiva em 32 Punhos - Projecto Carpo. XVII Jornadas Internacionais do Instituto Português de Reumatologia, Lisboa. Dezembro 2009 (Poster presentation)

Valadas, A. Meningioangiomatose. Caracterização clínica e neuropatológica. Neuro 2009, Algarve. May 14-16, 2009 (Oral presentation)

### Invited Lectures and Seminars

Conceição, I. Paramiloidose – diagnóstico clínico e neurofisiológico. Congresso de Neurologia, Lisboa, Portugal. November 2009.

Conceição, I. Polineuropatia amiloidótica familiar – como diagnosticar. 1º Congresso de Internacional de Paramiloidose, Barcelos, Portugal. June 2009.

de Carvalho, M. Actualizações em ELA. VIII Simposio Brasileiro de ELA/DNM, S. Paulo, Brasil. June 2009.

de Carvalho, M. Aspectos Neurofisiológicos nas Neuropatias Hereditárias. Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças Neuromusculares,

de Carvalho, M. Criteris Electrodiagnostics de la Malaltia de Neurona Motora XIII Reunio Anual de la Societa Catalana de Neurologia. Barcelona, Spain. March 2009.

de Carvalho, M. Electroneuromiografia diagnóstico precoce e diagnóstico de certeza. VIII Simposio Brasileiro de ELA/DNM, S. Paulo, Brasil. June 2009.

de Carvalho, M. Electrophysiological studies involving caffeine and the brain, Lisbon, Portugal. June 2009.

de Carvalho, M. Guidelines in ALS Xth Quantitative EMG Conference.,Veneza, Itália. May 2009

de Carvalho, M. Indices neurofisiológicos en ELA. Congresso Colombiano de Neurologia, St Marta. August 2009.

de Carvalho, M. Medidas neurofisiológicas en ELA : marcadores de progressión. Congresso Colombiano de Neurologia, St Marta. August 2009.

de Carvalho, M. Momento apropiado para ventilación no invasiva en pacientes con ELA. Congresso Colombiano de Neurologia, St Marta. August 2009

de Carvalho, M. Respuestas motoras evocadas por estimacion magnética transcraneal en ELA. Congresso Colombiano de Neurologia, St Marta. August 2009.

de Carvalho, M. Transcranial magnetic stimulation: 25 years of experience in Neurosciences. VII Encontro de Engenharia Biomédica, Lisboa, Portugal. October 2009.

Pinto, S. and de Carvalho, M. Respiratory Exercises in Amyotrophic Lateral Sclerosis (REALS). 20th International Symposium on ALS/MND, Berlin, Germany. December 2009.

### Organisation of National and International Conferences

Conceição, I. Co-organized the meeting “Reunião da Primavera da SPEDNM”, Sesimbra, Portugal. April 2009.

De Carvalho, M. Co-organizer and Chairman of the joint Meeting “Sociedade Portuguesa para Estudo das Doenças Neuromusculares”- “Sociedade Portuguesa de Neuropatologia”- “Associação Portuguesa de EEG e Neurofisiologia Clínica”, promoted by the “Sociedade Portuguesa de Neurologia”, Lisbon, Portugal. November 2009.

De Carvalho, M. Member of Scientific Committee of 7th European ALS Congress – research workshop and young investigators meeting, Turin, Italy. June 2009.

De Carvalho, M. Organizer and Chairman of the Electromyography Course, promoted by “Associação Portuguesa de EEG e Neurofisiologia Clínica”, Carcavelos, Portugal. June 2009.

Evangelista, T. Co-organized the meeting “Reunião da Primavera da SPEDNM”, Sesimbra, Portugal. April 2009.

### Prizes

De Carvalho, M. President of the International Juri of the “2nd International Prize to Medicine of the Instituto Paulo Gontijo”, São Paulo. October 2009.

### Advanced Teaching

Conceição, I. “Evoked Potentials”, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. April 2009.

De Carvalho, M. “Cortical Plasticity”, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. April 2009.

De Carvalho, M. “EMG and Clinical Neurophysiology, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. April 2009.

De Carvalho, M. “Investigation on Clinical Neurophysiology”, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. April 2009.

Evangelista, T. “Músculo – morfologia e metabolismo”, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. January 2009.

Evangelista, T. “Nervo – morfologia e metabolismo”, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. January 2009.

Pereira, P. “Biópsias de pele – Terminações nervosas intraepidérmicas”, Mestrado em Neurociências, Faculdade de Medicina de Lisboa, Lisboa. January 2009.

### Thesis

#### PhD Thesis

João Parracho Costa (2009) Evaluation of human subcortical circuits and pathways through stimulation and direct recording from electrodes implanted for chronic deep brain stimulation. Faculdade de Medicina da Universidade de Lisboa. Supervisor: M. de Carvalho

#### Master Degree Thesis

Nuno Vaz (2009) “Progressão Clínica e Neurofisiológica num Grupo de Doentes com Esclerose Lateral Amiotrófica”. Faculdade de Medicina da Universidade de Lisboa. Supervisor: M de

Carvalho.

Nuno Santos (2009) “Preliminary Biomechanical Studies on the Diaphragmatic Function in Controls and Patients with Loss of Motor Units”. Instituto Superior Técnico da Universidade Técnica de Lisboa and Faculdade de Medicina da Universidade de Lisboa. Co-supervisor: M. de Carvalho.

André Pereira (2009) Development of a Hill-Type Muscle Model with Fatigue for the Calculation of the Redundant Muscle Forces using Multibody Dynamics. Instituto Superior Técnico da Universidade Técnica de Lisboa and Faculdade de Medicina da Universidade de Lisboa. Co-supervisor: M de Carvalho.

Ana Rita Silvestre (2009) Alterações histológicas e moleculares no músculo esquelético de indivíduos com obesidade mórbida e sua relação com a resistência à insulina. Faculdade de Ciências e Faculdade de Medicina da Universidade de Lisboa. Co-supervisor: T. Evangelista.

## Autonomic Nervous System Unit

### Description and Objectives

The Unit of the Autonomic Nervous System (UANS) is devoted to the study of the autonomic nervous system in areas related with cardiovascular control. It assembles researchers of different origins: fundamental and clinical researchers with medical and non-medical degrees being the majority of them are also Faculty of Medicine of Lisbon. The research protocols embrace both ex-vivo and in vivo animal laboratory studies. These studies are complemented by the autonomic evaluation of human beings and the clinical evaluation of patients with dysautonomy that is performed at our human autonomic laboratory.

We work collaboratively with other colleagues of the University of Lisbon and Technical University of Lisbon with the goal of understanding how cells and body function and how we can modulate or interfere with the function in order to improve the health status.

Our staff at UANS also contributes to teaching of Medical Physiology at the Faculty of Medicine, in Lisbon, as well as in other academic undergraduate (eg, biomedical engineering, nutrition, microbiology, health sciences) and others that have physiology in their curricula (master/PhD program in Neurosciences, in Cognitive Sciences, in Sleep, in Neuro-ophthalmology, in Hyperbaric Medicine) involving around 800 students.

We also focus intensively on the study of the molecular basis of neurodegenerative disorders such as Alzheimer's and Parkinson's disease, with the goal of developing novel strategies for therapeutic intervention.

### Head of Unit

Isabel Rocha, PharmD, PhD and *Agregação* in Physiology, Associate Professor with *Agregação*, FMUL

### Principal Investigators

Pedro Freire Costa, MD, PhD, *Agregação* in Physiology, Full Professor of Physiology, FMUL

J.L. Ducla Soares, MD, PhD, *Agregação* in Medicine, Full Professor of Medicine, FMUL

### Research Team

Carlos Neves, MD, PhD	- Auxiliary Professor, FMUL
Esmeralda Delgado, DMV, PhD	- Auxiliary Professor, FMVL
Gabriela Postolache, PhD	- Post-Doc Fellowship, SorinCardio
Mario Oliveira, MD	- PhD Fellowship, SorinCardio
Mariana Santos, MD	- Assistant, FMUL
Fernando Domingos, MD	- PhD Student
Vera Geraldes, MSc	- PhD Fellowship, Merck/Foundation
Victor Silva, MSc	- Training Fellowship, FCT grant (August to November)
Cristiano Tavares, BsC	- Training Fellowship, FCT grant
Susana Raposo, BsC	- Training Fellowship, Merck Foundation (FCT, August to November)
Joana Alexandre, BsC	- Training Fellowship, FCT grant (July to October)
Sergio Laranjo, MD	- Training Fellowship, SorinCardio
Luisa Barreto, MD	- Training Fellowship

## Research Areas

Autonomic nervous system  
Signal processing  
Cell physiology  
Disautonomy

## Major Scientific Achievements

- Contribution to the clarification of the inner mechanisms that regulate ocular circulation, in particular, the choroidal circulation. We were able to show that intrinsic vasomotion is revealed in the ophthalmic artery in the absence of central nervous control and we established a controlled ex vivo model that can be used to study the effects of putative therapeutic drugs on ocular circular circulation, which is of major importance in ocular pathologies which have a vascular component.
- Study the influence of sublobule IX-b on cardiac function. These results have strong implications in the understanding of the central mechanisms that regulated blood pressure and respiratory control.
- Development of methodologies of data analysis on the time-scale domain applied to biological variables (e.g., heart rate, blood pressure, intraocular pressure, urinary bladder pressure) that are currently used to access autonomic function and disautonomy in a diagnostic perspective as well as to be used in the patients follow-up.
- Combination of the analysis of human pathology with rabbit and rat models that are contributing to clarify and elucidate the interference of autonomic nervous system in the genesis and maintenance of atrial fibrillation (PAF). Experimental tools have been used to demonstrate the effects of vagal stimulation on atrial electrical conduction and refractoriness properties and on the vulnerability for the initiation and maintenance of AF. In particular, the anesthetized rabbit model with preserved autonomic innervation has been useful to demonstrate the effects of vagal and sympathetic stimulation on atrial electrical conduction and refractoriness properties and on the vulnerability for the initiation and maintenance of AF and human studies on patients with PAF associated an increased dispersion of atrial refractoriness upon PAF induction. Genetic procedures applied to a rat model of tachyarrhythmia showed the importance of channel proteins remodelling in the modification of cells electrical activity

## Recent Most Relevant Publications

- Oliveira, M., Nogueira-da-Silva, M., Timoteo, A., Feliciano, J., Sousa, L., Santos, S., Silva-Carvalho, L. and Ferreira, R. (2009) Inducibility of atrial fibrillation during electrophysiologic evaluation is associated with increased dispersion of atrial refractoriness. *Int. J. Cardiol.* 136: 130-135 (Journal IF: 3.121)
- Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luis, J. and Silva-Carvalho, L. (2009) Intrinsic vasomotricity and adrenergic effects in a model of isolated rabbit eye. *Acta Ophthalmol. Scand.* 87: 443-449 (Journal IF: 2.138)
- Rocha, I., Gonçalves, V., Bettencourt, M.J. and Silva-Carvalho, L. (2008) Effect of stimulation of sublobule IX-b of the cerebellar vermis over cardiac function. *Physiol. Res.* 57: 701-707 (Journal IF: 1.653)
- Lima, P.A., Vicente, M.I., Alves, F.M., Dionísio, J.C. and Costa, P.F. (2008) Insulin increases excitability via a dose-dependent dual inhibition of voltage-activated K<sup>+</sup> currents in differentiated N1E-115 neuroblastoma cells. *Eur. J. Neurosci.* 27: 2019–2032 (Journal IF: 3.385)
- Ducla-Soares, J.L., Santos-Bento, M., Laranjo, S., Andrade, A., Ducla-Soares, E., Boto, J.P., Silva-Carvalho, L. and Rocha, I. (2007) Wavelet analysis of autonomic outflow of normal subjects on head-up tilt, cold pressure test, Valsalva manoeuvre and deep breathing tests. *Exp. Physiol.* 92: 677-686 (Journal IF: 2.910)



Ribeiro, M.A., Cabral, H.O. and Costa, P.F. (2007) Modulatory effect of NO on sodium currents in a neuroblastoma cell line: aspects of cell specificity. *Neurosci. Res.* 58: 361-370 (Journal IF: 2.473)

### Ongoing Research Projects

2008/2009 Unprotected Left Main Trunk Stenting-Safety and Efficacy, FMUL. Coordinator: I. Rocha (Ref 329.16/ EFFI/09)

2008/2009 Overlapping drug Eluting-Stents – Limus versus Paclitaxel, FMUL. Coordinator: I. Rocha (Ref 329.17/PACLI/09)

2008/2009 Efeitos da estimulação da insula e do hipotálamo posterior num modelo animal de acidente vascular cerebral, FMUL. Coordinator: I. Rocha (Ref 329.18/INSULA/09)

2008/2009 Alterações Autonómicas por défice de perfusão da artéria cerebral média, FMUL. Coordinator: I. Rocha (Ref 329.19/ARTE/09 )

2008/2009 Human autonomic evaluation after an ischemic insult at the level of the medial cerebral artery, FMUL. Coordinator: I. Rocha (Ref 329.38/HUMA/09)

2008/2010 Viral targeting of the paraventricular nucleus of the hypothalamus (PVN) – effect on normal blood pressure, I Rocha, MERCK FOUNDATION

2008/2012 The inner cellular and genetic mechanisms of paroxysmal atrial fibrillation, SORINCARDIO. Coordinator: I. Rocha

2009/2011 Autonomic nervous system influences on renal stone disease, FCT. Coordinator: I. Rocha (PIC/IC/83243/2007)

### Publications in Peer Review International Journals 2009

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho, L. (2009) L-Arginine and L-Nitroarginine Methyl ester effects on vasomotion in isolated rabbit eyes. *Ophthalmic Res.* 43: 113-121 (Journal IF: 1.317)

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho L. (2010) Amlodipine effects on vasomotion in rabbit external ophthalmic artery. *Graefes Arch. Clin. Exp. Ophthalmol.* 248: 213-221 (*Epub November 17, 2009*)(Journal IF: 1.770)

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho, L. (2009) Endothelin-1 effects on spontaneous oscillations in choroidal arterioles. *Acta Ophthalmol.* (*Epub August 6, 2009*)(Journal IF: 2.138)

Delgado, E., Marques-Neves, C., Rocha, I., Sales-Luís, J. and Silva-Carvalho, L. (2009) Intrinsic vasomotricity and adrenergic effects in a model of isolated rabbit eye. *Acta Ophthalmol.* 87: 443-449 (Journal IF: 2.138)

Galrinho, A., Branco, L., Oliveira, M.M., Silva, N., Abreu, J., Santos, S., Leal, A. and Ferreira, R. (2009) Cardiac resynchronization therapy- clinical and echocardiographic characteristics of responders and exceptional responders. *Rev. Port. Cardiol.* 28: 959-969

Galrinho, A., Branco, L.M., Oliveira, M.M., Da Silva, N., Abreu, J., Santos, S., Leal, A. and Ferreira, R.C. (2009) Cardiac resynchronization therapy--clinical and echocardiographic characteristics of responders and exceptional responders. *Rev. Port. Cardiol.* 28: 959-969 (Journal IF: NA)

Oliveira, M. (2009) Ablation of atrial fibrillation: what is the gold standard strategy? *Rev. Port. Cardiol.* 28 : 1043-1046 (Journal IF: NA)

Oliveira, M.M. (2009) Effect of dronedarone on cardiovascular events in atrial fibrillation. *Rev. Port. Cardiol.* 28: 345-347 (Journal IF: NA)

Oliveira, M.M., da Silva, N., Timóteo, A.T., Feliciano, J., Silva, S., Xavier, R., Rocha, I., Silva-Carvalho, L. and Ferreira, R. (2009) Alterations in autonomic response head-up tilt testing in paroxysmal atrial fibrillation patients: a wavelet analysis. *Rev. Port. Cardiol.* 28: 243-257

Oliveira, M.M., Silva, N., Timóteo, A., Feliciano, J., Silva, S., Xavier, R., Rocha, I., Silva-Carvalho, L. and Ferreira, R. (2009) Effects of stimulation and blockade of the autonomic nervous system on atrial refractoriness in patients with lone paroxysmal atrial fibrillation. *Rev. Port. Cardiol.* 28: 655-670 (Journal IF: NA)

Oliveira, M.M., Silva, N., Timóteo, A., Feliciano, J., Sousa, L., Santos, S., Silva-Carvalho, L. and Ferreira, R. (2009) Inducibility of atrial fibrillation during electrophysiological evaluation is associated with increase dispersion of atrial refractoriness. *Int. J. Cardiol.* 136: 130-135 (Journal IF: 3.121)

### **Communications in International Conferences**

Santos, N. et al. Brain natriuretic peptide profile in a population submitted to cardiac resynchronization therapy: can we use it as a marker of responders and non-responders? *Heart Failure 2009, Nice, France. May 2009* (Oral presentation)

Lousinha, A. et al. Is cardiac resynchronization therapy effective in patients with very dilated cardiomyopathy? *Heart Failure 2009, Nice, France. May 2009* (Poster presentation)

Hamad, H. et al. Late reverse remodeling evidence in congestive heart failure patients submitted to cardiac resynchronization therapy. *Heart Failure 2009, Nice, France. May 2009* (Poster presentation)

Hamad, H. et al. Reverse remodelling post CRT: 15% reduction of left ventricular end-systolic volume vs 15% reduction of left ventricular end-diastolic volume. *Heart Failure 2009, Nice, France. May 2009* (Poster presentation)

Ruben, R. et al. Cardiac resynchronization therapy: antiarrhythmic and mortality effect of reverse ventricular remodeling in hyperresponders. *Congresso Europeu de Cardiologia, Barcelona, Spain. September 2009* (Oral presentation)

Santos, N. et al. Prognostic value of microvolt T-wave alternans testing in patients with coronary artery disease submitted to cardiac rehabilitation program. *8<sup>th</sup> International Congress on Coronary Artery Disease, Prague, Czech Republic. October 2009* (Oral presentation)

Neves, C. et al. Sympathetic influence on intraocular pressure. *EVER, Slovenia. October 2009* (Poster presentation)

Neves, C. et al. Sympathetic variability of intraocular pressure of glaucoma patients evaluated by wavelets. *EVER, Slovenia. October 2009* (Poster presentation)

### **Communications in National Conferences**

Cunha, P. et al. Abordagem transeptal em eletrofisiologia de intervenção: experiência e complicações com técnica simplificada. *XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009* (Oral presentation)

Cunha, P. et al. Eficácia da monitorização à distância no seguimento de portadores de cardioversor-desfibrilhador. *XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009* (Poster presentation)

Leal, E. et al. Qualidade de vida em doentes com insuficiência cardíaca refractária submetidos a ressincronização cardíaca: persistência dos benefícios a médio prazo. *XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009* (Poster presentation)

Lima, P.A. et al. Into the role of Insulin in neuronal activity. *Sociedade Portuguesa de Neurociências, Braga. June 2009.*

Oliveira, M. et al. Condução eléctrica auricular em doentes com fibrilhação auricular paroxística: influencia da modulação aguda da actividade autonómica. XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009 (Oral presentation)

Oliveira, M. et al. Efeitos electrofisiológicos auriculares da estimulação vagal em coelhos anestesiados: impacto na inducibilidade e manutenção de fibrilhação auricular. XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009 (Poster presentation)

Santos, S. et al. Benefícios a longo prazo dum programa de treino de ortostatismo (Tilt training) no tratamento da síncope neurocardiogenica recorrente. XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009 (Poster presentation)

Timoteo, A. et al. Incidência de taquidismias ventriculares em doentes com disfunção sistólica grave: haverá benefício com ressincronização cardíaca? XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009 (Poster presentation)

Timoteo, A. et al. Preditores ecocardiográficos de terapêutica apropriada por desfibrilhador em doentes submetidos a ressincronização cardíaca combinada XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009 (Poster presentation)

### Invited Lectures and Seminars

Neves, C. Pressure and blood flow in glaucoma. EVER, Slovenia. October 2009.

Neves, C. Semiologia córnea – qualidade de visão, Curso EUPO – European University Professors of Ophthalmology- Colóquios de oftalmologia. February 2009.

Neves, C. Semiologia córnea – aberrometria, Curso EUPO – European University Professors of Ophthalmology- Colóquios de oftalmologia. February 2009.

Oliveira, M. A Morte Súbita ou Morrer de Repente – quando a causa é cardíaca, Vila Real. July 2009

Oliveira, M. Efeito da dronedarone no AVC em doentes com fibrilhação auricular resultados do estudo atheana: nova opção farmacológica. X Simpósio do Núcleo de Estudos de Doenças Cerebrovasculares da Sociedade Portuguesa de Medicina Interna, Porto. November 2009.

Oliveira, M. Effects of ANS electrical and molecular remodelling of the atria; experimental evidence for a pathophysiological role in atrial fibrillation. X Simpósio do Núcleo de Estudos de Doenças Cerebrovasculares da Sociedade Portuguesa de Medicina Interna, Porto. November 2009.

Oliveira, M. Fibrilhação auricular dos mecanismos à decisão clínica: mecanismos e substratos electrofisiológicos. XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009.

Oliveira, M. Síncope neurocardiogenica: abordagem diagnóstica e terapêutica, Simpósio Luso-Brasileiro, Lisbon Arrhythmia Meeting, Portugal. February 2009

Oliveira, M. Terapêutica da Fibrilhação auricular: novos antiarrítmicos na fibrilhação auricular na manutenção do ritmo sinusal: para quando? XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009.

Oliveira, M. Unusal procedures for LV lead replacement(case report). New Horizons in Heart Failure, Madrid, Spain. March 2009.

Rocha, I. Human autonomic evaluation – basic standard tests. NEUROCARD 2009: International Symposium on Neurocardiology, Belgrade, Serbia. October 2009.

Rocha, I. Novel approaches to analysis of human autonomic function. ISAN2009, Sydney, Australia. August 2009.

### Organization of National and International Conferences

Neves, C. and Rocha, I. Causa / Efeito das teorias vasculares no desenvolvimento fisiopatológico

do glaucoma, Workshop Glaucomas, Centro Cultural de Belém, Lisbon, Portugal. April 2009.

Neves, C. Curso de Glaucoma, Reunião Glaucoma Hospital da Feira, Feira, Portugal. May 2009.

Rocha, I. 3<sup>rd</sup> International Undergraduate Advanced Course on Autonomic Nervous System, Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal. July 23 – 30, 2009.

Oliveira, M. Management of cardiac resynchronization therapy, Hospital de Santa Marta, Lisbon. 2009

Rocha, I. Clinical evaluation of autonomic function: hands-on session, Workshop, ISAN 2009, Sydney, Australia. August 2009

Rocha, I. Avaliação da função muscular: sessão prática, Ciclo de Estudos Especiais em Neurofisiologia Clínica, Hosp Santa Maria, Lisbon, Portugal. November 2009.

Neves, C. Jornadas de Investigação da Sociedade Portuguesa de Oftalmologia. December 2009.

### **Advanced Teaching**

Oliveira, M. “Atrial fibrillation: Pathophysiology Insights and Role of Autonomic Nervous System” Master course in Neurosciences, Faculdade de Medicina da Universidade de Lisboa. February 2009.

Rocha, I. “Autonomic nervous system: anatomo-functional organization and central control” Master course in Neurosciences, Faculdade de Medicina da Universidade de Lisboa. February 2009.

Rocha, I. “Autonomic function: clinical evaluation and signal processing methodologies” Master course in Neurosciences, Faculdade de Medicina da Universidade de Lisboa. February 2009.

Rocha, I. “Autonomic nervous system: anatomo-functional organization and regulation of visceral activity” Master course in Cognitive Sciences, Faculdade de Medicina da Universidade de Lisboa. January 2009.

Rocha, I. “Autonomic function: clinical evaluation and signal processing methodologies” Master course in Cognitive Sciences, Faculdade de Medicina da Universidade de Lisboa. January 2009.

Santos, M. “Autonomic function and its clinical evaluation: hands on session” Ciclo de Estudos Especiais em Neurofisiologia Clínica, Hospital de Santa Maria. November 2009.

### **Public Perception of Science**

Oliveira, M. (2009) Acidente Vascular Cerebral, Bom Dia Portugal, Lisboa, Abril

Oliveira, M. (2009) Factores de Risco e Prevenção das Doenças Cardiovasculares, “Saúde +”, Cine-Teatro de Benavente, Maio

Oliveira, M. (2009) Campanha “Bate-Bate Coração” no âmbito da divulgação internacional da prevenção cardiovascular e risco de arritmias, Maio

### **Thesis Completed**

#### **Master Thesis**

Raluca Ivanov (2009) “Avaliação autonómica de uma população com alterações do metabolismo glucídico” Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa. Supervisor: I. Rocha

Andreia Luís (2009) “Prova de água. Efeitos oculares e cardiovasculares-análise integrada das respostas” Mestrado integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisor: C. Neves

Cláudia Gonçalves (2009) “Avaliação autonómica em doentes com glaucoma de baixa pressão”  
Mestrado em Neuroftalmologia, Faculdade de Medicina da Universidade de Lisboa. Supervisor:  
C. Neves

Diogo Gonçalves (2009) “Actividade física durante o crescimento e saúde do osso”, Mestrado  
integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisors: F.  
Sampaio and I. Rocha

## Neurological Clinical Research Unit

### Description and Objectives

The general aim of this Unit is to study the clinical epidemiology of diseases involving the nervous system. The work of the NCRU research will focus on large samples and on prevalent, disabling, chronic CNS conditions such as dementia, stroke and Parkinson's disease. Our aim is to identify risk factors, including ecological and genetic ones, and prognostic factors leading from an independent life to dependency or death, and on the development and assessment of therapeutical interventions that could be able to delay or prevent transition from a healthy, independent state to disability and death. Measures of disability will include neurophysiological and quality of life dimensions. The large samples of patients afflicted with such chronic conditions will offer a unique opportunity for detailed neuropsychological and neuroimaging studies and the development and testing of cognitive neuropsychological models, such range samples are instrumental for searching genetic determinants of these complex diseases.

NCRU has an additional aim of training young medical and non-medical clinical researchers and to disseminate an evidence-based and patient-oriented view of the research and practice in Clinical Neurosciences.

### Head of Unit

José M. Ferro, MD, PhD - Full Professor, FML/HSM

### Principal Investigators

Cristina Sampaio MD, PhD - Professor of Clinical Pharmacology and Therapeutics, FMUL

Alexandre Castro Caldas, MD, PhD - Full Professor of Portuguese Catholic University

Alexandre de Mendonça, MD, PhD - Investigator Principal, FMUL/IMM

Sofia A. Oliveira, PhD – Invited Auxiliary Professor, FMUL

Teresa Paiva, MD, PhD – FMUL and IST

Isabel Pavão Martins, MD, PhD - Auxiliary Professor, FMUL

José Pimentel, MD, PhD – Associate Professor with *Agregação*, FMUL

### STROKE GROUP

**PI:** José M. Ferro, MD, PhD – Associate Professor, FMUL/HSM

### Description and Objectives

This research group is dedicated to the clinical investigation of risk factors, prognosis, intervention and management of cerebrovascular disease and its consequences.

### Research Team

Vítor Oliveira, MD, PhD	- Auxiliary Professor, FMUL, HSM
Teresa Pinho e Melo, MD	- Assistant, FMUL, HSM
Patrícia Canhão, MD, PhD	- Assistant, FMUL, HSM
Filipa Falcão, MD	- HSM
Ana Verdelho, MD	- PhD Student, HSM
Sofia Madureira, PsyD	- FMUL
Lara Caeiro, PsyD	- PhD fellowship, FCT

Catarina Santos, PsyD	- PhD fellowship, FCT
Liliana Gouveia, MD	- MD, HSM
Ana Catarina Fonseca	- MD, HSM
Paulo Batista	- Technician, HSM
Fátima Soares	- Technician, HSM

### Research areas

Stroke

Vascular dementia / Vascular cognitive impairment

Neuropsychiatry, Neuropsychology of stroke

Cerebral venous thrombosis

Cardioembolic stroke

Transient neurological attack and transient ischemic attacks

### Recent Most Relevant Publications

Inzitari, D., Simoni, M., Pracucci, G., Poggesi, A., Basile, A.M., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J.M., Hennerici, M., Langhorne, P., O'Brien, J., Barkhof, F., Visser, M.C., Wahlund, L.O., Waldemar, G., Wallin, A., Pantoni, L.; LADIS Study Group (2007) Risk of rapid global functional decline in elderly patients with severe cerebral age-related white matter changes: the LADIS study. *Arch. Intern. Med.* 167: 81-88 (Times cited: 26) (Journal IF:9.110)

Bousser, M.G. and Ferro, J.M. (2007) Cerebral venous thrombosis: an update. *Lancet Neurol.* 6: 162-170 (Times cited: 51)(Journal IF: 14.270)

Einhäupl, K., Bousser, M.G., de Bruijn, S.F., Ferro, J.M., Martinelli, I., Masuhr, F. and Stam, J. (2006) EFNS guideline on the treatment of cerebral venous and sinus thrombosis. *Eur. J. Neurol.* 13: 553-559 (Times cited: 48)(Journal IF: 2.732)

Canhão, P., Ferro, J.M., Lindgren, A.G., Bousser, M.G., Stam, J., Barinagarrementeria, F.; ISCVT Investigators (2005) Causes and predictors of death in cerebral venous thrombosis. *Stroke* 36: 1720-1725 (Times cited: 36)(Journal IF:6.499)

Pantoni, L., Basile, A.M., Pracucci, G., Asplund, K., Bogousslavsky, J., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J.M., Hennerici, M., O'Brien, J., Scheltens, P., Visser, M.C., Wahlund, L.O., Waldemar, G., Wallin, A. and Inzitari, D. (2005) Impact of age-related cerebral white matter changes on the transition to disability -- the LADIS study: rationale, design and methodology. *Neuroepidemiology* 24: 51-62 (Times cited: 64)(Journal IF:1.737)

### Ongoing Research Projects

1998/2009 Impact of Age-related brain white matter changes on transition to disability in the elderly. **Leukoaraiosis And DISability**, European Commission. Co-Coordinator: J.M. Ferro (FP5-QLK6-2000-00446)

1998/2008 Tromboses venosas cerebrais - Estudo prospectivo multinacional, FCT. Coordinator: J.M. Ferro

2004/2010 Prospective registry of patients with transient neurological attacks (TNA). Classification of (TNA) in a consecutive cohort of patients referred to a TIA Clinic. Prognosis of TIA and non-ischemic TNA patients. Coordinator: P. Canhão

2009/2015 An international, Prospective, TIA-clinic based, Web-based registry with long-term follow-up. Coordinator: P. Canhão; International Coordinator: P. Amarengo

### CLINICAL NEUROPHARMACOLOGY GROUP

**PI:** Cristina Sampaio, MD, PhD, Professor of Clinical Pharmacology and Therapeutics,

FMUL

### Description and Objectives

The Clinical Pharmacology Group aims to contribute to the development of effective and safe therapeutic interventions for neurodegenerative diseases through the establishment of optimised methodologies and infrastructures for the assessment of the clinical impact of those interventions.

### Research Team

Joaquim Ferreira, MD, PhD	- Neurologist and Lecturer, HSM/FMUL
Mário Miguel Rosa, MD	- Neurologist and Lecturer, HSM/FMUL
Miguel Coelho, MD	- Neurologist and Researcher, HSM
João Costa, MD, PhD	- Neurologist and Lecturer, HSM/ FMUL
Ana Marta Teixeira Anes, MD- Pharmacist	
Leonor Correia Guedes, MD	- Resident and Researcher, HSM
Tiago Mestre, MD,	- Resident and Researcher, HSM
Ema Roque, Management Degree	- Technician
Ana Noronha, BSc	- Technician: 100%
Anabela Valadas, MD	- Resident and Researcher, HSM
Andreza Viegas, BSc	- Technician, 100%

### Research areas

Parkinson disease  
Movement disorders  
Clinical trials  
Systematic reviews  
Cochrane collaboration  
Pharmacoepidemiology

### Recent most Relevant Publications

Silveira-Moriyama, L., Guedes, L.C., Kingsbury, A., Ayling, H., Shaw, K., Barbosa, E.R., Bonifati, V., Quinn, N.P., Abou-Sleiman, P., Wood, N.W., Petrie, A., Sampaio, C., Ferreira, J.J., Holton, J., Revesz, T. and Lees, A.J. (2008) Hyposmia in G2019S LRRK2-related parkinsonism: clinical and pathologic data. *Neurology* 71: 1021-1026 (Times cited: 3)(Journal IF: 7.043)

Vellas, B., Andrieu, S., Sampaio, C., Wilcock, G; European Task Force group (2007) Disease-modifying trials in Alzheimer's disease: a European task force consensus. *Lancet Neurol.* 6: 56-62 (Times cited: 30)(Journal IF: 14.270)

Schrag, A., Sampaio, C., Counsell, N. and Poewe, W. (2006) Minimal clinically important change on the unified Parkinson's disease rating scale. *Mov. Disord.* 21: 1200-1207 (Times cited: 22)(Journal IF: 3.898)

Costa, J., Borges, M., David, C. and Vaz-Carneiro, A. (2006) Efficacy of lipid-lowering drug therapy for diabetic and non-Diabetic patients: a meta-analysis of randomized, controlled trials. *Brit. Med. J.* 332: 1115-1124 (Times cited: 85)(Journal IF: 12.827)

Emre, M., Aarsland, D., Alberto, A., Byrne, E.J., Deuschl, G., De Deyn, P., Durif, F., Kulisevsky, J., van Laar, T., Lees, A., Poewe, W., Robillard, A., Rosa, M.M., Wolters, E., Quarg, P., Tekin, S. and Lane, R. (2004) Rivastigmine for dementia associated with Parkinson's disease. *N. Engl. J. Med.* 351: 2509-2518 (Times cited: 266)(Journal IF: 50.017)



### Ongoing Research Projects

1996 Editorial Base of the Cochrane Movement Disorders Group: several financing sources negotiable annually, Fundação MDS, Fundação Grunenthal.

2003 Development of measurement instruments usable in clinical trials focused on Movement Disorders (part of a task force of the International Society for Movement Disorders).

2000 European Multiple System Atrophy Study Group (until 2005 funded by European Commission QLRT – 2000 - -00661)

1986/-- Movement Disorders Clinical Trials, funded by clinical trial contracts.

2002/-- Characterization of the incidence, typology and determining factors of adverse drug reactions in hospitals, funded by Pharmacovigilance Unit.

2003/-- Development of measurement instruments usable in clinical trials focused on Movement Disorders, task force of the International Society for Movement Disorders.

2003/-- Advanced stage Parkinson's Disease: transversal study on a hospital-based population of patients with Parkinson's disease in an advanced stage. Collaborative project with the Movement Disorders Unit of Hospital Clinic Barcelona, Spain.

2005/-- Avaliação do impacto na saúde das doenças neurológicas crónicas. Uma contribuição para a definição de resultados relevantes na perspectiva do doente e para a metodologia de avaliação dos serviços de saúde, FEDER and FCT.

#### Participation in collaborative projects and consortium

EURO\_HD, European. Huntington Consortium.

SLEEPMSA: Multicenter European study of the EMSA-SG (European MSA Study Group).

Vascular contacts in idiopathic hemifacial spasm, collaboration with the Department of Neuroimaging of Hospital Santa Maria.

Motivations and concerns of Parkinson's disease patients entering clinical trials.

### COGNITIVE NEUROSCIENCE GROUP

**PI:** Alexandre Castro Caldas, MD, PhD, Full Professor of the Catholic University of Portugal

#### Description and Objectives

The main interest of the group concerns language and the brain. Projects concerning aging, illiteracy, sign language are in progress.

#### Research team

Ana Mineiro, PhD	- Auxiliary Professor, ICS-UCP
Maria Vânia Nunes, PhD	- Auxiliary Professor, ICS-UCP
Ana Maria Abreu, PhD	- Pos-Doc fellowship, FCT
Sara Fernandes	- PhD fellowship, FCT

#### Research Areas

Illiteracy  
Cognitive Neuroscience  
Portuguese Sign Language

#### Recent Most Relevant Publication

Castro-Caldas, A., Nunes, M.V., Maestú, F., Ortiz, T., Simões, R., Fernandes, R., La Guia, E.,

Garcia, E. and Gonçalves, M. (2009) Learning orthography in adulthood: a magnetoencephalographic study. *J. Neuropsychol.* 3:17-30

Silva Nunes, M.V., Castro-Caldas, A., Del Rio, D., Maestú, F. and Ortiz, T. (2009) The ex-illiterate brain: the critical period, the cognitive reserve and the HAROLD model. *Dementia Neuropsychol.*, 222-227.

Rascol, O., Dubois, B., Castro-Caldas, A., Senn, S., Del Signore, S. and Lees, A. (2006) Parkinson REGAIN Study Group Early piribedil monotherapy of Parkinson's disease: A planned seven-month report of the REGAIN study. *Mov. Disorders.* 21: 2110-2115 (Times cited: 8)(Journal IF: 3.893)

DeRouen, T., Martin, M., Leroux, B., Townes, B., Leitão, J., Woods, Castro-Caldas, A., Luís, H., Bernardo, M., Rosenbaum, G. and Martins, I. (2006) Neurobehavioral Effects of Dental Amalgam in Children: A Randomized Clinical Trial. *JAMA* 295: 15 (Journal IF: 31.718)

Martins, I.P., Castro-Caldas, A., Townes, B.D., Ferreira, G., Rodrigues, P., Marques, S., Rosebaum, G., Benton, T.S., Leitao, J. and Derouen, T. (2005) Age and sex differences in neurobehavioral performance: a study of portuguese elementary school children. *Int. J. Neurosci.* 115: 1687-1709 (Journal IF:0.884)

### Ongoing Research Projects

2004 Caracterização de Perfis de Envelhecimento na População Portuguesa Normal, Fundação Calouste Gulbenkian.

2008/2010 Turma Piloto em Língua Gestual Portuguesa, Fundação Calouste Gulbenkian.

### DEMENTIA GROUP

**PI:** Alexandre de Mendonça, MD, PhD, Neurologist, Principal Investigator, FMUL and IMM

#### Description and Objectives

The Dementia Group of the IMM is member of the European Alzheimer Disease Consortium (EADC), a network of about 50 European centres of excellence working in the field of Alzheimer's disease, with the aim to increase the basic scientific understanding and to develop ways to prevent, slow, or ameliorate the primary and secondary symptoms of Alzheimer's disease, by facilitating large Europe wide research studies.

### Research team

Manuela Guerreiro, PhD	- Neuropsychologist, FMUL and IMM
Antonina Pereira, PhD	- Psychologist, Post-Doc IMM
Graça Melo	- Nurse, PhD student
Frederico Simões do Couto	- Psychiatrist, HSM, PhD student
Ana Verdelho,	- Neurologist, HSM, PhD student
Dina Silva	- Psychologist, HSM, PhD student
Manuela Lopes	- PhD student
Tiago Mendes, MSc	- Psychologist, HSM
Sandra Ginó, MSc	- Psychologist, Volunteer
Helena Pedrosa, MSc	- Psychologist, Volunteer
Carolina Maruta, MSc	- Psychologist, Volunteer
Erica Marcelino	- Psychologist, Master Degree student
Catarina Chester	- Psychologist, Master Degree student
Vanda Freitas	- Psychologist, Master Degree student

### Ongoing Research Projects

2009 Pesquisa de Novas Mutações em Genes Responsáveis por Demência Familiar, Grunenthal. Coordinator A. de Mendonça; Co-Coordinator: G. Miltenberger, GenoMed.

2005/2008 (extended to 2010) Cognitive Impairment in the Aged - A prospective clinical, neuropsychological, NMR and biochemical study, Fundação Calouste Gulbenkian. Coordinator A. de Mendonça. *Collaboration with Faculdade de Ciências e Tecnologia of Lisbon and Departamento de Biologia da Universidade de Aveiro.*

2007/2009 Biomarkers in Alzheimer's Disease: the Lipid Homeostasis / Oxidative Stress Connection Astra-Zeneca Coordinator: M. Martins; Co-Coordinator: A. de Mendonça. *Collaboration with Instituto Nacional de Saúde Ricardo Jorge and Instituto Gulbenkian de Ciência.*

2009-2011 Neuropsychological predictors of the outcome in non-demented subjects with cognitive complaints, FCT. Coordinator: A. de Mendonça

### GENOMICS OF COMPLEX DISEASES GROUP

**PI:** Sofia A. Oliveira, PhD – Invited Auxiliary Professor, FMUL

#### Description and Objectives

Our research focuses on understanding the genetic architecture of complex diseases such as Stroke, Parkinson's Disease (PD), Intracranial aneurysms, Behçet's Disease (BD) and Primary Spontaneous Pneumothorax (PSP). Common diseases result from the interaction of environmental and genetic factors, and an in-depth evaluation of their genetic underpinnings will not only unravel complex inheritance patterns but will also enable a better understanding of the environmental risks. We use both traditional (e.g. linkage and association) as well as new (e.g. genome-wide association studies, exome sequencing, microRNA expression profiling) approaches to identify novel susceptibility genes. We believe that studies with a multifaceted and multidisciplinary framework will have the greatest success in dissecting the complex etiology of common disorders and that this research will ultimately lead to the development of novel prevention strategies and targeted therapies.

#### Research Team

Madalena Martins, PhD	- Post-Doc fellowship, FCT
Patrícia Abrantes, PhD	- Post-Doc fellowship, FCT
Joana Xavier, MSc	- PhD fellowship, FCT
Tiago Krug	- PhD fellowship, FCT
Liliana Gouveia, MD	- Master student, HSM
João Sobral	- Research Technician, FCT grant (since November)

#### Research Areas

Genetics  
Genomics  
Complex diseases

#### Recent Most Relevant Publications

Li, Y.J., Scott, W.K., Zhang, L., Lin, P.I., Oliveira, S.A., Skelly, T., Doraiswamy, M.P., Welsh-Bohmer, K.A., Martin, E.R., Haines, J.L., Pericak-Vance, M.A. and Vance, J.M. (2006) Revealing the role of glutathione S-transferase omega in age-at-onset of Alzheimer and Parkinson diseases. *Neurobiol. Aging* 27: 1087-1093 (Journal IF: 5.96)

Oliveira, S.A., Li, Y.J., Noureddine, M.A., Züchner, S., Qin, X.J., Pericak-Vance, M.A. and

Vance, J.M. (2005) Identification of risk and age-at-onset genes on chromosome 1p in Parkinson disease. *Am. J. Hum. Genet.* 77: 252-264 (Times cited: 16)(Journal IF: 10.15)

Oliveira, S.A., Scott, W.K., Zhang, F.Y., Stajich, J.M., Fujiwara, K., Hauser, M., Scott, B.L., Pericak-Vance, M.A., Vance, J.M. and Martin, E.R. (2004) Linkage disequilibrium and haplotype tagging polymorphisms in the Tau H1 haplotype. *Neurogenetics* 5: 147-155 (Times cited: 19)(Journal IF: 3)

Li, Y-J., Oliveira, S.A., Xu, P., Martin, E.R., Stenger, J.E., Scherzer, C.R., Hauser, M.A., Scott, W.K., Small, G.W., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Pahwa, R., Stern, M.B., Hiner, B.C., Jankovic, J., Goetz, C.G., Mastaglia, F., Middleton, L.T., Roses, A.D., Saunders, A.M., Schmechel, D.E., Gullans, S.R., Haines, J.L., Gilbert, J.R., Vance, J.M., Pericak-Vance, M.A., Hulette, C., Welsh-Bohmer, K.A. et al. (2003) Glutathione S-Transferase omega 1 modifies age-at-onset of Alzheimer disease and Parkinson disease. *Hum. Mol. Genet.* 12: 3259-3267 (Times cited: 83)(Journal IF: 7.25)

Oliveira, S.A., Scott, W.K., Martin, E.R., Nance, M.A., Watts, R.L., Hubble, J.P., Koller, W.C., Pahwa, R., Stern, M.B., Hiner, B.C., Ondo, W.G., Allen, F.H. Jr, Scott, B.L., Goetz, C.G., Small, G.W., Mastaglia, F., Stajich, J.M., Zhang, F., Booze, M.W., Winn, M.P., Middleton, L.T., Haines, J.L., Pericak-Vance, M.A. and Vance, J.M. (2003) Parkin mutations and susceptibility alleles in late-onset Parkinson disease. *Ann. Neurol.* 53: 624-629 (Times cited: 77)(Journal IF: 9.94)

### Ongoing Research Projects

2007/2010 Genetic epidemiology of stroke in the post-genomic era, FCT. Coordinator: S.A. Oliveira (PTDC/SAU-GMG/64426/2006)

2007/2010 microRnomics and proteomics of Parkinson's disease, FCT. Coordinator: S.A. Oliveira (PTDC/SAU-GMG/64428/2006)

2009/2010 Genetics of Primary Spontaneous Pneumothorax (PSP), SPP/UCB Pharma. Coordinator: Feijó, Co-Coordinator: S.A. Oliveira

### SLEEP-EEG GROUP

**PI:** Teresa Paiva, MD, PhD - HSM, FMUL

#### Description and Objectives

The Sleep-EEG group is a multidisciplinary team with an important involvement and responsibility in health care and in pre and post graduate teaching. Pre graduate teaching includes the field of Neurology (mainly the areas of epilepsy and sleep) and Neurophysiology, while post graduate is centred in the Master Degree of Sleep Medicine, in the collaboration with the Master Degree in Neurosciences, and also in the high level Neurophysiology post graduate training. The main areas of research are epilepsy, sleep and dreams. Specific fields of epilepsy and sleep neurophysiology and in the area of dreams and their psycho and neurophysiological correlates are included. With these actions the group aims high level academic work, applied medical research and scientific dissemination. The ultimate objective is the formation of a critical mass of young scientists and healthcare professionals, able to invest in a multidisciplinary work in the target areas.

### Research Team

Carla Bentes, MD	- Assistant, FMUL, HSM
Ana Rita Peralta, MD, MSc	- HSM
Rosa Santos	- EEG Technologist, HSM
Joana Pires	- EEG Technologist, HSM
Lígia Ferreira	- EEG Technologist, HSM
Isabel Henriques	- Secretary, FMUL

## Research Areas

Neurophysiology  
Sleep  
Epilepsy.

## Ongoing Research Projects

2009/2011 Contribuição da resposta imunitária para a associação de doenças do sono (hipersonolência) e cardiovasculares, FCT. Coordinator: L. Moita. Co-Coordinator: T. Paiva, A. Bugalho de Almeida, R. Staats, S. Moreira, J. Valença, A. Rodrigues, C. Bentes, R. Gerales (PIC/IC/82991/2007)

## BEHAVIOURAL NEUROLOGY GROUP

**PI:** Isabel Pavão Martins, MD, PhD - Associated Professor of Neurology, FMUL

### Description and Objectives

The main objective of this Unit is to investigate the biological basis of human cognition and behaviour in particular Language and Memory and its changes with normal development and ageing. This is accomplished by the neuropsychological study of healthy subjects, patients with brain lesions and the rehabilitation and follow-up of patients with aphasia and dementia.

An additional line of clinical research includes the study of craniofacial pain and is undertaken in the Headache Outpatient Clinic of Hospital de Sta. Maria, coordinated by the PI. The main interest is the clinical characterization of the trigemino vascular and trigemino-autonomic cephalalgias.

## Research Team

Manuela Guerreiro, PhD	- Associated Professor, FMUL
Martin Lauterbach, MD, PhD	- Post-doc fellowship, FCT
Luísa Albuquerque, MD,	- Neurologist, HSM
Raquel Gil-Gouveia	- Neurologist, HSM
Clara Loureiro, MSc	- Psychologist, FMUL
Gabriela Leal	- Speech Therapist, HSM
Luisa Farrajota	- Speech Therapist, HSM
José Fonseca	- Speech Therapist, FMUL
Luz Rocha	- Speech Therapist, HSM
Catarina Chester	- Psychologist, Master student
Vanda Freitas	- Psychologist, Master student
Carolina Maruta	- Student Psychologist
Maurício Martins	- MD student
Joana Morgado, MD	- Master student

## Research Areas

Aphasia (in adults and childhood)  
Memory and Dementia  
Cognitive aspects of DBS surgery  
Migraine

## Ongoing Research Projects

2007/2010 Brain plasticity for language: evidence from aphasia in childhood, FCT. Coordinator: I.P. Martins (PTDC/PSI/74294/2006)

2005/2009 Cognition and ageing. Normative data on a sample of the Portuguese elderly population, Fundação Calouste Gulbenkian. Coordinator I.P. Martins (Projecto 0488)

2004/2012 Intensive speech therapy for patients with aphasia after stroke: a single-masked, parallel randomised controlled trial in aphasic stroke patients, Collaborative project with Cerebro-vascular disease Group and Centro de Medicina de Reabilitação de Alcoitão. Coordinator: G. Leal, L. Farrajota, J Fonseca, M Lauterbach, I.P Martins.

2007/2009 Writing Acquisition in a Bilingual Setting: German/Portuguese Collaborative project with the German School in Lisbon. Coordinator: M. Lauterbach, C. Loureiro, B. Dias, E. Menke.

2010/2012 Virtual Therapist for Aphasia treatment, FCT. Coordinator: A. Gareta, A. Serralheiro, I. Trancoso, J. Fonseca, G. Leal (RIPD/ADA/109646/2009)

2009/2011 Bateria Montreal de Avaliação da Comunicação – MAC. Coordinator: J Fonseca.

2009/2011 Lisling em imagens 3D. Coordinator: J Fonseca, Y Ryherczyk.

## **PATHOLOGY OF NERVOUS SYSTEM GROUP**

**PI:** José Pimentel, MD, PhD - Associate Professor, FMUL/HSM

### **Description and Objectives**

The Unit is positioned at the Laboratory of Neuropathology (LNP) of the Department of Neurology (Centro Hospitalar Lisboa Norte, Hospital de Santa Maria), and the staff is composed of three neuropathologists, three technicians and one secretary.

The LNP is particularly devoted to the hospital assistance concerning the diagnosis of nervous system tumours, the post-mortem diagnosis of nervous system diseases, the diagnosis of lesions resected from epilepsy surgeries and, finally, the diagnosis of neuro-muscular diseases.

Other activities are the academic and research ones, and, in what this last one is concerned, we are interested in: a) morphological, immunohistochemical and genetics characterization of novel glioneuronal tumour entities; b) oligodendrogliomas immunohistochemical characterization by using novel antibodies in order to establish correlations with morphological, anaplasia and genetics profiles, and to be contribute for the differential diagnosis with other gliomas; c) diagnosis of lesions resected from surgeries of epileptic patients; d) morbid obesity and muscle morphology changes; e) skin biopsies in small fibers neuropathies.

The time allocated for research activities is about 25% of the total time spent at the laboratory

### **Research Team**

Teresinha Evangelista, MD	- Assistant, FMUL / HSM
Cândida Barroso, MD	- Neurologist, HSM
Carla Firmo, MSc	-Technician, HSM
Pedro Pereira, Msc	-Technician, HSM
Ana Rita Silvestre, MSc	-Technician, HSM

### **Research Areas**

Neuropathology of epilepsy surgery  
Gliomas and glioneuronal tumours  
Human Prion Diseases  
Skin biopsies and polineuropathies  
Obesity and muscle morphology

**Recent Most Relevant Publications**

Viana-Pereira, M., Almeida, I., Sousa, S., Mahler-Araújo, B., Seruca, R., Pimentel, J. and Reis, R.M. (2009) Analysis of microsatellite instability in medulloblastoma. *Neuro. Oncol.* 11: 458-467

Zerr, I., Kallenberg, K., Summers, D.M., Romero, C., Taratuto, A., Heinemann, U., Breithaupt, M., Varges, D., Meissner, B., Ladogana, A., Schuur, M., Haik, S., Collins, S.J., Jansen, G.H., Stokin, G.B., Pimentel, J., Hwer, E., Collie, D., Smith, P., Roberts, H., Brandel, J.P., van Duijn, C., Pocchiari, M., Begue, C., Cras, P., Will, R.G. and Sanchez-Juan, P. (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt Jakob disease. *Brain* 132: 2659-2668 (Journal IF: 9.603)

**Ongoing Research Projects**

2008/2009 Gliomas malignos com longa sobrevida – caracterização genética e imunohistoquímica, Fundação AstraZeneca / FMUL. Coordinator: J. Pimentel, L. Roque

2008/2009 Caracterização quantitativa do núcleo basolateral da amígdala na esclerose mesial, GAPIC. Coordinator: J. Pimentel

2008/2009 Caracterização quantitativa do Gyrus Dentatus na esclerose mesial, GAPIC. Coordinator: J. Pimentel

**Publications in Peer Review International Journals 2009**

Abellan van Kan, G., André, E., Bischoff Ferrari, H.A., Boirie, Y., Onder, G., Pahor, M., Ritz, P., Rolland, Y., Sampaio, C., Studenski, S., Visser, M., Vellas, B. (2009) Carla Task Force on Sarcopenia: propositions for clinical trials. *J. Nutr. Health Aging* 13: 700-707 (Journal IF: 2.321)

Andrieu, S., Coley, N., Aisen, P., Carrillo, M.C., Dekosky, S., Durga, J., Fillit, H., Frisoni, G.B., Froelich, L., Gauthier, S., Jones, R., Jönsson, L., Khachaturian, Z., Morris, J.C., Orgogozo, J.M., Ousset, P.J., Robert, P., Salmon, E., Sampaio, C., Verhey, F., Wilcock, G. and Vellas, B. (2009) Methodological issues in primary prevention trials for neurodegenerative dementia. *J. Alzheimers Dis.* 16: 235-270 (Journal IF: 5.01)

Aziz, N.A., Jurgens, C.K., Landwehrmeyer, G.B., van Roon-Mom, W.M., van Ommen, G.J., Stijnen, T., Roos, R.A., Orth, M., Handley, O.J., Schwenke, C., Ho, A., Wild, E.J., Tabrizi, S.J. and Landwehrmeyer, G.B. (2009) EHDN Registry Study Group. Normal and mutant HTT interact to affect clinical severity and progression in Huntington disease. *Neurology* 73:1280-1285 (Journal IF: 7.043)

Benisty, S., Gouw, A.A., Porcher, R., Madureira, S., Hernandez, K., Poggesi, A., van der Flier, W.M., Van Straaten, E.C., Verdelho, A., Ferro, J., Pantoni, L., Inzitari, D., Barkhof, F., Fazekas, F. and Chabriat, H.; LADIS Study group (2009) Location of lacunar infarcts correlates with cognition in a sample of non-disabled subjects with age-related white-matter changes: the LADIS study. *J. Neurol. Neurosurg. Psychiatry* 80: 478-483 (Journal IF: 4.622)

Bentes, C., Costa, J., Pires, J. and Paiva, T. (2009) Dream Recall and Seizures in Patients with Temporal Lobe Epilepsy. *J. Neurol. Sci.* 285(S1): S264-265 (Journal IF: 2.359)

Blahak, C., Baezner, H., Pantoni, L., Poggesi, A., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J.M., Langhorne, P., O'Brien, J., Visser, M.C., Wahlund, L.O., Waldemar, G., Wallin, A., Inzitari, D., Hennerici, M.G.; LADIS Study Group (2009) Deep frontal and periventricular age related white matter changes but not basal ganglia and infratentorial hyperintensities are associated with falls: cross sectional results from the LADIS study. *J. Neurol. Neurosurg. Psychiatry* 80: 608-613 (Journal IF: 4.622)

Bousser, M.G., Amarenco, P., Chamorro, A., Fisher, M., Ford, I., Fox, K., Hennerici, M.G.,

Mattle, H.P., Rothwell, P.M.; PERFORM Study Investigators (2009) Rationale and design of a randomized, double-blind, parallel-group study of terutroban 30 mg/day versus aspirin 100 mg/day in stroke patients: the prevention of cerebrovascular and cardiovascular events of ischemic origin with terutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study. *Cerebrovasc. Dis.* 27: 509-518 (Journal IF: 3.041)

Castro-Caldas, A., Nunes, M.V., Maestú, F., Ortiz, T., Simões, R., Fernandes, R., La Guia, E., Garcia E. and Gonçalves, M. (2009) Learning orthography in adulthood: a magnetoencephalographic study. *J. Neuropsychol.* 3(Pt1): 17-30 (Journal IF: NA)

CLOTS Trials Collaboration, Dennis, M., Sandercock, P.A., Reid, J., Graham, C., Murray, G., Venables, G., Rudd, A. and Bowler, G. (2009) Effectiveness of thigh-length graduated compression stockings to reduce the risk of deep vein thrombosis after stroke (CLOTS trial 1): a multicentre, randomised controlled trial. *Lancet* 373: 1958-1965 (Journal IF: 28.409)

Connolly, S.J., Ezekowitz, M.D., Yusuf, S., Eikelboom, J., Oldgren, J., Parekh, A., Pogue, J., Reilly, P.A., Themeles, E., Varrone, J., Wang, S., Alings, M., Xavier, D., Zhu, J., Diaz, R., Lewis, B.S., Darius, H., Diener, H.C., Joyner, C.D., Wallentin, L.; RE-LY Steering Committee and Investigators (2009) Dabigatran versus warfarin in patients with atrial fibrillation. *N. Engl. J. Med.* 361: 1139-1151 (Journal IF: 50.017)

Coutinho, J.M., Ferro, J.M., Canhão, P., Barinagarrementeria, F., Cantú, C., Bousser, M.G. and Stam, J. (2009) Cerebral venous and sinus thrombosis in women. *Stroke* 40: 2356-2361 (Journal IF: 6.499)

Di Fonzo, A., Fabrizio, E., Thomas, A., Fincati, E., Marconi, R., Tinazzi, M., Breedveld, G.J., Simons, E.J., Chien, H.F., Ferreira, J.J., Horstink, M.W., Abbruzzese, G., Borroni, B., Cossu, G., Dalla Libera, A., Fabbri, G., Guidi, M., De Mari, M., Lopiano, L., Martignoni, E., Marini, P., Onofri, M., Padovani, A., Stocchi, F., Toni, V., Sampaio, C., Barbosa, E.R., Meco, G.; Italian Parkinson Genetics Network, Oostra, B.A. and Bonifati, V. (2009) GIGYF2 mutations are not a frequent cause of familial Parkinson's disease. *Parkinsonism Relat Disord.* 15: 703-705 (Journal IF: 1.907)

Dworkin, R.H., Turk, D.C., McDermott, M.P., Peirce-Sandner, S., Burke, L.B., Cowan, P., Farrar, J.T., Hertz, S., Raja, S.N., Rappaport, B.A., Rauschkolb, C. and Sampaio, C. (2009) Interpreting the clinical importance of group differences in chronic pain clinical trials: IMMPACT recommendations. *Pain* 146: 238-424 (Journal IF: 6.030)

European Heart Rhythm Association (EHRA); Heart Failure Association (HFA); Heart Rhythm Society (HRS); European Society of Emergency Medicine (EuSEM); European Federation of Internal Medicine (EFIM); European Union Geriatric Medicine Society (EUGMS); American Geriatrics Society (AGS); European Neurological Society (ENS); European Federation of Autonomic Societies (EFAS); American Autonomic Society (AAS), Moya, A., Sutton, R., Ammirati, F., Blanc, J.J., Brignole, M., Dahm, J.B., Deharo, J.C., Gajek, J., Gjesdal, K., Krahn, A., Massin, M., Pepi, M., Pezawas, T., Granell, R.R., Sarasin, F., Ungar, A., van Dijk, J.G., Walma, E.P., Wieling, W., Abe, H., Benditt, D.G., Decker, W.W., Grubb, B.P., Kaufmann, H., Morillo, C., Olshansky, B., Parry, S.W., Sheldon, R., Shen, W.K., ESC Committee for Practice Guidelines (CPG), Vahanian, A., Auricchio, A., Bax, J., Ceconi, C., Dean, V., Filippatos, G., Funck-Brentano, C., Hobbs, R., Kearney, P., McDonagh, T., McGregor, K., Popescu, B.A., Reiner, Z., Sechtem, U., Sirnes, P.A., Tendera, M., Vardas, P., Widimsky, P., Auricchio, A., Acarturk, E., Andreotti, F., Asteggiano, R., Bauersfeld, U., Bellou, A., Benetos, A., Brandt, J., Chung, M.K., Cortelli, P., Da Costa, A., Extramiana, F., Ferro, J., Gorenek, B., Hedman, A., Hirsch, R., Kaliska, G., Kenny, R.A., Kjeldsen, K.P., Lampert, R., Mølgard, H., Paju, R., Puodziukynas, A., Raviello, A., Roman, P., Scherer, M., Schondorf, R., Sicari, R., Vanbrabant, P., Wolpert, C., Zamorano, J.L. (2009) Guidelines for the diagnosis and management of syncope (version 2009): the Task Force for the Diagnosis and Management of Syncope of the European Society of Cardiology (ESC). *Eur. Heart J.* 30: 2631-2671 (Journal IF: 8.917)

Evatt, M.L., Chaudhuri, K.R., Chou, K.L., Cubo, E., Hinson, V., Kompoliti, K., Yang, C., Poewe, W., Rascol, O., Sampaio, C., Stebbins, G.T. and Goetz, C.G. (2009) Dysautonomia rating scales in Parkinson's disease: sialorrhea, dysphagia, and constipation-critique and recommendations by



- movement disorders task force on rating scales for Parkinson's disease. *Mov. Disord.* 24: 635-646 (Journal IF: 3.898)
- Ferro, J.M., Bacelar-Nicolau, H., Rodrigues, T., Bacelar-Nicolau, L., Canhão, P., Crassard, I., Bousser, M.G., Dutra, A.P., Massaro, A., Mackowiack-Cordiolani, M.A., Leys, D., Fontes, J., Stam, J., Barinagarrementeria, F.; ISCVT and VENOPORT investigators (2009) Risk score to predict the outcome of patients with cerebral vein and dural sinus thrombosis. *Cerebrovasc. Dis.* 28: 39-44 (Journal IF: 3.041)
- Ferro, J.M., Caeiro, L. and Santos, C. (2009) Poststroke emotional and behavior impairment: a narrative review. *Cerebrovasc. Dis.* 27(Suppl 1): 197-203 (Journal IF: 3.041)
- Ferro, J.M., Canhão, P., Stam, J., Bousser, M.G., Barinagarrementeria, F., Massaro, A., Ducrocq, X., Kasner, S.E.; ISCVT Investigators (2009) Delay in the diagnosis of cerebral vein and dural sinus thrombosis: influence on outcome. *Stroke* 40: 3133-3138 (Journal IF: 6.499)
- Fonseca, A.C., Costa, J., Cordeiro, C., Geraldês, R. and de Sá, J. (2009) Influence of climatic factors in the incidence of multiple sclerosis relapses in a Portuguese population. *Eur. J. Neurol.* 16: 537-539 (Journal IF: 2.732)
- Foreid, H., Barroso, C., Carvalho, H., Roque, L. and Pimentel, J. (2009) A 22-year-old man with intracranial hypertension and impaired sensation over the perineum and left foot. *Brain Pathol* 19: 735-738 (Journal IF: 5.576)
- Gil-Gouveia, R. and Goadsby, P.J. (2009) Neuropsychiatric side-effects of lidocaine: examples from the treatment of headache and a review. *Cephalalgia* 29: 496-508 (Journal IF: 3.686)
- Hauser, R.A., Bronzova, J., Sampaio, C., Lang, A.E., Rascol, O., Theeuwes, A., van de Witte, S.V.; Pardoprunox Study Group (2009) Safety and tolerability of pardoprunox, a new partial dopamine agonist, in a randomized, controlled study of patients with advanced Parkinson's disease. *Eur. Neurol.* 62: 40-48 (Journal IF: 1.897)
- Inzitari, D., Pracucci, G., Poggesi, A., Carlucci, G., Barkhof, F., Chabriat, H., Erkinjuntti, T., Fazekas, F., Ferro, J.M., Hennerici, M., Langhorne, P., O'Brien, J., Scheltens, P., Visser, M.C., Wahlund, L.O., Waldemar, G., Wallin, A., Pantoni, L.; LADIS Study Group (2009) Changes in white matter as determinant of global functional decline in older independent outpatients: three year follow-up of LADIS (leukoaraiosis and disability) study cohort. *BMJ* 339: b2477 (Journal IF: 12.827)
- Jokinen, H., Kalskam H., Ylikoski, R., Madureira, S., Verdelho, A., Gouw, A., Scheltens, P., Barkhof, F., Visser, M.C., Fazekas, F., Schmidt, R., O'Brien, J., Hennerici, M., Baezner, H., Waldemar, G., Wallin, A., Chabriat, H., Pantoni, L., Inzitari, D., Erkinjuntti, T.; LADIS group (2009) MRI-defined subcortical ischemic vascular disease: baseline clinical and neuropsychological findings. The LADIS Study. *Cerebrovasc. Dis.* 27: 336-344 (Journal IF: 3.041)
- Jokinen, H., Kalska, H., Ylikoski, R., Madureira, S., Verdelho, A., van der Flier, W.M., Scheltens, P., Barkhof, F., Visser, M.C., Fazekas, F., Schmidt, R., O'Brien, J., Waldemar, G., Wallin, A., Chabriat, H., Pantoni, L., Inzitari, D., Erkinjuntti, T.; LADIS group (2009) Longitudinal cognitive decline in subcortical ischemic vascular disease - the LADIS Study. *Cerebrovasc. Dis.* 27: 384-391 (Journal IF: 3.041)
- Martins, I.P., Loureiro, C., Ramos, S. and Moreno, T. (2009) Grammatical dissociation during acquired childhood aphasia. *Dev. Med. Child Neurol.* 51: 999-1002 (Journal IF: 2.561)
- Mestre, T., Ferreira, J., Coelho, M.M., Rosa, M. and Sampaio C. (2009) Therapeutic interventions for symptomatic treatment in Huntington's disease. *Cochrane Database Syst Rev.* (3): CD006456 (Journal IF: 5.182)
- Mestre, T., Ferreira, J.J. and Jain, S.A (2009) A Multimodal approach to physical therapy in Parkinson's disease: optimizing strategies. *Mov. Disord.* 24: 946 (Journal IF: 3.898)
- Mestre, T. and Ferreira, J.J. (2009) Eslicarbazepine acetate: a new option for the treatment of focal epilepsy. *Expert Opin. Investig. Drugs* 18: 221-229 (Journal IF: 4.058)

Olanow, C.W., Rascol, O., Hauser, R., Feigin, P.D., Jankovic, J., Lang, A., Langston, W., Melamed, E., Poewe, W., Stocchi, F., Tolosa, E.; ADAGIO Study Investigators (2009) A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *N. Engl. J. Med.* 361: 1268-1278 (Journal IF: 50.017)

Pimentel, J., Barroso, C., Miguéns, J., Firmo, C. and Antunes, J.L. (2009) Papillary glioneuronal tumor – prognostic value of the extension of surgical resection. *Clin. Neuropathol.* 28: 287-294 (Journal IF: 1.200)

Ropele, S., Seewann, A., Gouw, A.A., van der Flier, W.M., Schmidt, R., Pantoni, L., Inzitari, D., Erkinjuntti, T., Scheltens, P., Wahlund, L.O., Waldemar, G., Chabriat, H., Ferro, J., Hennerici, M., O'Brien, J., Wallin, A., Langhorne, P., Visser, M.C., Barkhof, F., Fazekas, F.; LADIS study group (2009) Quantitation of brain tissue changes associated with white matter hyperintensities by diffusion-weighted and magnetization transfer imaging: the LADIS (Leukoaraiosis and Disability in the Elderly) study. *J. Magn. Reson. Imaging.* 29: 268-274 (Journal IF: 2.658)

Ross, O.A., Spanaki, C., Griffith, A., Lin, C.H., Kachergus, J., Haugarvoll, K., Latsoudis, H., Plaitakis, A., Ferreira, J.J., Sampaio, C., Bonifati, V., Wu, R.M., Zabetian, C.P. and Farrer, M.J. (2009) Haplotype analysis of Lrrk2 R1441H carriers with parkinsonism. *Parkinsonism Relat. Disord.* 15: 466-467 (Journal IF: 1.907)

Sampaio, C. (2009) Biomarkers in Clinical Trials of Alzheimer's Disease (AD): what is expected from regulatory agencies? *J. Nutr. Health Aging* 13: 339-340 (Journal IF: 2.321)

Sampaio, C. (2009) Can focusing on UPDRS Part II make assessments of Parkinson disease progression more efficient? *Nat. Clin. Pract. Neurol.* 5: 130-131 (Journal IF: 6.979)

Silva, D., Santana, I., Simões do Couto, F., Maroco, J., Guerreiro, M. and de Mendonça, A. (2009) Cognitive deficits in middle-aged and older adults with bipolar disorder and cognitive complaints: Comparison with mild cognitive impairment. *Int. J. Geriatr. Psychiatry* 24: 614-631 (Journal IF: 2.128)

Silva Nunes, M.V., Castro-Caldas, A., Del Rio, D., Maestú, F. and Ortiz, T. (2009) The ex-illiterate brain: the critical period, the cognitive reserve and the HAROLD model. *Dement. Neuropsychol.* 3: 222-227 (Journal IF: NA)

Teodorczuk, A., Firbank, M.J., Pantoni, L., Poggesi, A., Erkinjuntti, T., Wallin, A., Wahlund, L.O., Scheltens, P., Waldemar, G., Schrotter, G., Ferro, J.M., Chabriat, H., Bazner, H., Visser, M., Inzitari, D. and O'Brien, J.T. (2009) Relationship between baseline white-matter changes and development of late-life depressive symptoms: 3-year results from the LADIS study. *Psychol. Med.* 12: 1-8 (Journal IF: NA)

Townes, B.D., Martins, I.P., Castro-Caldas, A., Rosenbaum, G. and DeRouen, T. (2009). The serial use of child neurocognitive tests: development versus practice effects. *Psychological Assessment*, 29 (4), 361-369 (Journal IF: NA)

Valadas, A., Bentes, C. and Ferro J. (2009) Generalized convulsive status epilepticus treatment: agreement of a standard is not sufficient. *Epilepsia* 50(Supp.10): 53-54 (Journal IF: 3.733)

Viana-Pereira, M., Almeida, I., Sousa, S., Mahler-Araújo, B., Seruca, R., Pimentel, J. and Reis, R.M. (2009) Analysis of microsatellite instability in medulloblastoma. *Neuro. Oncol.* 11: 458-467 (Journal IF: NA)

Zerr, I., Kallenberg, K., Summers, D.M., Romero, C., Taratuto, A., Heinemann, U., Breithaupt, M., Varges, D., Meissner, B., Ladogana, A., Schuur, M., Haik, S., Collins, S.J., Jansen, G.H., Stokin, G.B., Pimentel, J., Hewer, E., Collie, D., Smith, P., Roberts, H., Brandel, J.P., van Duijn, C., Pocchiarri, M., Begue, C., Cras, P., Will, R.G. and Sanchez-Juan, P. (2009) Updated clinical diagnostic criteria for sporadic Creutzfeldt Jakob disease. *Brain* 132: 2659-2668 (Journal IF: 9.603)

#### **Publications in Peer Review National Journals**

Borges, M., Gouveia, M., Costa, J., dos Santos Pinheiro, L., Paulo, S., Vaz Carneiro, A. (2009)

- The burden of disease attributable to smoking in Portugal. *Rev. Port. Pneumol.* 15: 951-1004
- de Mendonça, A. (2009) Em Memória. *Sinapse* 9: 43
- de Mendonça, A. (2009) Grupo de Demências – Consórcio Europeu sobre Doença de Alzheimer. *Alzheimer Portugal* 41: 11
- de Mendonça, A. (2009) Não aprendas depressa. *Sinapse* 9: 64
- de Mendonça, A. (2009) Os efeitos benéficos do café. *Café e Saúde*.
- de Mendonça, A. and Santana, I. Notícia. Página do Grupo de Estudos de Envelhecimento Cerebral e Demências, <http://www.geecd.org/>.
- de Mendonça, A. O Grupo de Estudos de Envelhecimento Cerebral e Demências. Página do Grupo de Estudos de Envelhecimento Cerebral e Demências, <http://www.geecd.org/>.
- Ferro, J.M. (2009) Management of Stroke. *Sinapse* 2: 5-6
- Fonseca, J. (2009) Linguagem e hemisfério direito. *Re(habilitar) - Revista da ESSA*, nº 8/9: 5-18.
- Martins, I.P. (2009) Enxaqueca. Da clínica para a etiopatogenia. Artigo de revisão. *Acta Medica Portuguesa* 22: 589-598
- Martins, M.J., Moura, B.M., Martins, I.P. and Figueira, M.L. (2009) Esquizofrenia e reconhecimento de expressões faciais de emoções e dor – correlações sintomáticas e influência da atenção. *Revista Faculdade de Medicina de Lisboa* 14: 97-98
- Morgado, J., Rocha, C.S., Maruta, C., Guerreiro, M. and Martins, I.P. (2009) Novos valores normativos do Mini-Mental State Examination. *Sinapse* 2: 10-16
- Rosa, M.M. (2009) Boas Práticas Clínicas (Good Clinical Practice). *Sinapse* 9: 51-55

### Books/Book Chapters

- Canhão, P., Ferro, J.M. and Stam, J. (2009) Cerebral venous thrombosis in *Handbook of clinical neurology, in Stroke. Part II: Clinical manifestations and pathogenesis*. Fisher, M. (ed). Elsevier Vol 93:809-822.
- Castro Caldas, A., Mineiro, A., Nunes, M.V., Silva, A.S. and Fernandes, S.A. (2009) Emoção. Um Vector Neurobiológico a Motivar a Emergência das Metáforas Náuticas dos Descobrimentos? *in Expressões da Analogia*. Edições Colibri.
- Coelho, M. and Ferreira, J.J. (2009) Non-motor symptoms in advanced Parkinson's disease *in The non-motor symptoms complex of Parkinson's disease*. Chaudhuri KR, Tolosa E, Schapira A, Poewe W (eds). Oxford University Press.
- Colaço, A., Mineiro, A., Leal, G. and Castro Caldas, A. (2009) The influence of literacy in paraphasias of fluent aphasic people, *in Linguistics: the Challenge of Clinical Application*. Marrero, V. and Pineda, I. (eds). Euphonia Ediciones.
- de Mendonça, A. (2009) Envelhecimento e Dependência – O Caso das Doenças Neurodegenerativas, *in O Tempo da Vida*. Principia, Lisboa,
- Oswald W (coordinator), Caramona M, Esteves, A P, Gonçalves J, Macedo T, Mendonça J, Pinheiro R L, Rodrigues A, Sampaio C, Sepodes, B, Teixeira AA (2009). *Prontuário Terapêutico* 8, Infarmed.

### Full Papers in Conference Proceedings

- Dores, A.R., Carvalho, I., Abreu, C., Nunes, J., Leitão, M. and Castro-Caldas, A. (2009) Virtual Reality: Application to cognitive rehabilitation after Acquired Brain Injury in Blashki K. (Org.). *Proceedings of 2<sup>nd</sup> International Conference on Software Development for Enhancing Accessibility and Fighting Info-Exclusion*, Lisboa, Portugal. Lisboa: DSAI, 68-72.

Dores, A.R., Carvalho, I.P., Guerreiro, S., Almeida, I., Romero, L., Abreu, C., Nunes, J., Leitão, M., Barbosa, J. and Castro Caldas, A. (2009) New answers for old questions in the domain of the cognitive rehabilitation. Proceedings of Interfaces and Human Computer Interaction 2009 and Game and Entertainment Technologies 2009. Algarve: IADIS, 91-96.

### **Communications in International Conferences**

Bentes, C. et al. Dream recall and seizures in patients with temporal lobe epilepsy. 19<sup>th</sup> World Congress of Neurology (WCN 2009), Bangkok, Thailand. October 24–30, 2009 (Poster presentation).

Caeiro, L. et al. Apathy in post stroke patients: a longitudinal study. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Poster presentation)

Caeiro, L. et al. Neuropsychiatric and neuropsychological indicators of post stroke depression. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Poster presentation)

Campos, A. et al. Surgery for Temporal Lobe Epilepsy Long-Term Seizures Follow-Up. EANS Joint Annual Meeting. Marseille, France. March 27-31, 2009 (Oral presentation)

Campos, A. et al. Epilepsy surgery of the temporal lobe. Long-term seizures outcome. 2nd International Epilepsy Colloquium – Pediatric Epilepsy Surgery. Lyon, France. May 3–6, 2009 (Oral presentation)

Canhão, P. and Fonseca, A.C. Clinical predictors of “TIA” or “mimic” diagnosis. 18th European Stroke Conference. Stockholm, Sweden. May 26-29, 2009 (Poster presentation)

Castro Caldas, A. et al. PRO\_LGP: an e-learning Project. Proceedings of the 1st Symposium in Applied Sign Linguistics, University of Bristol, Centre for Deaf Studie, Bristol, UK. October, 2009 (Oral presentation)

Chester, C. et al. Semantic Memory: Relations with demographic variables. INS-Year Meeting 2009. Helsinki, Finland and Tallinn, Estonia. July 29 – August 1, 2009 (Poster presentation)

Cortez-Pinto, H. et al. Liver disease is the main burden of illness attributable to alcohol drinking: results of a national Study. 44th Annual Meeting of the European Association for the Study of the Liver (EASL). Copenhagen, Denmark. April 22-26, 2009 (Oral presentation)

Costa, J. et al. Evaluation of oscillatory repetitive movements in Parkinson disease and essential tremor: implications for physiopathology and differential diagnosis. 19th World Congress of Neurology. Bangkok, Thailand. October 24 -30, 2009 (Oral presentation)

Coutinho, J.M. et al. Cerebral venous and sinus thrombosis in women. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)

Falcão, F. et al. Stroke mimics in RTPA-treated patients. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)

Ferreira, J.J. “Espresso coffee” for the treatment of excessive daytime sleepiness in Parkinson’s disease: Results of four pilot n-of-one clinical trials. 13th International Congress on Parkinson’s Disease and Movement Disorders. Paris, France. June 7-11, 2009 (Poster presentation)

Ferreira, J.J. et al. A systematic review of the incidence of fatigue as an adverse event in placebo-controlled trials for Parkinson’s disease. 13th International Congress of Parkinsons Disease and Movement Disorders, Paris, France. June 7-11, 2009 (Poster presentation)

Ferreira, L. et al. Cyclic alternating patterns in congenitally deaf subjects-quantitative and topographic analysis. 3<sup>rd</sup> International Congress on Sleep Medicine. 12<sup>th</sup> Brazilian Congress on Sleep Medicine – WASM, São Paulo, Brasil. November 7-11, 2009 (Poster presentation)

Ferro, J.M. et al. Safety of anticoagulation in cerebral venous thrombosis (CVT) with posterior fossa lesions. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Poster presentation)

Ferro, J.M. et al. Cerebral venous thrombosis (CVT) with non-hemorrhagic lesions: clinical

- correlates and prognosis. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)
- Fonseca, A.C. et al. Mitochondrial changes in patients with polymyositis. 13<sup>th</sup> Congress of the European Federation of Neurological Societies, Florence, Italy. September 12-15, 2009 (Oral presentation)
- Fonseca, A.C. et al. N-terminal pro-brain natriuretic peptide as possible biomarker of cardioembolic stroke. 18<sup>th</sup> European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)
- Fonseca, A.C. and Canhão, P. Classification of transient neurological attacks in a TIA clinic. 18<sup>th</sup> European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Poster presentation)
- Freitas, V. et al. Subjective memory questionnaire: an appraisal of executive functioning? INS-Year Meetin, Helsinki, Finland and Tallinn, Estonia. July 29 – August 1, 2009 (Poster presentation)
- Gil-Gouveia, L. and Silva-Carvalho L. Trigeminal activation and ocular autonómica dysfunction after stimulation of the posterior hypothalamus. 13<sup>th</sup> Congress of the European Federation of Neurological Societies, Florence, Italy. September 12-15, 2009 (Oral presentation)
- Gouveia, L. et al. Alternative therapies and Parkinson disease in Portugal. 13th Congress of the European Federation of Neurological Societies, Florence, Italy. September 12-15, 2009 (Poster presentation)
- Gouveia, L.O. et al. Alternative therapies and Parkinson's disease in Portugal. 13th International Congress of Parkinsons Disease and Movement Disorders, Paris, France. June 7-11, 2009 (Poster presentation)
- Krug, T. et al. Kalirin: A novel genetic risk factor for ischemic stroke. American Society for Human Genetics Meeting, Hawaii, USA. October 20-24, 2009 (Poster presentation)
- Krug, T. et al. Signature genes for Behçet's disease – Blood Genomic Expression Profile. MC-GARD 2009, Edimburgh, UK. April 1-5, 2009 (Oral presentation)
- Lima, A. et al. Embolia paradoxal cerebral associada a fistula arteriovenosa pulmonar isolada: utilidade do DTC no seu diagnóstico. II Congresso da Associação de Neurologia do Estado do Rio de Janeiro, Rio de Janeiro, Brasil. March 26-28, 2009 (Poster presentation)
- Lima, A. et al. Trombo livre na artéria carótida primitiva diagnosticada por ultrassonografia. II Congresso da Associação de Neurologia do Estado do Rio de Janeiro, Rio de Janeiro, Brazil. March 26-28, 2009 (Poster presentation)
- Lobo, P. et al. Cortical laminar necrosis with early involvement of the corpus callosum. 19<sup>th</sup> Meeting of the European Neurological Society, Milan, Italy. June 20-24, 2009 (Oral presentation)
- Madureira, S. et al. Sensibility and specificity of the neuropsychological evaluation on identifying dementia in a three year follow-up study of an independent elderly population with white matter changes: the LADIS Study. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)
- Manso, H. et al. Variants of the Matrix Metalloproteinase-2 (MMP-2) but not the Matrix Metalloproteinase-9 (MMP-9) genes significantly influence functional outcome after stroke. American Society for Human Genetics Meeting, Hawaii, USA. October 20-24, 2009 (Poster presentation)
- Martins, M.J. et al. Emotion recognition and sensitivity to expressions of pain by schizophrenia patients – differential cognitive strategies? 9th World Congress of Biological Psychiatry, Paris, France. June 28 – July 2, 2009 (Oral presentation)
- Maruta, C. et al. Cognition in migraine. INS-Year Meeting 2009, Helsinki, Finland and Tallinn, Estonia July 29 – August 1, 2009 (Poster presentation)
- Maruta, C. et al. The use of neuropsychological tests across Europe: the need for a consensus in

the use of assessment tools for dementia. 13<sup>th</sup> Congress of the European Federation of Neurological Societies, Florence, Italy. September 12-15, 2009 (Poster presentation)

Maruta, C. et al. Memory complaints and visual memory. INS-Year Meeting, Helsinki, Finland and Tallinn, Estonia. July 29 – August 1, 2009 (Poster presentation)

Morgado, J. et al. Cut-off scores in MMSE: a moving target. INS-Year Meeting, Helsinki, Finland and Tallinn, Estonia. July 29 – August 1, 2009 (Poster presentation)

Morgado, J. et al. Cut-off scores in MMSE: a moving target? 13<sup>th</sup> Congress of the European Federation of Neurological Societies, Florence, Italy. September 12-15, 2009 (Poster presentation)

Negre-Pages, L. et al. Predictive factors for PD progression preliminary results of PARKMIP/COPARK cohort after 24-months follow up. XVIII WFN World Congress on Parkinson's Disease and Related Disorders, Miami Beach, EUA. December 13-16, 2009 (Poster presentation)

O'Brien, J.T. et al. Relationship between baseline white matter changes and development of late life depressive symptoms: 3 year results from LADIS Study. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)

Oliveira, S.A. et al. From miRNomics to pathway analysis in Parkinson's Disease (PD). American Society for Human Genetics Meetin, Hawaii, USA. October 20-24, 2009 (Poster presentation)

Pimentel, J. et al. "Migralepsy" revisited. 28th International Epilepsy Congress, Budapest, Hungary. June 28 - July 2, 2009 (Poster presentation)

Pimentel, J. et al. Malignant status epilepticus: treatment persistence until when? The Innsbruck Colloquium on Status Epilepticus, Innsbruck, Austria. April 2-4, 2009 (Poster presentation)

Pires, J. et al. Auditory and verbal contents of dreams in congenital deaf and correlations with spectral EEG bands power. 3<sup>rd</sup> International Congress on Sleep Medicine – 12<sup>th</sup> Brazilian Congress on Sleep Medicine – WASM, São Paulo, Brasil. November 7-11, 2009 (Poster presentation)

Pires, J. et al. Relationship of EEG banda power during REM sleep to dream recall in congenital deaf and normal hearing individuals. 3<sup>rd</sup> International Congress on Sleep Medicine – 12<sup>th</sup> Brazilian Congress on Sleep Medicine – WASM, São Paulo, Brasi. November 7-11 2009 (Poster presentation)

Rodrigues, I.T. et al. Action verbal fluency in DP patients. 13th International Congress of Parkinsons Disease and Movement Disorders, Paris, France. June 7-11, 2009 (Poster presentation)

Soares, F. et al. Carotid dissection in a neonate diagnosed by ultrasound. XIV Meeting of the European Society of Neurosonology and Cerebral Hemodynamics, Riga, Latvia. May 23-25, 2009 (Poster presentation)

Soares, F. et al. Paradoxical brain embolism from pulmonary fistulae, the role of TCD. XIV Meeting of the European Society of Neurosonology and Cerebral Hemodynamics, Riga, Latvia. May 23-25, 2009 (Poster presentation)

Sousa, P.R. et al. Dream content involving pain and fatigue in fibromyalgia patients. 3<sup>rd</sup> International Congress on Sleep Medicine – 12<sup>th</sup> Brazilian Congress on Sleep Medicine – WASM, São Paulo, Brasil. November 7-11 2009 (Poster presentation)

Teles de Araujo, M. and Canhão P. Cost study of TIA patients in a TIA clinic. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Poster presentation)

Valadas, A. et al. Generalized convulsive statts epilepticus treatment: agreement of a standard is not sufficient. 28th International Epilepsy Congress, Budapest, Hungary. June 28 – July 2, 2009 (Poster presentation)

Valadas, A. et al. Motivations and concerns of Parkinson's disease patients to participate in

clinical trials. 13th International Congress of Parkinsons Disease and Movement Disorders, Paris, France. June 7-11, 2009 (Poster presentation)

Valadas, A. et al. Clinical trials: motivations and concerns of Parkinson's disease (PD) patients. 13th Congress of the European Federation of Neurological Societies, Florence, Italy. September 12-15, 2009 (Poster presentation)

Verdelho, A. et al. Depressive symptoms predict cognitive decline in an elderly non disabled population with white matter changes. Results of the LADIS study. 17th EPA Congress, Lisbon, Portugal. January 24-28, 2009 (Oral presentation)

Verdelho, A. et al. Relationship between baseline white matter changes and development of late life depressive symptoms: 3 year results from the LADIS study. 18<sup>th</sup> European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)

Verdelho, A. et al. White matter changes and diabetes predict cognitive impairment and dementia in independent elderly. Results of the LADIS Study. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)

Verdelho, A. et al. Previous stroke predict vascular dementia in independent elderly with cerebral white matter changes. Results of the LADIS Study. 18th European Stroke Conference, Stockholm, Sweden. May 26-29, 2009 (Oral presentation)

Wolf, P. et al. Non-Rasmussen, non-vascular epilepsy partialis continua (EPC). The Innsbruck Colloquium on Status Epilepticus, Innsbruck, Austria. April 2-4, 2009 (Poster presentation)

#### Communications in National Conferences

Costa, J. and Vaz-Carneiro, A. Caffeine and the brain. Coffee consumption and the risk of dementia and Parkinson's disease: a Systematic review of the literature. Lisbon, June 12-13, 2009 (Oral presentation).

Albuquerque, L. et al. Reconhecimento de emoções após cirurgia de estimulação do núcleo subtalâmico em Doença de Parkinson avançada. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

Almeida, V. et al. Biópsia estereotáxica seguida de exérese cirúrgica – concordância diagnóstica. Neuro 2009. Albufeira. May 14-16, 2009 (Oral presentation)

Antunes, A.P. et al. Hemossiderose superficial após lesão traumática do plexo braquial. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Poster presentation)

Barbosa, N. and Peralta, R. Cefaleias Diárias Matinais – Um caso com tratamento cirúrgico. Sociedade Portuguesa de Cefaleias, Reunião da Primavera 2009, Ponta Delgada. April 03-04, 2009 (Poster presentation)

Bentes, C. and Grupo da Cirurgia da Epilepsia. Epilepsia bitemporal. 1<sup>a</sup> Reunião dos Centros da Cirurgia da Epilepsia, Lisboa. November 27, 2009 (Oral presentation)

Campos, A. et al. Cirurgia da epilepsia em dois tempos: experiência de um centro. 21<sup>o</sup> Encontro Nacional de Epileptologia, Coimbra. March 13-14, 2009 (Oral presentation)

Campos, A. and Grupo da Cirurgia da Epilepsia do Hospital de Santa Maria. Um caso de Hemisferotomia. 1<sup>a</sup> Reunião dos Centros da Cirurgia da Epilepsia, Lisboa. November 27, 2009 (Oral presentation)

Campos, A. and Grupo de Cirurgia da Epilepsia do Hospital de Santa Maria. Discussão clínica de caso clínico. 21<sup>o</sup> Encontro Nacional de Epileptologia, Coimbra. March 13-14 (Oral presentation)

Canhão, P. and Fonseca, A.C. Preditores clínicos do diagnóstico de acidentes neurológicos transitórios. Crises, pseudo-crises e monitorização combinada EEG-ECG. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

presentation)

Coiteiro, D. et al. Revascularização directa (Bypass EC-IC) no tratamento da isquémia cerebral. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Falcão, F. et al. Falso AVC em doentes submetidos a terapêutica trombolítica. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Oral presentation)

Falcão, F. et al. Hemorragia subaracnoideia da alta convexidade cerebral numa unidade de AVC. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Poster presentation)

Faria, C. et al. Alterações genéticas associadas com baixa sobrevida livre de doença em doentes com tumores oligodendrogliais. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Fernandes, S. et al. Reconhecimento de emoções e Doença de Parkinson avançada. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Fonseca, A.C. and Canhão, P. Classificação de défices neurológicos transitórios numa consulta de Acidentes Isquémicos Transitórios. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Poster presentation)

Fonseca, A.C. et al. Leucoencefalopatia posterior reversível numa doente com Síndrome de Guillain-Barré. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Poster presentation)

Fonseca, A.C. et al. Necrose laminar cortical com envolvimento precoce do corpo caloso. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Fonseca, A.C. et al. N-terminal do pró-peptídeo natriurético cerebral como possível marcador de Acidente Vascular Cerebral isquémico de etiologia cardioembólica. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Poster presentation)

Fonseca, A.C. et al. Alterações mitocondriais em doentes com polimiosite. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5-8, 2009 (Oral presentation)

Fonseca, I. et al. Terapia da Fala intensiva versus Terapia da Fala convencional na afasia resultante de AVC isquémico: um ensaio multicêntrico randomizado e controlado. V Congresso Nacional da Associação Portuguesa de Terapeutas da Fala, Lisboa. November 20-21, 2009 (Oral presentation)

Fonseca, J. Avaliar a afasia ainda pode ser desafiante? V Congresso Nacional da Associação Portuguesa de Terapeutas da Fala, Lisboa. November 20-21, 2009 (Oral presentation)

Fonseca, J. et al. Estudar a eficácia da terapia da afasia. Que dificuldades? V Congresso Nacional da Associação Portuguesa de Terapeutas da Fala, Lisboa. November 20-21, 2009 (Oral presentation)

Fonseca, J. et al. Doente com padrão atípico de evolução do discurso afásico. Reunião Secção da Neurologia do Comportamento da Sociedade Portuguesa de Neurologia. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

Foreid, H. et al. Questões éticas respeitantes à utilização de testes de provocação no diagnóstico de pseudocrises. 21º Encontro Nacional de Epileptologia, Coimbra. March 13-14, 2009 (Poster presentation)

Foreid, H. et al. Paralisia periódica hipocaliémica tiorotóxica simulando clínica e electrofisiologicamente um Síndrome de Guillain-Barré. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Poster presentation)

Foreid, H. et al. Síndrome cerebeloso e alterações neuroradiológicas iatrogénicas ao metronidazol. Neuro 2009, Albufeira. May 14-16, 2009 (Poster presentation)

Foreid, H. et al. Hipercinésias por ferritinopatia. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)



Foreid, H. et al. Questões éticas respeitantes à utilização de testes de provocação no diagnóstico de pseudocrisis. 21º Encontro Nacional de Epileptologia, Coimbra. May 13-14, 2009 (Oral presentation)

Gouveia, M. et al. Impacto Sócio-económico da Diabetes em Portugal. Reunião de Outono - Os Desafios da Diabetes. Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo e Sociedade Portuguesa de Diabetologia, Lisbon. October 10, 2009 (Oral presentation)

Krug, T. et al. Genomic convergence approach applied to ischemic stroke. XXXIV Jornadas Portuguesas de Genética. April 28-30, 2009 (Oral presentation)

Krug, T. et al. Kalirin: A common genetic risk factor for vascular diseases? Congresso da Sociedade Portuguesa de Hipertensão, Vilamoura. February 12-15, 2009 (Oral and Poster presentation)

Lauterbach, M. et al. Avaliação de um novo tratamento de afasia num doente com afasia global crónica. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Lauterbach, M. et al. Polimicrogria perisúlvica bilateral - estudo clínico e imagiológico. Secção da Neurologia do Comportamento da Sociedade Portuguesa de Neurologia. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

Leal, G. et al. Defeito de nomeação de objectos e de faces públicas após cirurgia numa doente com epilepsia. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5-8, 2009 (Oral presentation)

Lobo, P.P. et al. Miopatia de doença crítica numa unidade de acidentes vasculares cerebrais. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Oral presentation)

Lobo, P.P. et al. Polineuropatia amiloidótica adquirida após transplante hepático sequencial. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5 - 8, 2009 (Oral presentation)

Lobo, P.P. et al. Hemorragia subaracnoideia perimesencefálica num doente anticoagulado. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Oral presentation)

Lobo, P.P. et al. Abscesso do tronco cerebral: terapêutica médica. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Lobo, P.P. et al. Romboencefalite fatal: caso anátomo-clínico. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5-8, 2009 (Oral presentation)

Lobo, P.P. et al. Topiramato e acidose metabólica hiperclorémica com “Gap” aniónico normal. 21º Encontro Nacional de Epileptologia, Coimbra. May 13-14, 2009 (Oral presentation)

Luzeiro, I. et al. Percepção do doente com enxaqueca em relação à sua doença. Reunião da Primavera da Sociedade Portuguesa de Cefaleias, Ponta Delgada. April 3-4, 2009

Martins M, Rosa A, Fonseca BV, Violante S, Guedes LC, Mestre T, Coelho M, Rosa MM, Ferreira J, Oliveira SA. From miRNomics to Pathway Analysis in Parkinson’s Disease. International Workshop on Genomic Disorders, Disease-Associated Chromosome Rearrangements and Position Effect. INSA. Lisboa, May 29-30, 2009 (Oral communication)

Martins, I.P. et al. Enxaqueca e Cognição. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Maruta, C. et al. Questionário Queixas Subjectivas de Memória: Uma Medida de Funções Executivas? Neuro 2009, Albufeira. May 14-16, 2009 (Poster presentation)

Maruta, C. et al. The use of Neuropsychological Tests across Europe: The need for a Consensus in the Use of Assessment Tools for Dementia”. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5-8, 2009 (Poster presentation)

Maruta, C. et al. Avaliação da memória em portugueses com idade superior a 50 anos: Validação e dados normativos para o CVLT-9 e para os subtestes Reprodução Visual e Faces da WMS – III. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-

8, 2009 (Oral presentation)

Melo, T.P. Via Verde Intra Hospitalar para o AVC – registo sistemático. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Oral presentation)

Mestre, T. et al. Indicações e escolhas terapêuticas na Doença de Huntington. Resultados do estudo observacional Registry. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

Gouveia, M. et al. Impacto Sócio-económico da Diabetes em Portugal. Reunião de Outono - Os Desafios da Diabetes. Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo e Sociedade Portuguesa de Diabetologia, Lisboa. October 10, 2009 (Oral presentation)

Morgado, J. et al. MMSE: Novos valores normativos. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Neto, L. et al. Hemangioblastoma do hipocampo. Neuro 2009, Albufeira. May 14-16, 2009 (Poster presentation)

Neutel, D. et al. Acidente Vascular Cerebral em doente com Cistinose. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Poster presentation)

Neutel, D. et al. Crises, pseudo-criises e monitorização combinada EEG-ECG. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

Neutel, N. et al. Estimativa de prevalência do tremor essencial em Portugal. Neuro 2009, Albufeira. May 14-16, 2009 (Poster presentation)

Neutel, N. et al. Prevalência do tremor essencial: meta-análise de estudos de base populacional. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Peralta, R. et al. EEG com prova de sono no diagnóstico de epilepsia. A que horas deve ser feito? Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)

Pimentel, J. et al. “Migralépsia” revisitada. 21º Encontro Nacional de Epileptologia, Coimbra. March 13-14, 2009 (Poster presentation)

Pimentel, J. Evolução da cirurgia da epilepsia no Hospital de Santa Maria. III Reunião de Trabalho da Comissão de Cirurgia da Epilepsia da Liga Portuguesa Contra a Epilepsia, Lisbon. November 5-8, 2009 (Oral presentation)

Pimentel, J. Terapêutica actual da epilepsia. XIII Jornadas Nacionais PC, Lisboa. February 12-13, 2009 (Oral presentation)

Ribeiro, S. et al. Estudo de eventos vaso-oclusivos intracraninos em doentes com drepancitose. XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009 (Poster presentation)

Rocha, L. Apresentação de dois casos. O teu problema é a parte do problema para onde tu olhas. V Congresso Nacional da Associação Portuguesa de Terapeutas da Fala, Lisboa. November 20-21, 2009 (Oral presentation)

Rodrigues, T. et al. Estudo de deleção 1p/19q em oligodendrogliomas anaplásicos primários e secundários. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)

Rosa, A. et al. Parkinson’s Disease miRNA profiling in PBMCs. XXXIV Jornadas Portuguesas de Genética, Lisboa. April 28-30, 2009 (Poster presentation)

Sancho, A. and Leal G. Tradução e validação de uma escala de avaliação da comunicação funcional para adultos: resultados preliminares. V Congresso da Associação Portuguesa de Terapeutas da Fala, Lisboa. November 20 -21, 2009 (Oral presentation)

Santos, P. and Pimentel J. Astrocitoma de células gigantes subependimário: estudo imunocitoquímico de 3 casos. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5-8, 2009 (Oral presentation)

- Silva, V. et al. Relação genotipo-fenotipo nos oligodendrogliomas. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)
- Teles de Araújo, M. and Canhão, P. Estudo de custos dos Acidentes Isquémicos Transitórios tratados em ambulatório, durante o 1º mês após ocorrência. 3º Congresso Português do AVC, Porto. February 5-7, 2009 (Oral presentation)
- Timóteo, A. et al. Meningiomas cordóides. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Oral presentation)
- Timóteo, A. et al. Um caso de schwannoma do IV ventrículos. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009 (Poster presentation)
- Valadas, A. et al. Meningioangiomas. Caracterização clínica e neuropatológica. Neuro 2009, Albufeira. May 14-16, 2009 (Oral presentation)
- Valadas, A. et al. Tratamento do estado de mal convulsivo generalizado: Não basta ter uma Norma de Orientação Terapêutica. 21º Encontro Nacional de Epileptologia, Coimbra. March 13-14, 2009 (Poster presentation)
- Valadas, A. et al. Ensaios clínicos: motivações e preocupações dos doentes com Doença de Parkinson. Neuro 2009, Albufeira. May 14-16, 2009 (Poster presentation)
- Valadas, A. et al. Estado de mal convulsivo generalizado maligno: persistir com a terapêutica até quando? 21º Encontro Nacional de Epileptologia, Coimbra. May 13-14, 2009 (Oral presentation)

#### Invited Lectures and Seminars

- Albuquerque, L. "Hallucinations in Parkinson's Disease". Brain and Cognition Joint Forces Workshop, Utrecht, The Netherlands. June 2009.
- Bentes, C. "Comments about Epileptic Seizures Semiology". V Reunión Hispano Lusa de Epilepsia, Sevilla, Spain. October 24, 2009.
- Canhão, P. "Condições que mimetizam o AIT". VI Reunião do Núcleo de Estudos das Doenças Cerebrovasculares do Hospital Distrital de Santarém, Santarém. May 8, 2009.
- Castro-Caldas, A. "A Doença de Alzheimer". Conferência ICS-UCP, Lisbon. May 13, 2009
- Castro-Caldas, A. "A Doença de Parkinson". XXX Congresso de Geriatria, Lisbon. October 27, 2009.
- Castro-Caldas, A. "A Justificação de um Prémio Nobel". Colóquio Egas Moniz, Coimbra. March 18, 2009
- Castro-Caldas, A. "A Neurobiologia da Língua Gestual" Jornadas: Diferentes Formas de Comunicação na Deficiência, Centro João Paulo II, Fátima. June 30, 2009.
- Castro-Caldas, A. "A Viagem ao Cérebro e a algumas das suas competências". Conferência ICS-UCP, Lisbon. March 11, 2009.
- Castro-Caldas A. "Biologia das Perturbações do desenvolvimento". 1º Congresso Internacional sobre Perturbações do Desenvolvimento, Cadin, Cascais. March 26, 2009.
- Castro-Caldas A. "Cérebro e Cognição". EnjoyMed, Faculdade de Medicina de Lisboa, Lisboa. November 8, 2009.
- Castro-Caldas, A. "Cognição e Movimento". 2º Curso Médico-Cirúrgico em Neurociências, Porto. January 23-24, 2009.
- Castro-Caldas, A. "Envelhecimento e problemas associados". 3º Encontro Temático sobre a Rede de Informação e Partilha de Saberes, Cruz Vermelha Portuguesa, Lisbon. October 20, 2009.
- Castro-Caldas, A. "Envelhecimento Saudável". 1as Jornadas de Gerontologia Social, Escola Superior de Educação João de Deus, Lisboa. November 28, 2009
- Castro-Caldas, A. "O Cérebro e a Cultura". I Jornadas de Psicologia, CMRA. October 29, 2009.

- Castro Caldas, A. “O Cérebro e o Canto”. Curso Saúde e Canto, Universidade Católica Portuguesa, Lisboa, April 15, 2009.
- Castro Caldas, A. “O Cérebro Emocional”. III Seminário do Espaço S “Emoções”, Centro Cultural Cascais, Cascais. November 19, 2009.
- Castro Caldas, A. “O Cérebro Social”. Communication, Cognition and Media – Communication Sciences International Congress, Universidade Católica Portuguesa, Braga. September 23, 2009.
- Castro Caldas, A. “Papel dos Estudos pós-graduados para profissionais de saúde”. Simpósio Infecção Associada aos Cuidados de Saúde: novas soluções para velhos problemas? Hospital da Luz, Lisbon. April 23, 2009.
- Castro Caldas, A. Chairman do módulo “Investigação em Demência”. Fórum Pfizer Neuroscience, Lisbon. March 28, 2009.
- de Mendonça, A. “Café e Demência”. Forum SPESM 2009, Lisbon. June 2009.
- de Mendonça, A. “Demências Rapidamente Progressivas”. Forum Futuro, Lisbon. April 2009.
- Ferreira, J.J. “Neurofarmacología de IMAO”. Neurofarmacologia de Transtornos del Movimiento IV, Madrid. May 29, 2009.
- Ferreira, J.J. “Quando e Como Começar a Tratar?” Workshop: Abordagem dos Estádios Iniciais da Doença de Parkinson, Fórum SNC, Lunndbeck, Tróia. June 5, 2009.
- Ferreira, J.J. “Relação entre sonolência diurna excessiva, ataques de sono, anti-parkinsonicos e Doença de Parkinson”. Conferência: Sono e Parkinsonismo, Congresso de Neurologia 2009, Lisboa. November 5-8, 2009.
- Ferreira, J.J. “Revisão das guidelines para o tratamento da dor neuropática”. Fórum Futuro, Grunenthal, Lisbon. April 18, 2009.
- Ferreira, J.J. “Update em Toxina Botulínica”. Fórum Futuro, Grunenthal, Lisbon. April 18, 2009.
- Ferro, J.M. “Advances in the secondary prevention of stroke”. Euro-brazilian Symposium of Neurology, Rio de Janeiro. November 27-28, 2009.
- Ferro, J.M. “Alterações psiquiátricas no AVC”. Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisboa. November 5-8, 2009.
- Ferro, J.M. “Antiagregantes plaquetares nas recomendações da ESO 2008 para a prevenção secundária do AVC”. 3º Congresso Português do AVC, Porto. February 5-7, 2009.
- Ferro, J.M. “Cerebral vasculitis”. Euro-brazilian Symposium of Neurology, Brasil, Rio de Janeiro. November 27-28, 2009.
- Ferro, J.M. “Cerebral venous thrombosis”. 2nd ENS Symposium in Índia, Chennai, Índia. July 25-26, 2009.
- Ferro, J.M. “Controvérsias do tratamento do AVC associado à fibrilhação auricular”. 3º Congresso Português do AVC, Porto. February 5-7, 2009.
- Ferro, J.M. “Educação, referenciação e diagnóstico”. 3º Congresso Português do AVC, Porto. February 5-7, 2009.
- Ferro, J.M. “Expanding the therapeutic window in acute stroke”. 2nd ENS Symposium in Índia. Chennai, India. July 25-26, 2009.
- Ferro, J.M. “Stroke and CNS infections”. 19th World Congress of Neurology, Bangkok, Thailand. October 24-30, 2009.
- Ferro, J.M. “Terapêutica cirúrgica do enfarte isquémico”. Neuro 2009, Albufeira. May, 2009.
- Ferro, J.M. “Vasculites do Sistema Nervoso Central”. 1º Congresso Nacional de auto-imunidade. XV Reunião anual do NEDAI, Torres Vedras. April 23-25, 2009.
- Ferro, J.M. Venous sinus thrombosis. 19th World Congress of Neurology, Bangkok, Thailand. October 24-30, 2009.

- Ferreira, J.J. Paralel session: “Investigação com medicamentos em Portugal: que prioridade?” A Inovação ao serviço da saúde e do doente, Lisbon. March 16, 2009.
- Leal, G. “Apresentação de um caso com defeito de linguagem após de cirurgia da epilepsia”. Reunião Conjunta das Consultas de Epilepsia da zona Sul, Hospital Garcia da Horta, Almada. May, 2009.
- Leal, G. “Avaliação da Comunicação Funcional na pessoa com afasia”. Workshop no V Congresso da Associação Portuguesa de Terapeutas da Fala, Lisboa. November, 2009.
- Madureira, S. “Construção de uma bateria de avaliação neuropsicológica: a experiência LADIS”. Instituto de Medicina Molecular, Lisboa. January 23, 2009.
- Madureira, S. “Tratamento da Demência Vasculiar, Intervenção não-farmacológica”. Fórum SNC, Tróia. June 5-6, 2009.
- Martins, I.P. “Cefaleias Primárias e Vertigem”. Otoneurologia Congress, Carvoeiro. June 26-27, 2009.
- Martins, I.P. “Elderness or disease?”. Update Course on Cognitive Dysfunction. Paths to the Future, New Clinical Perspectives, Lisbon. April 16-18, 2009.
- Martins, I.P. “O Reconhecimento das Faces”. VIII Colóquios de Neurologia do Hospital Garcia da Orta, Sesimbra. January 23, 2009.
- Melo, T.P. “Introduction to the Stroke Unit Hospital Santa Maria”, Neurology experts’ meeting on stroke and polyvascular disease, Lisbon. April 19, 2009.
- Melo, T.P. “Tratamento trombolítico”, 3º Congresso Português do AVC, Porto. February 5-7, 2009.
- Oliveira, S.A. “Genetics of Atherosclerosis (ATS) and ATS diseases”, Cardiometabolic Syndrome and Atherosclerosis Post-Graduate Course, Lisbon. February 27-28, 2009.
- Oliveira, V. “Avaliação ultrassonográfica das vertigens”, Congresso Otoneurologia 2009, Carvoeiro. June 27, 2009.
- Oliveira, V. “AVC e mulher: questões sem resposta”, Congresso da Sociedade Portuguesa de HTA, Vilamoura. February, 2009.
- Oliveira, V. “Deteccion de microembolios”, XII Congreso de la Sociedad Iberoamericana de Enfermedad Cerebro vascular, Assuncion, Paraguay. September 4, 2009.
- Oliveira, V. “É possível prevenir o AVC?” Encontro Renal 2009, Vilamoura. March 14, 2009.
- Oliveira, V. “Guidelines ESO: O que há de novo?” VI Jornadas do Hospital Santarém, Santarém. May 2009.
- Oliveira, V. “Política de saúde para o AVC.” X Congresso da Sociedade Portuguesa de Medicina Física e Reabilitação, Viseu. March 13, 2009.
- Oliveira, V. “Role of the ultrasound unit in evaluation of acute stroke”, Neurology experts’ meeting on stroke and polyvascular disease, Lisbon. April 19, 2009.
- Paiva, T. et al. “Dream bizarreness in Neurologic patients with fronto temporal dysfunction”, ESF Exploratory Workshop on The dreaming mind-brain, consciousness and psychosis: bridging the gap from the phenomenology of mentation to neurones, Challand Saint Anselme (Aosta), Italy. May 25–28, 2009.
- Peralta, A.R. “Dreams in sensory dysfunctional subjects”, ESF Exploratory Workshop on The dreaming mind-brain, consciousness and psychosis: bridging the gap from the phenomenology of mentation to neurones, Challand Saint Anselme (Aosta), Italy. May 25–28, 2009.
- Peralta, A.R. “Neurocognitive deficits in Sleep Apnea patients”, Curso Pós graduado em Síndrome da Apneia do Sono, Funchal. October 8-10, 2009.
- Peralta, A.R. “Problemas comuns em Neurologia – patologia de sono”, XIII Jornadas Nacionais Patient Care, Centro de Congressos de Lisboa – FIL, Lisboa. 2009.

Pimentel, J. Chairman: “Cirurgia da Epilepsia – uma oportunidade de mudança”, Simpósio Satélite “UCB” - Neuro 2009, Albufeira. May 14-16, 2009.

Pimentel, J. Chairman: “Neurofisiologia e neuropatologia das lesões epileptogénicas do lobo temporal”, Congresso de Neurologia 2009 & Encontro Luso Brasileiro de Neurologia, Lisbon. November 5-8, 2009.

Pimentel, J. “Patologia do pescoço de vertigem”, Congresso de Otoneurologia, Carvoeiro. June 26-27, 2009.

Pimentel, J. “Os desafios de um Centro de Cirurgia de Epilepsia em Portugal”, Simpósio Satélite “UCB”, Neuro 2009, Algarve. May 14-16, 2009.

Rocha, L. “Tratamento dos disfágicos adultos em Portugal. A abordagem do Terapeuta da Fala”, Workshop no Seminário de Investigação em Disfagia, Introdução à EMG de superfície para a reabilitação da disfagia, Aveiro. February 19, 2009.

Rosa, M.M. “Emergências na Doença de Parkinson: Psicoses, Alucinações, Fadiga, Impulsividade, Flutuações Motoras e Discinésias”, Curso de cuidadores da Associação de Doentes de Doença de Parkinson, Lisbon. May 27, 2009.

Rosa, M.M. “Fármacos analgésicos”, Fórum Futuro, Lisbon. April 18, 2009.

Rosa M.M. “How to manage depression, psychosis and dementia in Parkinson’s Disease”, UNIC-MDS course, Lisbon. May 31, 2009.

Rosa, M.M. “Investigação clínica em neurociências: Desafio em Portugal”. Ciência 2009-Encontros com a Ciência, Lisboa. July 30, 2009.

Sampaio, C. “Early management of Parkinson’s Disease”, 13<sup>th</sup> International Congress on Parkinson’s Disease and Movement Disorders, Paris, France. June 5-11, 2009.

Sampaio, C. Chairpersons: Aisen P, Sampaio C, Vellas B. 2nd Conference: clinical trials on Alzheimer’s disease, Task force in Early Clinical trial in AD, Las Vegas, Unites States. October 28-30, 2009.

Sampaio, C. “Metodologia da investigação clínica na doença de Alzheimer”. 2º Curso Médico-Cirúrgico em Neurociências, Porto. January 24-25, 2009.

Sampaio, C. “Biomarker in Clinical Trials” 9<sup>th</sup> International Conference on AD/PD 2009, Prague, Czech Republic. March 11-15, 2009.

Sampaio, C. Alzheimer Disease Strategy Expert meeting, Windsor, England. January 26, 2009.

Sampaio, C. “Alzheimer’s Disease Roundtable, Regulatory view”, European Medicines Agency, Washington DC, USA. April 1-2, 2009.

Sampaio, C. “Use of Biomarkers of Brain Disorders in Clinical Trials Intended for Drug Licensing”, Biomarkers in Brain Disease, Oxford, UK. January, 26-28, 2009.

Sampaio, C. “Biomarkers in Clinical Drug development”, British Neuroscience Association Annual Meeting, Liverpool, UK. April 19-22, 2009.

Sampaio, C. “Drug Development: questions and answers”, Asent 11<sup>th</sup> Annual Meeting, Arlington, USA. March 5-7, 2009.

Sampaio, C. “Enlightening the dark sides of Parkinson’s disease; Is neuroprotection a possible approach for new drugs in Parkinson’s disease?”, Paris, France. June 8, 2009.

Sampaio, C. Chairperson: “European Ropinirole Release Advisory Board”, London, UK. January 8-9, 2009

Sampaio, C. “Future of Neurology Think Tank on Parkinson’s Disease”, Divonne les Bains, France. May 17-18, 2009.

Sampaio, C. “Impact –XII, Research methods and outcomes for human experimental and clinical studies involving abuse liability of analgesic medications”, Maryland, USA. October 1-2, 2009.

Sampaio, C. “International conference on Alzheimer’s Disease, Per-Protocole Analysis Versus

ITT in Alzheimer's Preventive Trials", Vienna, Austria. July 11-16, 2009.

Sampaio, C. "Limpe Seminar, Old and New Dopamine Agonists in Parkinson's Disease: a Reappraisal, Dopamine Agonists in Clinical Development", Pisa, Italy. February 26 -28, 2009.

Sampaio, C. Chairperson: "Preladenant Advisory Board Meeting", Rome, Italy. April 5, 2009.

Sampaio, C. "Regulatory issues in analgesia development", Symposium on Pain Research, Cambridge, UK. September 3-4, 2009.

Sampaio, C. "What is expected from agencies what we have learned from recent trials? What is expected?", Task Force on Biomarkers in Alzheimer's Trials. January, 29- 30, 2009

Sampaio, C. Workshop on "Clinical trials on hypnotics and antidepressant drugs", Rome, Italy. May 8-9, 2009.

Sampaio, C. Redes de Investigação em Portugal: PTECRIN – o futuro, XXX Congresso Português de Cardiologia, Vilamoura. April 19-22, 2009.

Sampaio, C. Chairperson: Cell Therapy roundtable, XXII Conference of the Spanish Society for clinical pharmacology and XXVII Portuguese meeting of clinical pharmacology, Spain, Badajoz. October 13-16, 2009.

Verdelho, A. As alterações da substância branca cerebral: o que sabemos depois do estudo LADIS, Forum Bial, Lisbon. November 2009.

Verdelho, A. Delirium numa Unidade de Demências, Forum Futuro, Lisbon. April 2009.

### **Organisation of National and International Conferences**

Canhão, P. Member of the Cientific Committee of "Congresso da Sociedade Portuguesa do AVC", Porto. 2009.

Ferro, J.M. Member of the Programm Committee of the European Stroke Conference, Stockholm, Sweden. May 26-29, 2009.

Castro Caldas, A. "Sign Languages Around the World", Universidade Católica Portuguesa, Lisbon. September 8-10, 2009.

Castro Caldas, A. 8º Simpósio da Fundação BIAL "Aquém e Além do Cérebro", Porto. April 7–10, 2010.

Castro Caldas, A. "Sign Languages in UCP", Universidade Católica Portuguesa, Lisbon. January 29, 2009

Ferreira, J.J. I Teaching Course in Movement Disorders of the Neurological Clinical Research Unit, Instituto de Medicina Molecular, Lisbon. May 30, 2009.

Ferro, J.M. Chairman. Neurology experts' meeting on stroke and polyvascular disease, Lisbon. April 17-19, 2009.

Costa, J. International congress - G-I-N – Guidelines International Network, Lisbon. November 1-4, 2009

Martins, I.P. Organizer of Congress of Neurological Portuguese Society, Lisboa. November 05-07, 2009.

Martins, I.P. Organizer Fórum of Neurological Portuguese Society, Algarve. May 14-16, 2009.

Peralta, R. Co-organized the "Síndrome de Apneia Obstrutiva do Sono" – Curso de Pós-Graduação, Funchal. October 8-10, 2009.

### Prizes, Honours and Awards

Thomé Villar 2009 award to M. Borges, M. Gouveia, J. Costa, L. Pinheiro, S. Batista, A. Vaz-Carneiro. Custos e Carga da Doença atribuída ao Tabagismo em Portugal – granted by Sociedade Portuguesa de Pneumologia –.

Corino Andrade Prize from Sociedade Portuguesa de Neurologia 2009 award to M. Lauterbach, I.P. Martins, M. Carvalho, B. Townes, P. Slade, T. DeRouen, A. Castro-Caldas. Neurobases of Neurological Softsigns: a correlation study.

Corino Andrade Prize from Sociedade Portuguesa de Neurologia 2009 award to D. Silva, I. Santana, F.S. Couto, J. Maroco, M. Guerreiro, A. de Mendonça. Cognitive deficits in middle-aged and older patients with bipolar disorder: Comparison with mild cognitive impairment.

Distinguished Career Award of the International Neuropsychology Society award to A. Castro-Caldas.

Verdades de Faria Award 2009 of Santa Casa da Misericórdia de Lisboa award to A. Castro-Caldas.

António Flores Poster Prize (2009) award to A.C. Fonseca and P. Canhão for the presentation of “Classificação de défices neurológicos transitórios numa consulta de Acidentes isquémicos transitórios” at Congresso de Neurologia de 2009, Lisboa, Portugal.

Prof. Doutor Aurélio Quintanilha award to Tiago Krug from the Sociedade Portuguesa de Genética, 2009.

### Advanced Teaching

Anes, A.M. “Avaliação Benefício-Risco, Mecanismos e Factores de risco”, Estudos farmacoepidemiológicos, Curso Intensivo de Farmacovigilância, Lisbon. January 16-17, 2009.

Anes, A.M. “Farmacovigilância em Ensaios Clínicos”, X Masters in Neurosciences, Faculdade de Medicina de Lisboa, Lisbon. June 23, 2009.

Anes, A.M. “Métodos de Monitorização de Segurança de Medicamentos”, Mestrado em Regulamentação Farmacêutica, Faculdade de Farmácia da Universidade de Lisboa, Lisbon. April 22, 2009.

Anes, A.M. “Notificação espontânea de Reacções Adversas a Medicamentos”, Mestrado em Regulamentação Farmacêutica, Faculdade de Farmácia da Universidade de Lisboa, Lisbon. April 29, 2009.

Anes, A.M. “Notificação espontânea de Reacções Adversas a Medicamentos”. Acção de Sensibilização em Farmacovigilância. Centro de Saúde de Loures - Ext. Sto. António dos Cavaleiros, Lisbon. May 26, 2009.

Anes, A.M. “Notificação espontânea de Reacções Adversas a Medicamentos”, Acção de Sensibilização em Farmacovigilância, Farmácia da Pontinha, Lisbon. March 18, 2009.

Anes, A.M. “Notificação espontânea de Reacções Adversas a Medicamentos”, Acção de Sensibilização em Farmacovigilância, Farmácia Leitão Ribeiro, Lisbon. March 5, 2009.

Anes, A.M. “Notificação espontânea de Reacções Adversas a Medicamentos”, Mestrado em Cuidados Farmacêuticos, Universidade Lusófona de Humanidades e Tecnologia, Lisbon. January 16, 2009.

Anes, A.M. “Notificação espontânea de Reacções Adversas a Medicamentos”. Sessão prática, Mestrado em Cuidados Farmacêuticos, Universidade Lusófona de Humanidades e Tecnologia. Lisbon, January 17, 2009.

Bentes, C. and Paiva, T. Development of “Farmacologia do Sono” Module, Mestrado do Sono e Learning programme, Faculdade de Medicina da Universidade de Lisboa, Lisboa. 2009.

Bentes, C. “Dreams in Epilepsy”, Dreams Module, IV Mestrado de Ciências do Sono IV,



Faculdade de Medicina da Universidade de Lisboa, Lisboa. November 20, 2009.

Bentes, C. “Epilepsia temporal não lesional”, Sessão Clínica, Serviço de Neurologia, Hospital de Santa Maria, Lisboa. November 26, 2009.

Bentes, C. “MSLT and MWT”, PSG Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. October 13, 2009.

Bentes, C. “Other parasomnias”, Sleep Disorders and Sleep Regulation Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. April 17, 2009.

Bentes, C. “Sleep in Neurology”, Sleep Functions Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. October 17, 2009.

Bentes, C. “Sleep staging and reporting”, PSG Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. October 12, 2009.

Bentes, C. “Test of the Master Degree”, Module of new features of AASM classification, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa, February 27, 2009.

Bentes, C. Lecturer Ciclo de Estudos Especiais de Neurofisiologia Clínica, Hospital de Santa Maria. 2007/2011

Bentes, C. “MSLT and MWT”, Sleep Laboratory Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. June 26, 2009.

Caeiro, L. “Descrição de um caso neuropsicológico”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisboa. May 13, 2009.

Caeiro, L. “Neurobehavioral changes in acute stroke patients”, 13th ESO Stroke Summer School, Lille, France. September 3, 2009.

Canhão, P. “Estudos de coorte. Factores de risco e factores de prognóstico”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisboa. May 19, 2009

Canhão, P. “Testes de Diagnóstico”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa. June 2, 2009.

Castro Caldas, A. “Memória”, Curso de Pós-Graduação em Neurociências, Cofanor, Porto. 2009.

Castro Caldas, A. “Viagem ao Cérebro”, Curso de Verão, Universidade Católica Portuguesa, Porto. July 17, 2009.

Castro Caldas, A. Formação Avançada em Geriatria, Santa Casa da Misericórdia de Montemor-o-Novo. 2009.

Castro Caldas, A. Lecturer Master Course in Neuroscience, Faculdade de Medicina da Universidade de Lisboa, Lisboa. 2009.

Castro Caldas, A. Lecturer in Master course in Rehabilitation and Master course in Speech Therapy of Escola Superior de Saúde do Alcoitão (ESSA).

Castro Caldas, A. Workshop de Neurodesenvolvimento, Faculdade de Medicina do Porto, Porto. 2009.

Costa, J. “Investigação Clínica”, Unidade de Saúde Familiar da Amora, September 18, 2009.

Costa, J. “Medicina Baseada na Evidência”, V Congresso Nacional do Médico Interno, Ordem dos Médicos, Lisbon. February 19 – 21, 2009.

Costa, J. “Revisões sistemáticas”. X Masters in Neurosciences, Faculdade de Medicina de Lisboa. Lisbon, May 26, 2009.

Costa, J. Mestrado de Investigação Clínica, Forpoint e Universidade Pompeu Fabra de Barcelona, Barcelone, Spain. 2009.

Evangelista, T. “Nervo – morfologia e metabolismo”, Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 19, 2009.

Evangelista, T. “Músculo – morfologia e metabolismo”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 19, 2009.

Ferreira, J.J. “Apresentação de casos clínicos, com vídeo e televoto”, 2º curso médico-cirúrgico em neurociências, Porto. January 24-25, 2009.

Ferreira, L. “Mounting the PSG”, PSG Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa: October 15, 2009.

Ferro, J.M. “Anxiety and disorders of emotional control”, European Master in Stroke Medicine, Donau University, Krems, Austria. October 7, 2009.

Ferro, J.M. “As ferramentas básicas da investigação clínica em Neurociências”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisboa. May 5, 2009.

Ferro, J.M. “Cerebral vasculitis”, 13th ESO Stroke Summer School, Lille, France. September 1, 2009.

Ferro, J.M. “Consciência e conceito de pessoa: problemas éticos do estado vegetativo persistente”, Iº Curso Pós-Graduado de actualização de decisões éticas em fim de vida, Faculdade de Medicina da Universidade de Lisboa, Lisboa. March 19-21, 2009.

Ferro, J.M. “Delirium and related disorders”, European Master in Stroke Medicine, Donau University, Krems, Austria. October 7, 2009.

Ferro, J.M. “Estado vegetativo”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 13, 2009.

Ferro, J.M. “Estudos casos-controlo”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisboa. May 12, 2009.

Ferro, J.M. “Executive deficits”, European Master in Stroke Medicine, Donau University, Krems, Austria. October 7, 2009.

Ferro, J.M. “Memory disorders”, European Master in Stroke Medicine, Donau University, Krems, Austria. October 7, 2009.

Ferro, J.M. “Mood disorders”, European Master in Stroke Medicine, Donau University, Krems, Austria. October 7, 2009.

Ferro, J.M. “Visual agnosias”, European Master in Stroke Medicine, Donau University, Krems, Austria. October 7, 2009.

Martins, I.P. “Aphasia”, European Master in Stroke Medicine, Donau University, Krems, Austria. October, 2009.

Martins, I.P. “Memory Disorders”, European Master in Stroke Medicine, Donau University, Krems, Austria. October, 2009.

Martins, I.P. “Neglect”, European Master in Stroke Medicine, Donau University, Krems, Austria. October, 2009.

Martins, I.P. “Reading and writing disorders”, European Master in Stroke Medicine, Donau University, Krems, Austria. October, 2009.

Oliveira, S.A. “Epidemiologia genética”, X Masters in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Lisbon. May 7, 2009.

Oliveira, S.A. Mestrado em Doenças Metabólicas, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 17 and 30, 2009.

Peralta, R. “Dreams in sensory deprivation”, “Dreams” Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. November 20, 2009.

Peralta, R. “Insomnia-Clinical Presentation”, Sleep Disorders and Sleep Regulation Modules, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. April 17, 2009.

Peralta, R. “Pathophysiology of insomnia” Sleep Disorders and Sleep Regulation Modules, IV

- Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. April 18, 2009.
- Peralta, R. “Quantitative Analysis of Dreams”, Dreams Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. November 20, 2009.
- Peralta, R. “Scoring Dreams”, Sleep Laboratory, Sleep, Age and Gender Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. June 26, 2009.
- Peralta, R. “Sleep and Gender”, Sleep, Age and Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. October 30, 2009.
- Pereira, P. “Biópsias de pele. Terminações nervosas intraepidérmicas”, Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 25, 2009.
- Pimentel, J. “Epilepsia do lobo temporal”, Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 18, 2009.
- Pimentel, J. “Tumores cerebrais - gliomas”, Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 17, 2009.
- Pimentel, J. “Tumores gliais e mixtos”, Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 18, 2009.
- Pires, J. “Mounting the PSG”, PSG Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. October 15, 2009.
- Rosa, M.M. “Use of Humans in Biomedical Experimentation”, Scientific Integrity and Ethics Module, Programa de Formação Médica Avançada, Faculdade de Medicina da Universidade de Lisboa and IMM, Lisboa. February 24, 2009.
- Sampaio, C. “Entrada do Medicamento no Mercado – Eficácia e Segurança”, IV Curso Pós-Graduado de Atualização em Ensaios Clínicos, Instituto de Medicina Preventiva, Faculdade de Medicina da Universidade de Lisboa, Lisboa. February 12-16, 2009.
- Sampaio, C. “Fases no Estudo de um Novo Medicamento”, IV Curso Pós-Graduado de Atualização em Ensaios Clínicos, Instituto de Medicina Preventiva, Faculdade de Medicina da Universidade de Lisboa, February 12-16, 2009
- Santos, R. “Mounting the PSG”, PSG Module, IV Mestrado de Ciências do Sono, Faculdade de Medicina da Universidade de Lisboa, Lisboa. October 15, 2009.
- Silvestre, A.R. “Obesidade e alterações musculares”, Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa, Lisboa. January 25, 2009.
- Verdelho, A. “Depression after stroke”, 13th ESO Stroke Summer School, Lille, France. September 4, 2009.
- Verdelho, A. “Sintomas depressivos no idoso, quando a depressão não é só depressão”, Mestrado de Gerontopsiquiatria, Universidade Nova de Lisboa, Lisbon. February 2009.
- Members of the Behavioural Neurology Group are involved in pre graduate and pos graduate teaching (Master in Neurosciences and Doctoral programme Voice, Language and Communication).
- Cognitive Neuroscience Group organizers and lectures in Master courses from Instituto Ciências da Saúde, Universidade Católica de Portugal: Medical Education, Nursing, Paliative Care and Portuguese Sign Language and Education of the deafs.
- Neuropharmacology Group Lecturers in Clinical Pharmacology and Therapeutics, Farmacocinética e Nutrição, Neurology, Evidence Based Medicine, Faculdade de Medicina da Universidade de Lisboa.
- Clinical Neuropharmacology Group Lecturers in Neuroanatomy, Neurophysiology and Physiophatology, Escola Superior de Alcoitão.
- Clinical Neuropharmacology Group Lecturers in Ensaios Clínicos, Faculdade de Farmácia da Universidade de Lisboa.

Clinical Neuropharmacology Group Lecturers in Neurofisiologia clínica, Instituto Superior Técnico, Universidade Técnica de Lisboa, Lisboa.

### Public Perception of Science

Ferreira, J.J. “Terapêutica da Doença de Parkinson”, Curso de Cuidadores, Associação Portuguesa de Doentes de Parkinson, Lisbon. May 27, 2009.

Martins, I.P. Co-Organizer of a Round table at Pavilhão do Conhecimento on Brain Awareness week. European Brain Council, a joint initiative of the Portuguese Societies of Neuroscience and Neurology. March 2009.

Oliveira, V. “Memória e criação musical. A música e a ciência”, Évora. November, 2009

Peralta, R. “Apneia do sono” – Programa: Consultório – TVI, Outubro 2009.

Peralta, R. “Apneia do sono” – Programa: Jornal da Manhã – TVI, Outubro 2009.

Rebelo Pinto, H. and Peralta, R. “Programa de terapêutica e ensino da insónia” – Programa: Mais cedo ou mais tarde – TSF, Março 2009.

Sampaio, C. “Gripe A” - Serviço de Saúde, RTP1. October 21, 2009.

### Thesis Completed

#### PhD Thesis

João Costa (2009) “Effects of subcortical activation of deep brain pathways and recording from deep brain structures through the electrodes implanted for deep brain stimulation in patients with Parkinson's disease”. Supervisors: C. Sampaio and M. de Carvalho

Joaquim Ferreira (2009) “Anti-Parkinsonian drugs and sleep disorders in Parkinson's disease”, Faculdade de Medicina da Universidade de Lisboa. Supervisors: C. Sampaio and O. Rascol

Maria Vânia Rocha da Silva Nunes (2009) “Estudo das alterações do sistema mnésico com a idade. Uma abordagem integrada no âmbito das neurociências do envelhecimento”, Faculdade de Medicina da Universidade de Lisboa. Supervisor: A. Castro Caldas

Miguel José de Carvalho Viana Baptista (2009) “Alterações da substância branca cerebral relacionadas com o envelhecimento”, Faculdade de Ciências Médicas da Universidade Nova de Lisboa. Supervisor: J.M. Ferro

Patrícia Martins Canhão Rodrigues (2009) “Doença trombótica das veias e seios venosos cerebrais”, Faculdade de Medicina da Universidade de Lisboa. Supervisor: José M. Ferro

#### Master Thesis

Ana Catarina Gaspar Fonseca (2009) Peptídeo natriurético cerebral como possível marcador de acidente isquémico de etiologia cardioembólica. Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa. Supervisor: J.M. Ferro

Ana Rita Teixeira Silvestre (2009) Alterações histológicas e moleculares no músculo esquelético de indivíduos com obesidade mórbida e sua relação com a resistência à insulina. Mestrado em Biologia Molecular Humana, Faculdade de Ciências da Universidade de Lisboa. Supervisor: H. Cortez Pinto, Co-Supervisor: T. Evangelista.

Carina Pinto (2009) Nomeação e evocação de nomes e verbos - Estudo na população Portuguesa. Mestrado em Neurociências. Faculdade de Medicina da Universidade de Lisboa. Supervisor: I.P. Martins

Carolina Maruta (2009) Avaliação da memória em portugueses com idade superior a 50 anos: validação e dados normativos para CVLT - 9 e para os subtestes reprodução visual e faces da WMS - III. Mestrado Integrado em Psicologia, Instituto Superior de Psicologia Aplicada. Supervisor: I.P. Martins.

Indira Andrade (2009) fMRI Sparse Design for Auditive paradigms: Toward a Signal and Stimulus Perception Improvements. Mestrado Integrado em Engenharia Electrotécnica e de Computadores. Supervisor: M. Lauterbach.

Inês Tello Rodrigues (2009) Fluência Verbal na doença de Parkinson. Universidade Católica Portuguesa. Supervisor: A. Castro Caldas

Joana Morgado (2009) MMSE: Novos Valores Normativos. Mestrado Integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisor: I.P. Martins.

Joana Pires (2009) Análise do conteúdo onírico dos sonhos em surdos congénitos e correlação com as bandas espectrais do EEG. Mestrado em Medicina do Sono, Faculdade de Medicina da Universidade de Lisboa. Supervisor: T. Paiva

Leonor Correia Guedes (2009) Diários electrónicos para avaliação de Flutuações Motoras e Discinésias na Doença de Parkinson. Mestrado em Neurociências, Faculdade de Medicina da Universidade de Lisboa. Supervisor: C. Sampaio.

Maria de Fátima Horta Soares (2009) Avaliação da doença venosa dos membros inferiores em pilotos de aviação comercial. Mestrado em Engenharia da Saúde, Faculdade de Engenharia da Universidade Católica Portuguesa. Supervisor: V. Oliveira

Mauricio Martins (2009) Sensitivity to Expressions of Pain in Schizophrenia patients: A comparison with emotion recognition in static expressions. Mestrado Integrado em Medicina, Faculdade de Medicina da Universidade de Lisboa. Supervisor: I.P. Martins.

Miguel Rebocho (2009) Aprendizagem baseada em problemas no desenvolvimento profissional contínuo médico: Uma experiência. Universidade Católica Portuguesa. Supervisor: A. Castro Caldas

Rui Manuel Fernandes (2009) Identificação e Caracterização de potenciais E-Learners Um Contributo para a Aplicação do E-Learning à Educação Médica Contínua. Universidade Católica Portuguesa. Supervisor: A. Castro Caldas

Tiago Pereira (2009) Consolidação da Memória Motora em Atletas e Sedentários. Faculdade de Medicina da Universidade de Lisboa. Supervisor: A. Castro Caldas

### **Diploma Thesis**

Alexandre Sargaço (2009) Validação de Provas de Decisão Sintáctica. Licenciatura Bietápica em Terapia da Fala. Escola Superior de Medicina e Reabilitação do Alcoitão. Supervisor: M. Lauterbach.

Madalena Graça (2009) Teste de decisão semantica em frases: aferição dos valores normativos para a população portuguesa. Licenciatura Bietápica em Terapia da Fala. Escola Superior de Medicina e Reabilitação do Alcoitão. Supervisor: M. Lauterbach.

## Cell and Molecular Neuroscience Unit

### Description and Objectives

UNCM (Unidade de Neurociência Celular e Molecular) was started in June 2007, with the goal of understanding the molecular basis of disorders intimately associated with protein misfolding and aggregation, mostly those which affect the brain, such as Parkinson's, Huntington's, or Alzheimer's disease. Because the molecular pathways involved in protein homeostasis are highly conserved, we employ a wide variety of model organisms, from the simple but powerful budding yeast to mammalian cell culture and mice.

Our ultimate goal is to develop novel therapeutic approaches for these and other related disorders. We are working closely together with clinicians in order to accelerate drug discovery efforts, translating basic research into clinical applications that will improve the lives of patients.

### Head of Unit

Tiago Fleming Outeiro, Ph.D., Auxiliar Professor, FMUL

### Principal Investigators

Teresa Pais, Ph.D., Staff Scientist

### Research Team

Sandra Tenreiro	- Postdoc fellowship
Rita Oliveira	- Postdoc fellowship
Joana Matos	- Postdoc fellowship
Mónica Cunha	- Postdoc fellowship
Leonor Fleming	- PhD Student, FCT
Hugo Miranda	- PhD Student, FCT
Susana Gonçalves	- PhD Student, AXA Research Fund
Oldriska Marques	- PhD Student, FCT
Olga Sin	- Master student
Sónia Oliveira	- Technician
Ana Oliveira	- Technician
Patrícia Guerreiro	- Technician

### Research Areas

- Protein misfolding, oligomerization and aggregation
- Yeast models for Parkinson's and Huntington's disease
- Modulators of protein aggregation and toxicity
- Microscopy-based methods for investigating the molecular nature of the toxic proteinaceous species in Parkinson's disease
- In vivo imaging using multi-photon microscopy

### Recent Most Relevant Publications

Outeiro, T.F. and Ferreira, J. (2009) Current and future therapeutic strategies for Parkinson's disease. *Curr. Pharm. Des.* 15: 3968-3976 (Times cited: 1)(Journal IF: 4.399)

Outeiro, T.F., Klucken, J., Bercury, K., Tetzlaff, J., Putcha, P., Oliveira, L.M., Quintas, A., McLean, P.J. and Hyman, B.T. (2009) Dopamine-induced conformational changes in alpha-

synuclein. PLoS One.4:e6906 (Journal IF: NA)

Mollenhauer, B., Cullen, V., Kahn, I., Krastins, B., Outeiro, T.F., Pepivani, I., Ng, J., Schulz-Schaeffer, W., Kretschmar, H.A., McLean, P.J., Trenkwalder, C., Sarracino, D.A., Vonsattel, J.P., Locascio, J.J., El-Agnaf, O.M. and Schlossmacher, M.G. (2008) Direct quantification of CSF alpha-synuclein by ELISA and first cross-sectional study in patients with neurodegeneration. *Exp. Neurol.* 213: 315-25 (Times cited: 7)(Journal IF: 3,974)

Outeiro, T.F., Kontopoulos, E., Altmann, S.M., Kufareva, I., Strathearn, K.E., Amore, A.M., Volk, C.B., Maxwell, M.M., Rochet, J.C., McLean, P.J., Young, A.B., Abagyan, R., Feany, M.B., Hyman, B.T. and Kazantsev, A.G. (2007) Sirtuin 2 inhibitors rescue alpha-synuclein-mediated toxicity in models of Parkinson's disease. *Science* 317: 516-519 (Times cited: 102)(Journal IF: 28.103)

Bodner, R.A., Outeiro, T.F., Altmann, S., Maxwell, M.M., Cho, S.H., Hyman, B.T., McLean, P.J., Young, A.B., Housman, D.E. and Kazantsev, A.G. (2006) Pharmacological promotion of inclusion formation: a therapeutic approach for Huntington's and Parkinson's diseases. *Proc. Natl. Acad. Sci. USA* 103: 4246-4251 (Journal IF: 9.380)

Outeiro, T.F. and Lindquist, S. (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science* 302: 1772-1775 (Times cited: 149)(Journal IF: 28.103)

### Ongoing Research Projects

2007/2009 SirT2 as a Drug Target in Parkinson's Disease, Michael J. Fox Foundation, USA. Coordinators: T.F. Outeiro and A. Kazantsev

2007/2009 Unraveling the genetic basis for alpha-synuclein oligomerization in living cells using bimolecular fluorescence complementation, Michael J. Fox Foundation, USA. Coordinator: T.F. Outeiro.

2009/2011 Deciphering the Molecular Role of DJ-1 in Parkinson's disease, UK Parkinson's Disease Society. Coordinators: T.F. Outeiro and F. Giorgini

2007/2011 NEUROFOLD, Uncovering the Molecular Basis of Neurodegenerative Disorders, European Commission. Coordinator: T.F. Outeiro.(MIRG-CT-2007-203312)

2009/2011 Slow and Rapid Progression Parkinson's Disease: Molecular Determinants and Biomarkers, FCT. Coordinator: T.F. Outeiro (PTDC/SAU-GMG/099655/2008)

### Publications in Peer Review International Journals 2009

da Costa, G., Gomes, R., Correia, C.F., Freire, A., Monteiro, E., Martins, A., Barroso, E., Coelho, A.V., Outeiro, T.F., Ponces Freire, A., Cordeiro, C. (2009) Identification and quantitative analysis of human transthyretin variants in human serum by Fourier transform ion-cyclotron resonance mass spectrometry. *Amyloid*. 16: 201-207 (Journal IF: 1,714)

Outeiro, T.F. and Ferreira, J. (2009) Current and Future Therapeutic Strategies for Parkinson's Disease. *Curr. Pharm. Des.* 15: 3968-3976 (Journal IF: 4.399)

Outeiro, T.F., Klucken, J., Bercury, K., Tetzlaff, J., Putcha, P., Oliveira, L.M., Quintas, A., McLean, P.J. and Hyman, B.T. (2009) Dopamine-induced conformational changes in alpha-synuclein. *PLoS One* 4: e6906 (Journal IF: NA)

Tenreiro, S., Sin, O., Miranda, H.V. and Outeiro, T.F. (2009) Yeast as a model to investigate the role and regulation of alpha-synuclein phosphorylation in Parkinson's disease. 24th International Conference on Yeast Genetics and Molecular Biology, Manchester, UK, July 19-24. *Yeast* 26: 189 (Journal IF: 2,622)

### **Books/Book Chapters**

Gitler, A. and Outeiro, T.F. (2009) Unravelling the Molecular Basis of Parkinson's Disease Using Yeast Models.

### **Communications in International Conferences**

Machado de Oliveira, R. et al. Aging and neurodegeneration: common targets, common hopes. WFN Congress in Parkinson's disease, Miami, USA. December 2009 (Poster presentation)

Outeiro, T.F. Genetic modifiers of  $\alpha$ -synuclein oligomerization. International Symposium on Synucleinopathies, Beijing, China. August 29, 2009.

Outeiro, T.F. Yeast models of neurodegeneration. 27<sup>th</sup> Issy Pasteur's Legacy – Yeasts for Health and Biotechnology, Institut Pasteur, Paris, France. August 27, 2009.

Outeiro, T.F. Modifying  $\alpha$ -synuclein aggregation in living cells. Benzon Symposium, Denmark. August 24-27, 2009.

Outeiro, T.F. The molecular basis of neurodegenerative disorders. EMBO YIP Meeting, Istanbul, Turkey. May 2009.

Outeiro, T.F. The molecular basis of neurodegenerative disorders, Ankara, Turkey. May 2009.

Outeiro, T.F., Sirtuins in Aging and Neurodegeneration: Eisai Symposium, UCL, London. April 22, 2009.

Outeiro, T.F. Modifying  $\alpha$ -synuclein aggregation in living cells. ESN, Leipzig. August 2009.

Outeiro, T.F. Unravelling the molecular basis of neurodegeneration: from cells to drugs. Endo-Neuro-Psycho meeting, Amsterdam, The Netherlands. June 4-6, 2009.

Outeiro, T.F. Sirtuins in aging and neurodegeneration. AD/PD Meeting, Prague. March 11-15, 2009.

Gonçalves, S.A. et al. Genetic modifiers of  $\alpha$ -synuclein oligomerization in living cells, Annual meeting of the Society for Neurosciences, Chicago, USA. October 17-21, 2009 (Poster presentation)

Pais, T.F. et al. Sirtuins: a link between ageing and microglial cell neurotoxicity. Annual meeting of the Society for Neurosciences, Chicago, USA, October 17-21, 2009 (Poster presentation)

Tenreiro, S. et al. Yeast as a model to investigate the role and regulation of  $\alpha$ -synuclein phosphorylation in Parkinson's disease. 24<sup>th</sup> International Conference on Yeast Genetics and Molecular Biology, Manchester, UK. July 19-24, 2009 (Poster presentation)

Gonçalves, S.A. et al. Dangerous attractions: modifying  $\alpha$ -synuclein dimerization in living cells. AXA Talent Day on Longevity and Long-Term Care, Paris, France. October 6-7, 2009 (Poster presentation)

### **Communications in National Conferences**

Gonçalves, S.A. et al. Dangerous attractions: modifying  $\alpha$ -synuclein dimerization in living cells. 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon, Portugal. October 12-13, 2009 (Poster presentation)

Gonçalves S.A. and Outeiro T.F. Novel insights into  $\alpha$  synuclein intracellular dynamics, 11<sup>th</sup> Portuguese Meeting for Neurosciences, School of Health Sciences, Universidade do Minho, Braga. June 3-6, 2009 (Poster presentation)

Marques, O. et al. The role of  $\alpha$ -synuclein secretion in microglia activation and neurodegeneration in Parkinson's disease. 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon. October 12-13, 2009 (Poster presentation)



Vicente Miranda, H. et al. Protein Glycation and Methylglyoxal Metabolism in Parkinson's Disease. 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon. October 12-13, 2009 (Oral presentation)

Sin, O. et al. Unraveling the Role of Alpha-Synuclein Phosphorylation in the Etiology of Parkinson's Disease. 11th Meeting of the Portuguese Society for Neurosciences, Braga. June 4-6, 2009 (Poster presentation)

Miller-Fleming, L. et al. Molecular Genetic Characterisation of Kynurenine 3- Monooxygenase, a Candidate Therapeutic Target for Huntington. 11th Meeting of the Portuguese Society for Neurosciences, Braga. June 4-6, 2009 (Poster presentation)

Miller-Fleming, L. et al. Deciphering the role of DJ-1 in the etiology of Parkinson's disease using yeast as a model. 3<sup>rd</sup> IMM PhD Students Meeting, Lisbon. October 12-13, 2009 (Poster presentation)

### **Invited Lectures and Seminars**

Outeiro, T.F. From molecules to cures: the molecular basis of Parkinson's disease, Bayreuth University, Germany. December 2009.

Outeiro, T.F. From molecules to cures: the molecular basis of Parkinson's disease, Centro Nacional de Biotecnologia, Madrid, Spain. December 2009.

Outeiro, T.F. From molecules to cures: the molecular basis of Parkinson's disease, Aachen University, Germany. November 2009.

Outeiro, T.F. Signaling pathways in Parkinson's disease. SINAL 2009, Universidade de Aveiro. May 2009.

Outeiro, T.F. Protein misfolding in biology and disease, Faculdade de Farmácia da Universidade de Lisboa. April 3, 2009.

Outeiro, T.F. From molecules to cures: the molecular basis of Parkinson's disease, Centro de Neurociências, Universidade de Coimbra. Abril 2009.

Outeiro, T.F. Parkinson's disease and alpha-synuclein, ITQB. March 19, 2009.

Outeiro, T.F. Unravelling the molecular basis of neurodegeneration, University of Leuven, Belgium. March 2, 2009.

Outeiro, T.F. Porque morrem os neurónios? APDPk, Madeira. March 26, 2009.

Outeiro, T.F. Neurodegeneration in Alzheimer's disease, Pfizer Symposium, Lisbon. March 28, 2009.

### **Organisation of National and International Conferences**

Outeiro, T.F. Organizer of the Lisbon Area Neuroscience Meetings throughout the year of 2009. Lisbon, Portugal.

### **Prizes, Honours and Awards**

Young Investigator Travel Award to Tiago F. Outeiro, AD/PD Meeting, Prague, Czech Republic.

Young Investigator Travel Award to Tiago F. Outeiro, WFN Congress on Parkinson's disease, Miami, USA.

IMM PhD Program Oral Presentation prize 2009 to Hugo V. Miranda, Lisbon, Portugal.

### **Advanced Teaching**

Outeiro, T.F. "Protein Folding and Misfolding", GABBA PhD Program, IBMC, Porto. January

2009.

Outeiro, T.F. “Neurodegenerative disorders”, PFMA and IMM PhD Program, IMM, Lisbon. February 2009.

Outeiro, T.F. “Protein Misfolding in Biology and Disease”, CNC PhD Program, University of Coimbra, 2009.

Outeiro, T.F. “Protein misfolding in biology and disease”, PENS Summer School, Gunzburg, Germany, July 2009

Outeiro, T.F. “Protein Misfolding in Biology and Disease”, Master Course in Neurosciences, Faculdade de Medicina da Universidade de Lisboa, Portugal. December 2009.

Pais, T.F. “Neuron-glia interactions” Master Course in Molecular Genetics and Biomedicine. Universidade Nova de Lisboa, Caparica, Portugal. December 2009.

Pais, T. F. “A flow cytometry approach to study inflammation in the Central Nervous System” Applications of flow cytometry in biomedical research, Escola de Ciências da Saúde da Universidade do Minho, Braga, Portugal. June 15-17, 2009.

### **Public Perception of Science**

Outeiro, T.F, “Neurodegenerative disorders”, Brain Awareness Week, Lisbon. March 2009.

Outeiro, T.F. “Novos avanços na investigação na doença de Parkinson”, Associação Portuguesa de Doentes de Parkinson, Porto. September 2009.

Outeiro, T.F. “Porque morrem os neurónios?”, Alzheimer Portugal, Coimbra. November 2009.

Outeiro, TF “Há ciência em Portugal?”, School, Odivelas. December 2009.

### **Thesis Completed**

#### **Master Thesis**

Olga Sin (2009) The role of a-synuclein phosphorylation in Parkinson’s disease models, Universidade de Lisboa. Supervisor: T.F. Outeiro

## **FACILITIES AND SUPPORT**

Alongside with research, IMM has a core of resource units, which complement and maintain the high quality scientific environment. Among these are the Management, the Information Systems, Communication & Training and Lab Management. In addition to these transversal units, and in the spirit of shared resources, IMM offers recently equipped state-of-the-art Facilities: Flow Cytometry, Bioimaging, Animal House and Fish, as well as a Biosafety level 3 Laboratory and a Histology Service. All Resource Units have dedicated staff that provides support to IMM community and to develop initiatives that promote research.

Management	Margarida Pinto Gago, Manager
Communication and Training	Marta Agostinho, PhD
Information Systems	Tito Santos Silva, PhD
Lab Management	Alexandra Maralhas
Bioimaging	José Rino, PhD
Flow Cytometry	Maria Soares, PhD
Animal House	Domingos Henrique, PhD
Zebrafish Facility	Leonor Saúde
Histology Service	Sandra Casimiro



## Management Unit

### Description and Objectives

The Management Unit is responsible for the overall IMM legal, administrative and financial support. At IMM, funding for research projects comes from many different sources – national and international, public and private – requiring the skills of experienced administrative staff. We manage Human Resources, undertaking all tasks necessary to hire new staff and terminate contracts (advertising, selection, contracts preparation, all legally required registrations). Also, the unit is responsible for Accounts and budget management – performing all the required activities to achieve the IMM legal accounts as well as the cost accounting per project/activity and budget control; and, Project Management, which deals with all administrative and financial aspects of research projects, from the moment of funding approval to the date of final report.

Moreover the Management Unit is responsible for tax reporting, a considerable task, given the specifications of the legal status of IMM combined with the number and variety of activities and collaboration protocols contracted. In addition, within our unit we have a Lawyer who provides legal support to researchers, namely intellectual property issues and consortium agreements

### Head of Unit

Margarida Pinto Gago, Management Degree, Instituto Superior de Economia e Gestão da Universidade Técnica de Lisboa

### Team

André Fialho	- Lawyer
Cláudia Soeiro	- Front desk
Sandra Duarte	- Accounts (since May)
Isabel Roque;	- Accounts
Inês Bernardes;	- Accounts
Filipa Proença	- Accounts (left in October)
Vera Rego	- Accounts
Andreia Vaz	- Project Management
Maria José Antunes	- Project Management

### Service Areas

Human Resources;  
Accounts and Budget;  
Project Management;  
Legal and Tax

### Major Achievements

Effective response to a continually increasing number of scientific collaboration protocols and HR contracts, namely related to new European Commission projects, under the FP7. This work is performed mostly, by Dr. André Fialho, as legal support for IMM.

Improvements were made to the finance and management software. New features will enable greater interaction between different areas and give better access to information by different researchers/administrative support. Also a new module was developed to facilitate the preparation of periodic project reporting.

Staff functions and procedures were reviewed, aiming to give all research and support units a

**254 | 2009 IMM Activity Report**

more effective, efficient and quicker response to daily needs, within the legal, fiscal and financing entities requirements.

Organization and participation in periodic meetings with IMM Board and the other support units for a better common and coordinated response to IMM daily issues.

## Communication and Training Unit

### Description and Objectives

The Communication & Training Unit was created in 2008 to develop a coordinated framework for Science Communication, Advanced Training and Science Funding.

The Unit has dedicated staff to address Science in Society activities, in collaboration with national and international partners. A science communication programme has been developed, which builds on researchers' initiatives as well as projects aiming at developing two-way interaction between scientists and a wide range of audiences. Activities include workshops, hands-on activities for younger generations, meetings involving scientists and different publics, the development of educational resources and communication training activities for scientists. Schools and the media are among privileged partners/audiences.

At UCOM we are responsible for the overview and coordination of Advanced Training initiatives. Moreover, we provide the link with the "Centro Académico de Medicina de Lisboa" and foster collaborations amongst national and international education and/or research institutions. The core of our educational programmes is the IMM PhD Programme, for which we oversee and guarantee the progress of all its activities, namely: course organization; students' events; promotion of the programme; and record management.

We provide assistance and advice on funding and grant management issues. IMM researchers can find support in applying for national and international funding, from provision of funding information to application and contract conclusion.

In addition to these three core areas, the Unit also serves a range of transversal areas including External Relations, Internal Communication, IMM Events, and Support to the Direction.

### Head of Unit

Marta Agostinho, PhD, University of Lisbon, Staff Scientist

### Team

Margarida Trindade, PhD	- Science Funding Coordinator, Staff Scientist
Inês Crisóstomo, PhD	- Advanced Training Coordinator, Staff Scientist (since July)
Joana Costa, PhD	- Science and Management Post-Doc fellowship, FCT
Cheila Almeida, MsC	- Communication assistant, IMM
Ana Catarina Rebelo, MsC	- Training fellowship, IEFP

### Research/Service Areas

#### Communication

- Promote IMM corporate image and provide public relations support to IMM bodies
- Place IMM and Portuguese Science in the media agenda
- Promote two-way dialogue between researchers and a wide range of audiences, and establish partnerships with key players (schools, national agencies, etc.)
- Promote inquiry-based literacy and positive images of science and scientists
- Ensure efficient dissemination of events and other relevant issues
- Help strengthening bridges between IMM and special partners
- Build on team building and internal communication

#### Advanced Training

- Oversee and coordinate the IMM PhD Programme and ensure its proper functioning
- Establish and Link with partner research and education institutions and programmes
- Promote advanced training initiatives at the IMM

#### Science Funding

Foster an environment for additional Science Funding at IMM through the provision of updated and timely information/advice on research funding opportunities, and support researchers on national and international grant application and contract negotiation procedures.

#### Transversal activities

- Support IMM Direction and researchers
- Support to events organized by IMM researchers

### Major Achievements

#### Communication

In 2009, the new IMM website was developed and launched, institutional materials were produced, such as the IMM Reports, and regular collaborations were established (e.g., participation at FMUL Newsletter). The internal and external dissemination of IMM events was reorganized and an internal weekly newsletter was launched. Collaboration with the media was strengthened, including the production and delivery of 9 press releases, which resulted in a constant presence of IMM research and researchers in the media. This presence is monitored by the maintenance of a press clipping, which showed an increase in IMM presence in the media as well as an increasing tendency for commentary/opinion making participations, when compared to 2008.

UCOM coordinated the awareness package of the EU project *Researchers Night 2009 - Setting the Stage*, participating also in the design, development and delivery of science-related theatre plays and outreach activities. This event reached more than 13.000 people across Portugal and constituted a truly collaborative science communication project among several research centres, universities and smes in Portugal.

The IMM School Visits Programme, created in 2008, was consolidated, assuring 16 school visits, in collaboration with several research units and facilities, reaching students from all levels of schooling, from 4 year-olds to undergraduate students (total students 617). Regular collaboration with the national agency *Ciência Viva* was assured.

#### Advanced training

In 2009 the PhD Programme was improved, not only did we increase our nanocourse offer (IMM organized 7 nanocourses) but also we set-up a booklet of rules and regulations. Also, the students commission, with our support, initiated a new activity – the students seminar series: “Onboard talks” (More details on the PhD Programme section).

In collaboration with the Faculdade de Medicina da Universidade de Lisboa and Hospital de Santa Maria we submitted an accreditation request for a joint PhD Programme.

During 2009 we diversified our advanced training initiatives by developing a course aimed at experienced researchers (Effective Grant Writing).

#### Science Funding

Science Funding support at IMM started in 2008 and became full operational in 2009. In a universe of approximately 350 researchers, we recorded 243 national and 29 international funding applications by IMM researchers. During 2009, we have provided the IMM community with funding information by the weekly delivery of a «Tip of the Week – funding opportunities» section for the IMM newsletter, the weekly update of a table of funding opportunities on IMM Intranet, the organization or hosting of specific information sessions (total: 6), and by directly contacting researchers. In parallel, this information was gathered from different sources, such as attendance to funding session (total: 7), email and Internet. General support with grant application processes was provided by means of on-line resources on IMM Intranet and email information. In addition, individual case-by-case support was provided, which included grant writing and reviewing in a minor number of cases. Finally, we have undertaken



contract negotiations for 9 European projects, of which 6 are now concluded. In the national scenario, 2009 was characterized by the opening of two FCT calls for projects (104 and 82 IMM applications, respectively) and the launching of the Portugal-Harvard Program. This has resulted in a substantial increase in national IMM applications, with a corresponding increase in pre and post-submission support.

#### Transversal activities

In addition to its core activities, UCOM has also undertaken a series of other activities as a response to institutional needs identified by the Directors or as direct requests from researchers. In this group we include: the production of activity/other reports (total: 3), the organization of visits from external delegations (total: 5) and its respective follow-up, the organization of events (total: 4), the organization of internal retreats, the coaching of fundraising opportunities (total: 1), the definition and implementation of internal communication procedures, internal events and support to recruitment processes (total: 10), the participation in the IMM quality certification and participation in the IMM induction sessions.

#### **Ongoing Projects**

2009 SettingTheStage—Setting the Stage in Portugal: researchers and the public come together through theatr, European Commission. Coordinator at IMM: M. Agostinho (FP7-PEOPLE-2009-NIGHT).

2009/2010 LiveAlife - Consultancy on the development of storyboards on primary health care, QREN. Coordinator at IMM: M. Trindade and M. Agostinho. Scientific.

#### **Invited Lectures and Seminars**

Agostinho, M. *Looking far beyond*. Course “New frontiers, new careers: science outside academia”, held at IMM, March 9.

Agostinho, M. and Trindade, M. “An insight into communication and funding at IMM”, IMM Seminar, July 20.

Agostinho, M. and Almeida, C. Organized the session “Escolho eu! – Welcome to IMM”, EnjoyMed Meeting, November 7.

Trindade, M. and Jacinto, A. Chairing of session on “The Role of Private Foundations in Science Funding”, Gameets 2009 Meeting, held at IGC, December 28.

#### **Training Actions**

Agostinho, M. and Almeida, C. organized the course "Scientists and the media: how and why", held at IMM12 - 13 February. (12 participants, limited number)

Trindade, M. Organization of the information session on the 4<sup>th</sup> FP7 Health Call and on Cost Actions, held at IMM, April 23 (13 participants).

Trindade, M. Hosting of session organized by GPPQ on “2010 FP7 funding on Health-related subjects”, June 25

Crisóstomo I., Costa J. Organization of the information session on on the 2009 FCT call for Doctoral and Post-Doctoral Grants, held at IMM , July 10 (19 participants)

Trindade, M., Vidal, S. Organization of the information session on the ERC Starting Grant 2009 call, held at IMM and at IGC, September 3 (19 participants).

Trindade, M. Organization of the information session on the Marie Curie Initial Training Networks 2009 call, held at IMM, October 22 (5 participants)

Trindade, M. and Vidal, S. Organization of the information session on the ERC Advanced Grant 2009 call, held at IMM and at IGC, November 2 (15 participants).

Crisóstomo, I., Trindade, M. and Vidal, S. Organization of the course “Effective Grant Writing” by consultant Jane Fraser (UK), held at IMM 3-4 November and IGC 5-6 November. (36 participants, limited number)

### Public Perception of Science

Agostinho, M. *Fazer Ciência, promover a vida*, ciclo de conferências "O poder e os limites da ciência: que desafios para o século XXI", Escola Secundária Artística António Arroio, Lisbon, May 8. (80 students from 10-12<sup>o</sup> year.)

IMM visit - of 15 MD students from the 2nd and 3rd years; Twinning Programme: students exchange from Holland, 12 January.

Visit to the school - Escola Secundária Vila Franca de Xira - 30 students from 10<sup>o</sup> and 11<sup>o</sup> years, Vila Franca de Xira, Presentation and debate about: genetic engineering, 14 January.

IMM Visit - 5 students from 12<sup>o</sup> year from school Escola Secundária de Santiago do Cacém, Santiago do Cacém; Project Área Escola about Technology in research, 16 February.

IMM Visit - 55 students from 8<sup>o</sup> year from school Escola S Prof. Mendes dos Remédios, Nisa; about Research models – fly and chicken, 4 March.

IMM Visit – 150 students from 12<sup>o</sup> year from the FML Candidate Day; visit to the laboratories, 12 March.

IMM Visit / Open day - 120 students from 10<sup>o</sup> until 12<sup>o</sup> year; Brain Week about “Brain and the senses”, 20 March.

IMM Visit – 10 students from the 2<sup>o</sup> e 3<sup>o</sup> year of Biomedical Engineering degree, Universidade Católica Portuguesa; about biomedical research, 26 March.

IMM Workshop - from the Project Setting the stage - Teatro Fórum; 20 participants, 18 April and 19 April.

IMM Workshop - from the Project Setting the stage – Stand Up Comedy; 20 participants, 25 April.

IMM Workshop - from the Project Setting the stage – Teatro do Gesto; 20 participants, 26 April.

IMM Visit – 39 students from 8<sup>o</sup> year from Escola EB 2,3 Abade Correia da Serra, Serpa; about Research models – fly and chicken, 28 April.

IMM Visit – students from 12<sup>o</sup> year from Escola Secundária Cardoso Pires; Project Área Escola about Genetic manipulation, 28 May.

Theatre performance - from the project Setting the stage; general public; Stand-up Comedy and Forum theatre; 50 participants. Festas do Concelho de Oeiras: Celebrar Oeiras, 20 June.

Ciência Viva IMM internships- 6 students from high school. July and August.

IMM Visit - for candidates to artistic residences from Ciência Viva / DGArtes - 10 candidates, 8 e 16 September.

Researcher’s Night / Setting the stage event - in Fundação Calouste de Gulbenkian, open to the general public, 25 September.

IMM Stand - Restaurante "O IMM"; Researcher’s Night / Setting the stage event in the Fundação Calouste de Gulbenkian gardens, open to the general public, 25 September.

IMM Visit - 23 students from Medicine, EnjoyMed Event, 7 November.

IMM Visit - 25 students from 12<sup>o</sup> year from school Escola Secundária Vergílio Ferreira, about Immunology, 26 November.

IMM Visit - 4 students from 12<sup>o</sup> year, from school Escola Secundária Miguel Torga, Project Área Escola – Oncology patient nutrition, 4 December.

IMM Visit - 25 students from Preschool, Associação de Protecção à Infância da Ajuda, about

Exploring the human body and a disease – Malária, 16 December.

IMM Visit - 27 students from 12<sup>o</sup> year, from school Escola Secundária Vergílio Ferreira, about Immunology, 16 December.

### **FMUL Newsletter - 18 Articles published**

*Nº4 March 2009*

Teste as suas raízes

Bolsa EMBO Installation Grant para Tiago Outeiro

Prémios Pfizer distinguem duas equipas do IMM nas áreas do VIH e cancro

Oncology Series – A investigação em oncologia aplicada à clínica

Ser estudante de doutoramento no Instituto de Medicina Molecular

GenoMed - A Ciência ao Serviço da Clínica

Reportagem / Perfil - José Rino - Unidade de Bioimagem

IMM-Biobank – Um banco de amostras biológicas para a investigação biomédica clínica em Portugal

Alimentação no Doente Oncológico

*Nº6 May/June 2009*

Semana do Cérebro

*July 2009*

Candidaturas ao Programa Harvard Medical School-Portugal abrem em breve

Instituto de Medicina Molecular tem novo website

*August/September 2009*

Dopamina e doença de Parkinson: investigadores do IMM e de HMS revelam pistas moleculares

*October 2009*

Formação Avançada no IMM

Prémio Grunenthal Dor 2008

Avaliadores externos visitam IMM

*November/December 2009*

Subir ao palco e partilhar a aventura da investigação

Bruno Silva-Santos vence Prémio Pfizer de Investigação Clínica

### **9 Press Releases**

1-Jan-09 | Investigadoras do IMM publicam livro sobre Alimentação no Doente Oncológico

5-Mar-09 | Linfócitos T: defensores contra infeções ou promotores de doenças autoimunes?

30-Mar-09 | Investigadores portugueses identificam novo mecanismo viral de manipulação do hospedeiro associado ao desenvolvimento de linfomas

19-May-09 | Investigadores portugueses desvendam mecanismos moleculares envolvidos na imunoterapia contra o cancro

20-Jul-09 | Da célula estaminal ao neurónio: cientistas Portugueses desenham mapa que levam à geração de neurónios in vitro

31-Aug-09 | Dopamina e doença de Parkinson: cientistas Portugueses e Norte-americanos revelam pistas moleculares

21-Sep-09 | Centros de investigação e empresas de biotecnologia unem-se para criar rede europeia de Imunoterapia no sudoeste europeu

13-Oct-09 | Prémio Grunenthal Dor 2008 – Investigação básica atribuído a trabalho que descreve novo fármaco com propriedades analgésicas

11-Nov-09 | Tecnologia desenvolvida por equipa portuguesa do Instituto de Medicina Molecular premiada no Texas em competição internacional

## Thesis Completed

### Postgraduation

Marta Elisa Dias Agostinho (2009) Postgraduate Diploma in Science & Society, The Open University, UK.

## Information Systems Unit

### Description and Objectives

The Information Systems Unit provides, manages and develops Information Technology Resources in order to facilitate the work of IMM researchers increasing their productivity. It also enforces process compliance with international and internal norms and procedures. The Information Systems Unit is designing and implementing several projects that aim to pursue its main goals. These projects intervene in the areas of 1) Information System Design; 2) Integration of Existing and New Information Systems; 3) Web Site (in collaboration with the Communication and Training Unit); 4) Documentation and Information Workflow; 5) Physical Support (Backups, Storage, Network Reliability, Network Performance, Security); 6) Knowledge Management. Support to IMM researchers includes the provision of a help-desk support service. A help-desk team is scheduled in order to respond all working days of the year. This help-desk team receives all help requests from the users, and acts as their only contact. If necessary, the help-desk team works in cooperation with the Information Technology Unit of the Medicine Faculty of Lisbon. In 2009, the projects were mainly focused on the Information Systems, Web Site and Storage Projects.

### Head of Unit

Tito Santos Silva, PhD, Consultant, Associate Professor, Universidade Católica Portuguesa

### Team

Daniel Silva	- PhD Fellowship, IMM
Pedro Eleutério	- Training Fellowship, IMM
Ruben Alves	- Technician, IMM

### Research/Service Areas

Information Systems;  
Databases;  
Computer Network maintenance;  
Helpdesk.

### Ongoing Projects

1. IMM Information System. Objectives: integration of IMM databases whenever useful for data coherence and productivity; simplifying routine tasks and processes.
2. Implementation of an Enterprise Content Management System
3. Implementation of a Backup System for IMM data
4. Increasing Data Storage Capacity for IMM
5. Implementation of an automatic Self-Testing System for accessing Infra-Structure performance

### Major achievements

1. A new IMM Website went live;
2. A first version of the Information System is implemented and is currently used to

## 262 | 2009 IMM Activity Report

automatically feed some internal services, like some features within the Website;

3. Data Storage System was re-designed, and partially implemented;
4. A Testing System was implemented and is now working, aiming servers and network structure.

## Lab Management Unit

### Description and Objectives

The main Lab Management mission is the maintenance and safety of the institute's infrastructures and equipments, in order to ensure high standards of usability and quality to all the researches unites.

It is also responsible for the supply of carbon dioxide, dry ice, liquid nitrogen; the management of chemical products; the design of new laboratory infrastructures, the development of standard operating procedures and the implementation, at the Institute, of good health and safety practices in work, according to the National Legislation.

The Lab Management unit is also responsible for the Purchasing Office and the Washing Room. The Purchasing Office, which includes the Supply Center and Internal Storehouse is responsible for all institute acquisitions (products, services and equipments), including import / export services and provides information on prices, ongoing promotions, new products and delivery conditions and other acquisition requirements. In addition, it maintains a storehouse for the most used consumables.

The Washing Room provides researchers with clean and sterilized material, according to each research specific requirement and it is equipped for the preparation and sterilization of all types of materials and solutions.

### Head of Unit

Luís Moita, Scientific Adviser

Alexandra Maralhas, Lab Manager

### Team

Ana Lúcia Barbosa	- Lab Manager Assistant (left October 2009)
Sandra Lopes	- Purchasing Office
Ana Rita Vicente	- Purchasing Office
Edna Gomes	- Washing Room technician
Paula Correia	- Washing Room technician (half-time)
Fernanda Vila-Chã	- Washing Room technician
Rita Soares	- Lab Manager Assistant (since December)
Alexandre Jesus	- Lab Manager Assistant (since December)

### Research/Service Areas

Infrastructure and equipments maintenance  
 Health and Safety  
 Laboratory Design  
 Purchases

### Major Achievements

In 2009 the Lab management implemented, within the Intranet, better communication system to be used between the support units and the research groups. This system supplies information on: legislation, laboratory safety, environment management system, procedures and rules of hygiene, health and safety at work. The Lab Management intranet page also includes the acquisition and booking system.

The unit is responsible for the implementation of a Quality Management System, according to ISO9001:2008 in the Institute. This system will allow a better basis and control for the existing procedures, implement new standards and procedures and an easier access to forms and procedures (Standard Operation Procedures, Forms, Equipment Manuals, Equipments User's Log Books and date of upcoming meetings and training sessions).

During 2009, a major effort was dedicated to improving the "maintenance and repair", both of equipment and facilities, with new or renegotiated contracts. Also during this year, the construction works for installation of new laboratories and other remodeling works were carried out in order to achieve better working conditions and more efficient usage of office and laboratory spaces.

Regarding the Purchasing Office, there was a significant increase of the daily orders. They also took on the responsibility for importations and international shipment of biological material, dealing with all matters associated with Portuguese customs.

A great effort is being made to increase the support and interaction with all research and support units in areas regarding maintenance, equipment repair, safety and efficiency. With this in mind a new scheme of meetings with the lab responsible's was initiated during the current year.

The lab Management unit worked actively with the Animal Facility / P3 team in defining needs, improvements and adjustments regarding the construction works and the purchase of equipment. There were held meetings once a week to discuss the functioning of these facilities.

Two surveys are conducted daily to about twenty common equipments and rooms, where is verified, for example, the state of operation, cleaning, usage logs, etc.

Various meetings were carried out with the Management Unit of the Building Egas Moniz – Faculty of Medicine – for the assessment of technical feasibility and level of safety of the facilities.

Luís Moita, the Lab Management Scientific Adviser, provides simultaneously scientific and technical input, contributing to the continuous improvement of IMM Lab Management.

With these tools and more resources, the Lab Management, can achieve high standards of communication, accessibility and quality planned for all the Research Units.



## BioImaging Unit

### Description and Objectives

The BioImaging Unit constitutes the core microscopy facility of the IMM, acting as a support structure to carry out and nurture research done with Light Microscopy inside the institute. The unit presently manages 4 laser scanning confocal microscopes (including one spinning disk system), 3 widefield epifluorescence systems and other imaging resources such as brightfield microscopes and a bioluminescent imaging system. Besides managing resources, the unit provides IMM scientists and visitors with excellence in scientific know-how and expertise in using advanced light microscopy methods for their research. The unit assists in planning microscopy-oriented projects, provides advice and support on sample preparation, image analysis and processing and further assists in preparing and writing research papers with microscopy data. Together with continuous training of new users, the unit organizes regular courses and workshops which range from basic to more advanced training on the most recent microscopy techniques and systems. The unit's research is strongly focused on the development of novel microscopy techniques in collaboration with research units with challenging biological problems. Thus, besides fostering interactions and collaborations between microscopy users at the IMM, the Bioimaging unit is also committed to disseminate and apply its own developed microscopy tools and technology to research groups within the institute. The unit also provides advice and acts as a communication and decision making platform for groups interested in the joint acquisition of high-cost top-of-the-line microscopy systems, also providing assistance in writing grant applications for that purpose.

### Head of Unit

José Rino, PhD in Biophysics at Faculdade de Ciências da Universidade de Lisboa

### Team

António Temudo

- Research Technician, IMM

### Research/Service Areas

Fluorescence Microscopy  
 Laser Scanning Confocal Microscopy  
 Image Processing and Analysis  
 Quantification of protein dynamics and interactions

### Major achievements

#### Core Facility

In 2009 the BioImaging Unit services were used by 133 users from 25 different research units (4 of them from outside the IMM), which corresponds to an increase of 14% in the total number of users relative to 2008, yet keeping a total number of circa 8000 hours of usage for all the systems. With the support of USI, a central data storage service has been implemented in all computer-controlled microscopy systems as well as administrator remote access for technical support and quality control. Two microscopy courses were organized for internal and external users: an introductory course on microscopy and an advanced optical sectioning imaging course in collaboration with Zeiss Campus which provided the latest state-of-the-art Zeiss imaging systems for the event.

#### Research

In collaboration with the Signal and Image Processing Group from Instituto Superior Técnico, novel tools for automated microscope operation and remote control via internet access were successfully implemented (Master thesis of students Hugo Pinto and Diogo Loureiro, November 2009). This work will now constitute the basis for the development of data driven image acquisition software for specific research applications.

### **Ongoing Research Projects**

#### Participation in projects from other units:

2008/2010, Identification and characterization of the supercomplexes from the bacterial aerobic respiratory chain of *Escherichia coli* and *Bacillus subtilis*, FCT. Coordinator: A. Melo (PTDC/BIA-PRO/67105/2006)

### **Advanced Teaching**

The BioImaging Unit organized two courses in 2009:

#### Advanced Optical Sectioning Microscopy Course. January 20-22, 2009 (40 participants)

Speakers: António Jacinto (IMM), Gabriel Martins (FCUL), José Rino (IMM), Kirsten Hanson (IMM), Mathias Koeppen (IGC), Monika Marx (Zeiss), Nuno Moreno (IGC), Ricardo Henriques (IMM), Soren Prag (IMM), Sven Laarman (Zeiss), Paula Sampaio (IBMC) and Thorsten Kues (Zeiss)

#### BioImaging NanoCourse on Microscopy 2009. October 27-28, 2009 (35 participants)

Speakers: António Temudo (IMM), Carina Santos (IMM), Gabriel Martins (FCUL), Henrique Rosário (IMM), José Rino (IMM) and Kirsten Hanson (IMM).

Rino, J. Lecturer “Introduction to Confocal Microscopy” Module, Workshop on Basic Techniques in Multiphoton and Confocal Laser Scanning Microscopy, Instituto Gulbenkian de Ciência, Oeiras, Portugal. July 21-22, 2009.

Rino, J. Lecturer “FRAP and FRET – looking at protein dynamics and interactions inside living cells” module, Course on Multifluorescence Microscopy, Centro de Neurociências de Coimbra, Universidade de Coimbra, Coimbra. Portugal. September 2-4, 2009.

### **Public Perception of Science**

J. Rino participated and provided technical support for microscopy systems during the event “Noite dos Investigadores – Researchers’ Night”, Fundação Calouste Gulbenkian, Lisboa, September 25 2009.

## Flow Cytometry Unit

### Description and Objectives

The Flow Cytometry Unit at the IMM is a core facility that provides support and training to IMM and external researchers who require this technology in their research projects. The Unit is currently equipped with three Becton Dickinson analyzers: two FACSCaliburs and one FACSCanto. The High Speed Cell Sorter, BD FACSAria equipped with 3 lasers enables the purification of cell populations and is operated by dedicated staff only. The cell analysers are operated by researchers after training provided by our staff. The Flow Cytometry Unit provides regular training to users by organizing Flow Cytometry workshops or at an individual basis whenever required. We aim to train researchers in the adequate use of the instruments and also on flow cytometry principles, experiment design, as well data analysis and interpretation. Data analysis can be performed at our Mac stations using CellQuest or FlowJo. UCF staff are also involved in research projects in collaboration with other IMM Units.

### Head of Unit

Paul Hutchinson, PhD, Monash University, Melbourne, Australia (Until June)

Maria Soares, PhD, University College London, London, UK (Since July 2009)

### Team

Ana Luisa Caetano	- Technician, FMUL
Alexandre Varela	- Technician (since July)

### Research/Service Areas

Training users in flow cytometry.  
 Advice on new techniques and methodologies available.  
 Support in experiment design.  
 Training in flow cytometry data analysis using FlowJo and CellQuest.  
 Purification of cell populations by High Speed Cell Sorting.

### Major Achievements

In 2009 the services provided by the Flow Cytometry Unit at the IMM have been used by almost every Unit at the IMM. We currently have a users list of 140 researchers. Following from previous years we continued to grow in the number of hours of use of our flow cytometers. In 2009 we increased the number of hours of use of flow cytometers per week in 27 %. Increased machine usage has prompted the implementation of new daily routines for instrument maintenance to ensure adequate service. Quality control protocols for monitoring instrument performance and stability have been implemented on all machines.

A data storage system has been fully implemented.

### Ongoing Research Projects

2008/2011 Immune Reconstitution after Unrelated and Haplotype-Mismatched Stem Cell Transplantation, FCT. Coordinator: Maria Soares (PTDC/SAU-MII/67662/2006). This project is ongoing at the Clinical Immunology Unit at the IMM, please see UIC report for details.

Participation in projects from other Units:

## 268 | 2009 IMM Activity Report

2007/2010 Molecular and Functional Effects of TAL1 and LMO2 Overexpression in Normal Human Hematopoietic Stem Cells: Impact on T-cell leukemogenesis, FCT. Coordinator: J.T. Barata (PTDC/SAU-OB/69974/2006)

2007/2010 HIV-2 infection and thymus, FCT. Coordinator: A.E. Sousa (PTDC/SAU-MII/66248/2006)

2009/2012 Long-term impact of thymectomy performed in early infancy, FCT. Coordinator: A.E. Sousa (PIC/IC/83068/2007)

2009/2012 Clinical implications of activation of PI3K/Akt signaling pathway in primary adult acute lymphoblastic leukemia, FCT. Coordinator: J.F. Lacerda (PIC/IC/83023/2007)

### Publications in Peer Review International Journals 2009

Henson, S.M., Franzese, O., Macaulay, R., Libri, V., Azevedo, R.I., Kiani-Alikhan, S., Plunkett, F.J., Masters, J.E., Jackson, S., Griffiths, S.J., Pircher, H.P., Soares, M.V. and Akbar, A.N. (2009) KLRG1 signaling induces defective Akt (ser473) phosphorylation and proliferative dysfunction of highly differentiated CD8+ T cells. *Blood* 113: 6619-6628 (Journal IF: 10.9)

Azevedo, R.I.\*, Soares, M.V.\*, Barata, J.T., Tendeiro, R., Serra-Caetano, A., Victorino, R.M., Sousa, A.E. (2009) IL-7 sustains CD31 expression in human naïve CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. *Blood* 113: 2999-3007 \*joint first authors (Journal IF: 10.9)

### Communications in International Conferences

Azevedo, R.I. et al. IL-7 Sustains CD31 Expression in Human Naïve CD4+ T Cells and Preferentially Expands the CD31+ Subset in a PI3K-Dependent Manner. 2nd European Congress of Immunology, Berlin, Germany. September 13 – 16, 2009 (Poster presentation)

Tendeiro, R. et al. Evidence for increased replicative rate of the naïve CD4+ T-cell pool during HIV-2 infection, a naturally occurring attenuated form of HIV disease. 2nd European Congress of Immunology, Berlin, Germany. September 2009 (Poster presentation)

### Communications in National Conferences

Tendeiro, R. et al. Evidence for increased replicative rate of the naïve CD4+ T-cell pool during HIV-2 infection, a naturally occurring attenuated form of HIV disease. XXXV Annual Meeting of Sociedade Portuguesa de Imunologia, Lisbon. September 28-30, 2009 (Poster presentation)

### Invited Lectures and Seminars

Tendeiro, R. et al. Telomere Length by Flow FISH as a tool to assess immune replicative senescence. XI Congresso da Sociedade Ibérica de Citometria, Covilhã, Portugal. May 7-9, 2009

Soares, M. IL-7 sustains CD31 expression in human naïve CD4+ T cells and preferentially expands the CD31+ subset in a PI3K-dependent manner. IGCxIMM Immunology Meeting, Instituto Gulbenkian de Ciência, Oeiras. Portugal. May 20, 2009.

### Advanced Teaching

The Flow Cytometry Unit organized two Workshops in 2009

10<sup>th</sup> IMM Flow Cytometry Workshop. April 20-23, 2009

11<sup>th</sup> IMM Flow Cytometry Workshop. November 23-26, 2009

These courses were attended by a total of 24 participants and comprised theoretical on

Fundamentals of Flow Cytometry (Rui Gardner, IGC), Multicolour Calibration (Maria Soares, IMM), Flow Cytometry Applications (Maria Soares, IMM), Basic notions, techniques and applications of Fluorescence-Activated Cell Sorting (Ana Luísa Caetano, IMM). The practical modules were focused on three-color calibration/acquisition- FACSCalibur (Maria Soares), introduction to Diva. Six-color calibration/acquisition - FACSCanto (Ana L. Caetano) and an introduction to CellQuest and FlowJo Software (Alexandre Varela, IMM)

Soares, M. Lecturer “Immunology” Module, VI Mestrado em Doenças Infecciosas Emergentes. IMM, Lisbon, Portugal. January 19 - February 2, 2009

Soares, M. Lecturer “Immunology” Module, VII Mestrado em Doenças Infecciosas Emergentes. Faculdade de Medicina de Lisboa, Lisbon, Portugal. November 16-30, 2009

## Animal Facility

### Description and Objectives

The IMM has currently one conventional animal facility, and is starting a new, larger barrier Facility, including a P3 facility. The conventional facility is currently used at 100% of its capacity by IMM researchers. All husbandry and manipulation procedures are performed according to high standards of biocontainment and bioexclusion, in order to ensure the best possible conditions in terms of health and safety. The new barrier facility, with an area of 800m<sup>2</sup>, will host mice and rats in microisolators and individually ventilated cages, and will be available for both IMM and external researchers. The new animal quarters include one Production facility, one Experimental Facility, one Quarantine room, two Transitional Animal Rooms, one Surgical Suite, two Procedure Rooms and one gamma irradiation suite. The new quarters will also include a 70m<sup>2</sup> P3 facility with two tissue culture rooms, one animal room and one procedure room. The Animal Facility provides both internal and external services for academia, pharma, biotech and other fields of research. These services will run from animal hosting to procedure performance such as: experimental procedures, surgical procedures, *in vivo* imaging and assisted reproduction techniques such as timed pregnancies, *in vitro* fertilization, embryo & cesarean derivation, and cryopreservation.

This unit is highly committed to follow the 3Rs principles – *Replace, Reduce and Refine* – for a conscious and responsible use of laboratory animals, while supporting high quality science. For this reason, it provides education, training and guidance for researchers, according to Portuguese and international law and recommendations for good practices and animal welfare.

### Head of Unit

Domingos Henrique, PhD – Investigator, FML

### Team

Alina Costa	- Unit Manager
Dolores Bonaparte	- Veterinarian
Yuri Leite	- Technician
Joel Filipe	- Technician
Olena Pinho	- Animal caretaker
Nuno Inácio	- Animal caretaker
José Vila-Chã	- Animal caretaker

### Research/Service Areas

Animal husbandry;  
Management of genetically modified colonies;  
Animal project management;  
Education and training of researchers in animal experimentation;  
Ethical review of research projects involving animals.

## **Zebrafish Facility**

### **Description and Objectives**

The zebrafish (*Danio rerio*) is a representative of the vertebrate species, its genome is sequenced, a large number of synchronously developing embryos can be produced and the optical clarity of embryos and larvae allow real-time imaging. In addition, zebrafish embryos are permeable to drugs and can be easily manipulated using well-established genetic and molecular approaches.

Currently, the zebrafish model is used for the study of human genetic diseases such as, cancer, cardiovascular disorders, neurological diseases, inflammation, angiogenesis, muscle-associated diseases and osteoporosis. Unlike humans, the zebrafish is one of the few vertebrate species that can fully restore the shape, structure and function of body parts lost after severe injury or amputation. Therefore, it has become a powerful model for regenerative medicine.

The IMM has decided to foster this powerful model system to complement research on vertebrate development, physiology and disease mechanisms relevant to human diseases. The objective was to implement a facility that could be used by a number of IMM research units to undertake a diverse range of experimentation.

### **Head of Unit**

Leonor Saúde, PhD      Invited Auxiliary Professor, FML

### **Team**

Lara Carvalho	- Unit Manager
Inês Matos	- Part-time Technician
Andreia Pinto	- Part-time Technician (Left in August 2009)
Ana Catarina Pronto	- Part-time Technician (Left in October 2009)
Patrícia Matos	- Part-time Technician

### **Research/Service Areas**

Zebrafish husbandry  
 Management of zebrafish line stocks  
 Identification of transgenic and mutant lines  
 Electroporation DNA and Morpholino oligos of adult fish  
 Embryo culture  
 Microinjections of DNA, RNA and Morpholino oligos into embryos  
 Cell/tissue transplantation in embryos

### **Major achievements**

- Acquisition of a stand alone Zebra Fish Housing System that operates in an independent mode. This system will allow to perform regeneration experiments in a controlled environment.
- Production of transgenic lines
- Setting up of a chemical screen protocol

## **Histology Service**

### **Description and Objectives**

Starting on 1<sup>st</sup> September 09, a new Histology Service was implemented in IMM, resulting from collaboration with the Histology Institute of FML. The Histology Service has a permanent technician, with a degree in Technical Pathology, Anatomy and Histology, and is located in the Histology facilities of the Histology Institute of FML.

The main objectives of the Histology Service are: processing tissue samples for routine histochemical procedures; training new users in sample preparation with the available equipment in the laboratory; and provide tutorship in the design and implementation of immunostaining and other procedures (not performed in the Histology Lab).

### **Head of Unit**

Sandra Casimiro, PhD – Post-Doc Researcher, IMM

### **Team**

Andreia Pinto – Technician, IMM

### **Research/Service Areas**

Processing tissue samples for routine histochemical procedures.  
Training in sample preparation and equipment utilization.  
Provide tutorship in the design and implementation of histology procedures.

### **Major achievements**

The implementation of the Histology Service represents a major achievement in IMM. A survey conducted in the beginning of 2009 indicated that most research Units considered necessary to have such facilities available in-house. In fact, since the Service implementation several research Units have used the available services in a regular basis. Researcher's formation in standard procedures had also a high demand, demonstrating that training and tutorship should continue to be one of our main goals.



## **COMPANIES**

The IMM collaborates with start-up and biotechnology companies in areas of biomedical technologies and sophisticated health care delivery, located at the Edifício Egas Moniz. The IMM exchanges with these companies scientific know-how, supplies access to facilities, equipments and services.



## GenoMed

### Description and Objectives

GenoMed is a spin-off company of IMM created in 2004. The mission of GenoMed is to provide cutting-edge technology transfer services relevant for medical applications. Currently, GenoMed offers over 150 different tests that are used in diagnosis, prognosis and therapeutic follow up. The main medical areas covered include Oncology, Infectious Diseases, Genetic diseases and Pharmacogenetics. Additionally, GenoMed provides parental and ancestry DNA testing.

### Directors

Maria Carmo-Fonseca, MD, PhD - Executive Director

Teresa Porta Nova, MSc - Technical Director

### Team at IMM

#### Genetic Diseases and Pharmacogenetics Unit:

Gabriel Miltenberger-Miltenyi, MD, PhD	- Coordinator, IMM staff scientist
Ana Coutinho, PhD	- Technician
Sónia Pereira, BSc	- Technician
Rosário Nolasco, BSc	- Technician, IMM Fellow
Catarina Silveira, Lic. Clinical analysis and public health	- Technician
Marina Costa, PhD	- Technician, Post-doctoral fellowship, IMM (left in November)

#### Virology Unit:

Rute Marcelino, MSc	- Coordinator
André Janeiro, BSc	- Technician
Paula Mimoso, BSc	- Technician, IMM fellow

#### Oncology Unit:

Sónia Santos, PhD	- Citogenetics Unit Coordinator
Paula Costa, BSc	- Citogenetics Unit Technician
Rosana Rocha, MSc	- Citogenetics Unit Technician
Sónia Matos, MSc	- Hemato-Oncology Unit Coordinator
Ana Carla Sousa, BSc	- Hemato-Oncology Unit Technician
Sara Malveiro, BSc	- Hemato-Oncology Unit Technician

#### Management Unit:

João Pedro Rebelo	- Business Manager
Filipe Fialho	- Administrative officer (left in December)
Ana Flores	- Management Assistant

### Research/Service Areas

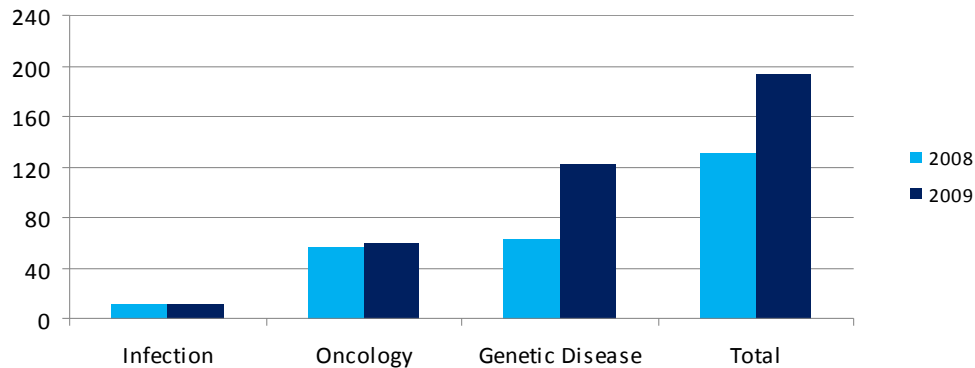
In vitro molecular diagnostics have established themselves as effective tools for all aspects of disease management, especially in areas of unmet clinical need. Such tests have been developed for screening and prognosis as well as for applications, such as determination of genetic predisposition to disease, detection of presymptomatic disease, and prediction of individual drug response. Molecular diagnostics are the basis of pharmacogenomics, enabling the evolution of personalized medicine.

### Major scientific achievements

Certification by ISO 9001:2008 in March 2009.

Increased number of tests available.

#### Total Number of tests available



#### Recent Most Relevant Publications

Antunes, J., Filipe, P., André, M., Fraga, A., Miltenyi, G. and Marques Gomes, M. (2009) Livedoid vasculopathy associated with plasminogen activator inhibitor-1 promoter homozygosity (4G/4G) and prothrombin G20210A heterozygosity. *Acta Dermato-Venereologica Accepted* (Journal IF: 2.456)

Cardoso, B.M., Castanhinha, S., Dupont, J., Albuquerque, M., Pereira, S.V., Miltenberger-Miltenyi, G. and G Oliveira, G. (2009) Cleidocranial Dysplasia with Severe Parietal Bone Dysplasia: a new (p.Val124Serfs) RUNX2 mutation (accepted by *Clinical Dysmorphology*) (IF [2008]: 0.521, cited: 0)

Miltenberger-Miltenyi, G., Schwarz, T., Auer-Grumbach, M., Janecke, A.R. (2009) Identification and in-silico analysis of 14 novel GJB1, MPZ and PMP22 gene mutations. *epub: Eur J Hum Genet.* 2009 17:1154-9 (IF [2008]: 3.925, cited: 0)

Marcelino, R. (2009) How to deal with viral resistance? *Rev. Farmácia Portuguesa* 183: 32-34

Carmo-Fonseca, M. (2009). Who were our ancestors. *Rev. Farmácia Saúde* 155: 32-34

Carmo-Fonseca, M. (2009). Cancer is a genetic disease. *Rev. Farmácia Saúde* 154: 36-38

Carmo-Fonseca, M. (2009) Genetic tests of ancestry. *Rev. Farmácia Portuguesa* 182: 22-23

Carmo-Fonseca, M. (2009) I will suffer from Alzheimer's? *Rev. Farmácia Saúde* 153: 54-56

Carmo-Fonseca, M. (2009) Biological Research Kinship. *Rev. Farmácia Portuguesa* 181: 42-44

Carmo-Fonseca, M. (2009) Human papillomavirus - the virus that causes cancer. *Rev. Farmácia Saúde* 151: 40-42

Carmo-Fonseca, M. (2009) The uncertainty of the genetic susceptibility. *Rev. Farmácia Portuguesa* 180: 38-39

Carmo-Fonseca, M. (2009). Genes and Health. *Rev. Farmácia Saúde* 149: 40-41

Carmo-Fonseca, M. (2009) The pharmacy in the post genomics. *Rev Farmácia Portuguesa* 179: 32-33

Cortez-Dias, N., Correia, M.J., Coutinho, A., Fernandes, C., Nunes Diogo, A., Lopes, M.G. (2009) Pharmacogenetics and anticoagulant therapy: two cases of genetically determined response to warfarin. *Rev. Port. Cardiol.* 28: 995-1004

### Ongoing Research Projects

2006/2009 Study of Prognostic Factors in Chronic Lymphocytic Leukaemia Patients (LLC Project), Schering Lusitana Foundation and Private Donation. Coordinator: J. Perdigão e S. Matos

2008/2009 Diabetic nephropathy - analysis of polymorphisms of the TCF7L2 gene in susceptibility to diabetic nephropathy in patients with type 2 diabetes mellitus, Department of nephrology of the Hospital de Santa Maria. Coordinators: G. Miltenberger-Miltenyi and S. Gonçalves.

2008/2009 Impact of intreratrial setum anomalies in stroke of young adults (Factor II e XII), GAPIC/AIDFM/Department of cardiology of the Hospital de Santa Maria. Coordinators: G. Miltenberger-Miltenyi and A. Almeida.

2008/2009 Study of the gene for vitamin D binding protein (DBP) in the DNA of patients with amyotrophic lateral sclerosis, GAPIC/AIDFM. Coordinators: G. Miltenberger-Miltenyi and M. de Carvalho.

2008/2009 Genetics of alcohol - a study of polymorphisms, IMM and Department of Hepatology of the Hospital de Santa Maria. Coordinators: G. Miltenberger-Miltenyi and H. Cortez Pinto.

2009/2009 Multicenter comparison of assays for cerebrospinal fluid markers for Alzheimer's Disease. Department of Neurology and Clinical Chemistry of VU University Medical Center. Coordinators: N. Verwey.

2009/2010 FUS gene analysis in Amyotrophic Lateral Sclerosis patients, Department of Neurology of the Hospital de Santa Maria. Coordinators: G. Miltenberger-Miltenyi and M. de Carvalho

2009/2010 Comparison of methods of mutation analysis of the K-ras gene in colorectal cancer (multicenter study). Nijmegen Centre for Molecular Life Sciences (NCMLS), University Medical Centre St Radboud. Coordinators: J. Han and J.M. van Krieken.

2009/2011 Development of a pyrosequencing method to detect resistance mutations to nucleosidic and non nucleosidic polimerase inhibitors and to protease inhibitors, in chronic infection of Hepatits C Virus (HCV), Roche/APEF Funding. Coordinator: R. Marcelino

### Communications in International Conferences

Gonçalves, S. et al. Analysis of the association of TCF7L2 polymorphism rs7903146 with susceptibility to diabetic nephropathy on type 2 diabetic patients. American Society of Nephrology, Renal Week 2009, San Diego, CA, USA. October 27 - November 1, 2009 (Poster presentation)

Cardoso, B. et al. Cleidocranial dysplasia with severe calvarial phenotype in a patient with a new RUNX2 mutation. 9th World Congress of Perinatal Medicine, Berlin, Germany. October 24-28, 2009 (Poster presentation)

Miltenberger-Miltenyi, G. and Pereira, S.P. Molecular genetical findings of familial Alzheimer's disease and familial frontotemporal lobar degeneration in a patient cohort in Portugal - description of five novel mutations. Congress of the European Society of Human Genetics, Vienna, Austria. May 23-26, 2009 (Oral presentation)

### Communications in National Conferences

Robusto-Leitão, O. et al. Pharmacogenetic study (P450) of a sample of patients with inadequate

response to psychotropic drugs. V Congress of the Portuguese Society of Psychiatry and Mental Health, Porto. November 24-28, 2009 (Oral presentation)

Santos-Sousa, P. et al. Leucemia aguda en células de dador post-transplante de progenitores hematopoyéticos en un caso de linfoma no-hodgkin folicular. LI National Meeting of AEHH and 25° National Congress of SETH, Barcelona. November 12-14, 2009 (Poster presentation)

Miltenberger-Miltenyi, G. et al. Preliminary data from genetic analysis in Portuguese children with nephrotic syndrome, description of three new mutations. Days of Pediatric, Lisbon. November 11-14, 2009 (Oral presentation)

Miltenberger-Miltenyi, G. Genotype-phenotype correlations in familial dementia in a Portuguese patient cohort – identification of seven novel mutations. Congress of Neurology, Lisbon. November 5-7, 2009 (Oral presentation)

Santos-Sousa, P. et al. Leucemia aguda em células do dador pós-transplante alogénico de progenitores hematopoiéticos num caso de linfoma não-Hodgkin folicular. Annual Meeting of SPH, Tróia. October 29-31, 2009 (Poster presentation)

Santos-Sousa, P. et al. Terapêutica da leucemia mielóide crónica com imatinib: valor preditivo da resposta citogenética completa aos 12 meses na obtenção de resposta molecular e progressão de doença. Annual Meeting of SPH, Tróia. October 29-31, 2009 (Oral presentation)

Guerra, L. et al. Terapêutica com dasatinib ou nilotinib em doentes com leucemia mielóide crónica resistentes ou intolerantes ao Imatinib. Annual Meeting of SPH, Tróia. October 29-31, 2009 (Poster presentation)

Rodrigues, T. et al. Study 1p/19q deletion in primary and secondary anaplastic oligodendroglioma. Neuro 2009 – Congress of the Portuguese Society of Neurology and Neurosurgery, Algarve. May 14-16, 2009 (Oral presentation).

Porta Nova, T. The retail sale of genetic tests. Scientific congress of ANL, Porto. May 1-2, 2009 (Oral presentation)

### **Invited Lectures and Seminars**

Miltenberger-Miltenyi, G. Molecular genetic diagnostics from the patient to the laboratory: basics, applications and new developments. Seminars of Biomedical Sciences (Licenciatura), University of Algarve, Faro, Portugal. October 21, 2009.

Marcelino, R. Detection Methods of Hepatitis B Virus Mutants Against Nucleos(t)ide Analogues—certainties and problems. Viral Hepatitis Monothematic Reunion, Guimarães, Portugal. January 24, 2009.

### **Prizes, Honours and Awards**

Roche/APEF Research Prize award to Marcelino, R., Janeiro, A., Marinho, R., Velosa, J., Serejo, F., Baldaia, C., Nunes, J. for the project Development of a pyrosequencing method to detect resistance mutations to nucleosidic and non nucleosidic polimerase inhibitors and to protease inhibitors, in chronic infection of Hepatitis C Virus (HCV).

### **Advanced Teaching**

Marcelino, R. “Molecular Biology Module”, Emergent Infectious Diseases Master Course. Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal. January 7-14, 2009

### **Public Perception of Science**

Miltenyi, G. and Matos, S. “GenoMed – What we do?”, Encontro de Estudantes de Medicina –

EnjoyMed, Lisbon, Portugal. 7 November 2009

Marcelino, R. “Ciência Hoje: A Flu: 67,4% of samples collected on IMM were positive – sample collection covers children and adults between 25 and 45 years old”. December 2009.







## TechnoPhage

TechnoPhage, SA is a Biotechnology company focused on the discovery and development of biopharmaceuticals, using different proprietary technologies. TechnoPhage is engaged in three main R&D programs:

- R&D of novel bacteriophage-based products for the treatment and diagnosis of bacterial infections caused by antibiotic-resistant bacteria. Ongoing projects aim the specific elimination of topical infections, using bacteriophages or phage lytic enzymes.
- R&D of recombinant single domain antibody fragments (sdAbs), for therapeutic and diagnostic applications. Ongoing projects in the areas of inflammation, oncology and thrombosis.
- Drug discovery using the zebrafish as an in vivo screening system on skeletal formation and regeneration. Ongoing projects in the area of bone disorders.

The company laboratories are located at the Institute of Molecular Medicine (IMM), under a scientific collaboration protocol.





