LSHB-CT-2006-036813

PROLIGEN

Hypoxic renal proliferation

Specific targeted research or innovation project

FP6-2005-lifescihealth-7, Program: Life sciences and biotechnology for health

Publishable final activity report

Period covered: from 31-October-20086to 31- October-2009
Date of preparation: 31-October-2009

Duration: 18 months

Project coordinator name: Georgina Hotter
Project coordinator organisation name: CSIC draft 1

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1. PROJECT EXECUTION

SUMMARY DESCRIPTION OF PROJECT OBJECTIVES

PROLIGEN aims to enhance the endogenous regenerative capacity of injured kidneys based on information derived from genomics/proteomics and functional genomics.

The approach of PROLIGEN is:
• To define and identify set of genes/proteins associated with functional recovery from renal injury.
• To build up high throughput test systems to follow the basic biological process involved in regeneration and to use these read out systems for functional genomics.
• To develop biologicals and cell based therapy to foster proliferation.

The project focuses on macrophages as the centre of a complex regulatory network receiving and distributing signals from all biological process involved from renal injury to recovery.

PROLIGEN particularly focuses on use of functional genomic technology, which provides a new network of gene function and platforms to deliver innovative cellular based therapy strategy.

The generated knowledge will also contribute to improve the regeneration in other tissues, being an alternative to the use of stem cells

Experiments will be carried out in vivo and in vitro using rodent, tubular cell and macrophage culture. Genomics, functional genomics and recombinant protein synthesis SMEs and prominent experts in fundamental biological process associated to the renal ischemia/reperfusion and regeneration processes will provide the necessary knowledge and critical mass to reach the goal of this project; namely and foremost, the development of intervention strategies for amelioration of acute renal failure and promotion of regeneration and healing.

The resulting gene function and new cell therapy information may provide the PROLIGEN consortium the necessary tools to deliver new biologicals and cell-based therapies for kidney regeneration. The generated knowledge will also contribute to improve the regeneration in other tissues avoiding the use of stem cells.

CO-ORDINATOR CONTACT AND CONTRACTORS

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RESULTS ACHIEVED AND EXPECTED END RESULTS. INTENTION FOR USE AND IMPACT

Little is known about the temporal and spatial mechanisms that promote tissue healing after injury. A better understanding of this process would give rise to new diagnostic (perhaps even prognostic) approaches and indicate a basis for new treatment strategies, as there is a desperate need for market introduction of new drugs for this disease.

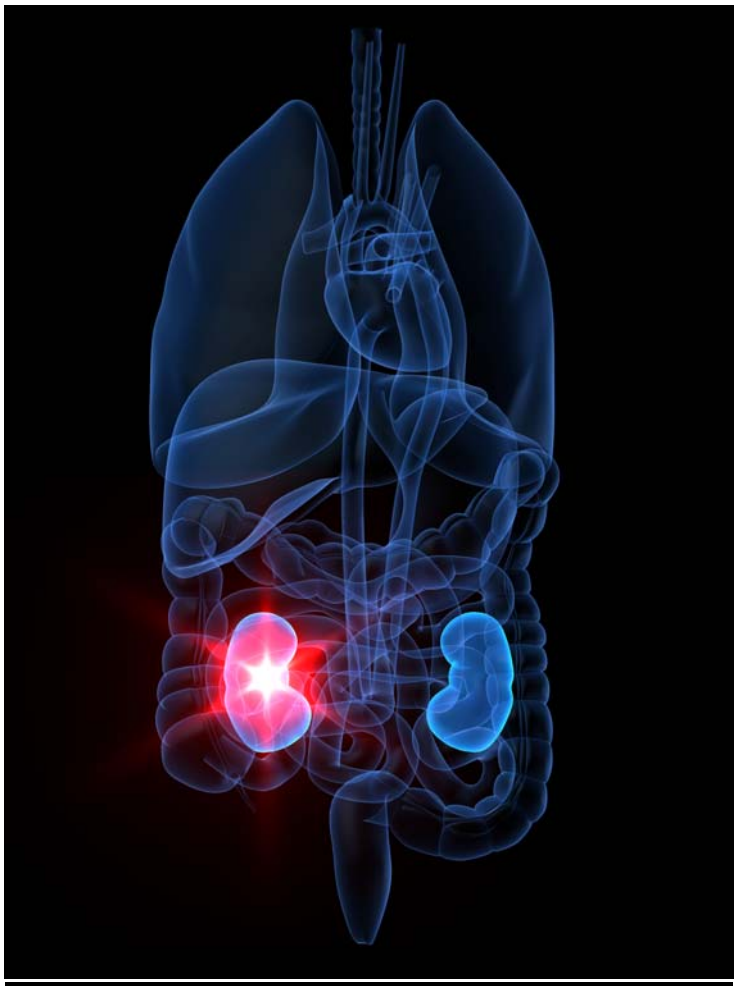
PROLIGEN seeks to address this severe and debilitating societal problem for both patient and public health care by developing new strategies to understand, prevent and treat acute renal failure.

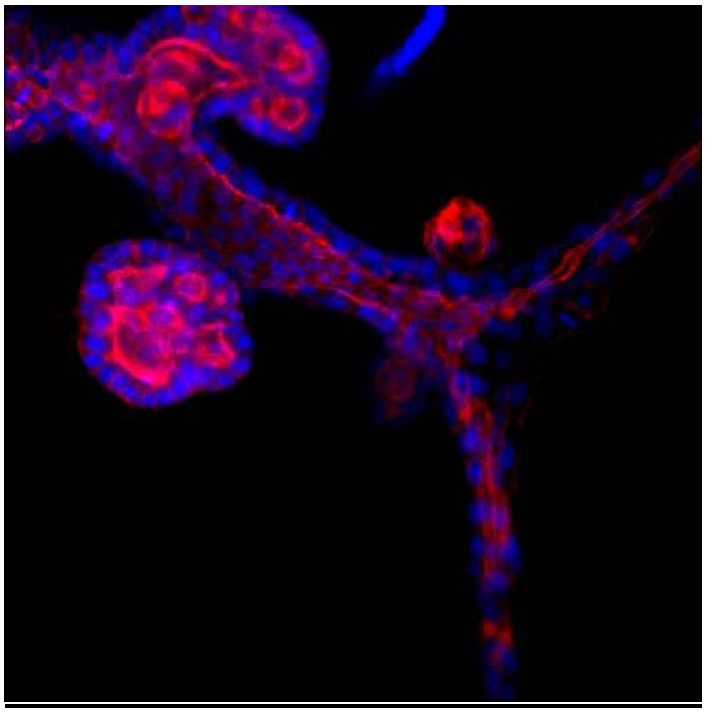
PROLIGEN has achieved the information to foster kidney regeneration by providing knowledge on the gene control of mechanisms involved in regeneration.

In fact we have obtained a list of genes and proteins that defines regeneration vs injury stage after ischemia/hypoxic insults. We have validated the genes in “in vitro” systems using high troughput test systems to follow tubulogenesis and the change of macrophage phenotype. We have validated 14 genes and one protein in “in vivo models and delivered a new cell theraphy using genetically modified macrophages to overexpress NGAL.

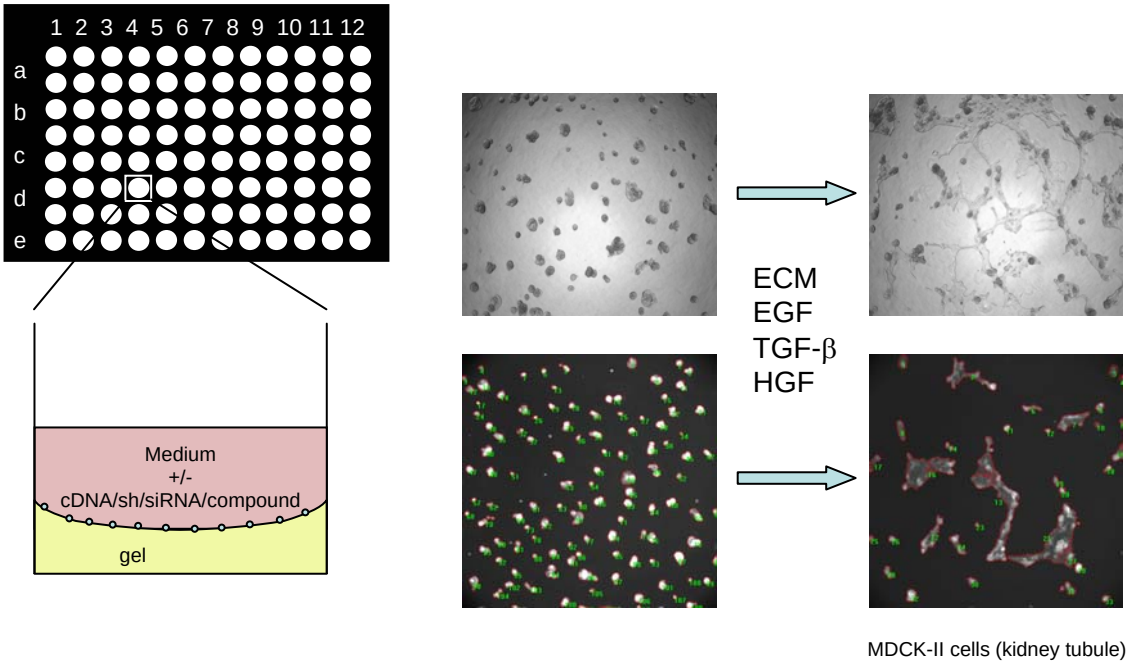
In this sense, the resulting gene function and the new cell therapy information has provided the PROLIGEN consortium with the necessary tools to deliver new biological and cell-based therapies for kidney regeneration.


GeneChip

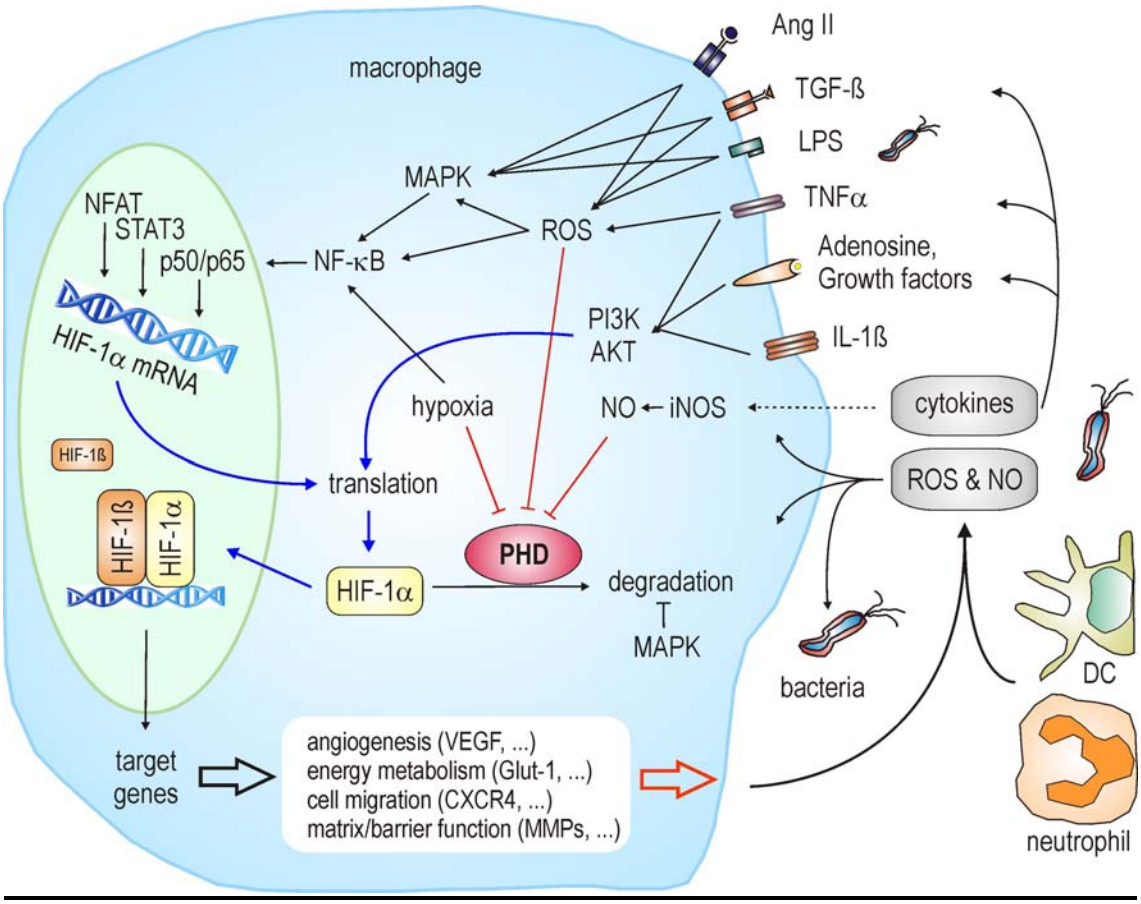




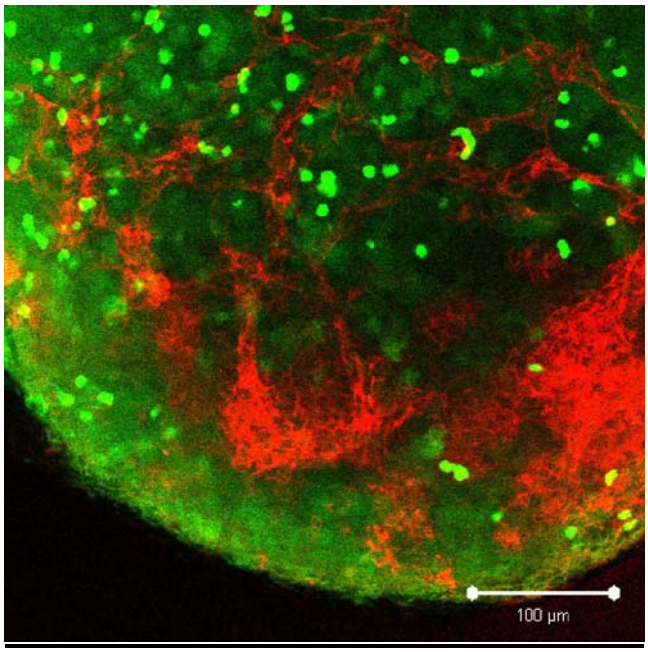
Picture of renal tubule epithelial cells cultured in 3D, stained for f-actin (red) and nuclei (blue)



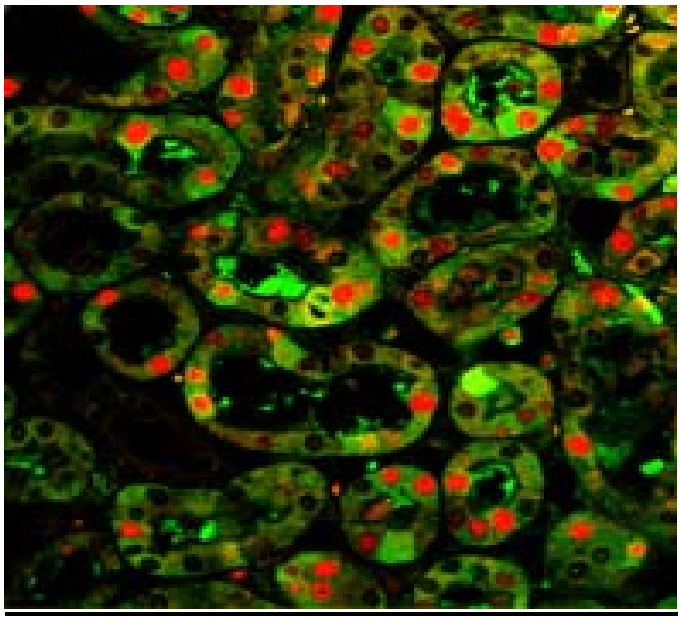
High throughput 3D morphogenesis assay – 96 well



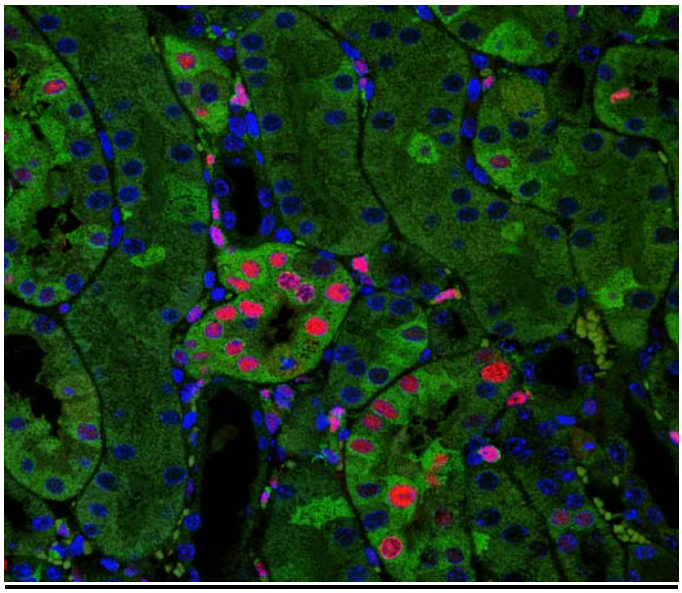
Multiple pathways provoking HIF-responses



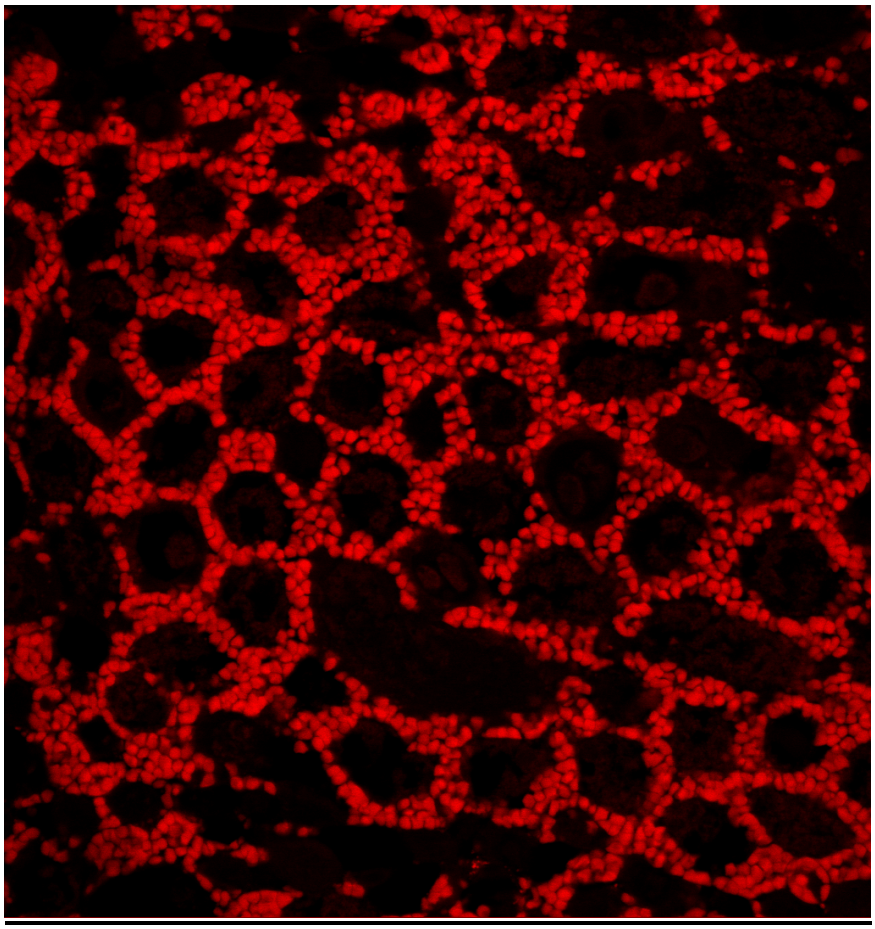
Confrontation assay of embryonic stem cells forming vessel structures in tumor spheroids (red: CD31 staining)



Picture of renal tissue regeneration, stained for PCNA (red) and stathmin (green)



Picture of renal tissue regeneration, stained for PCNA (red), stathmin (green) and dapi (blue).



Picture of macrophage infiltration into renal tissue.

2.- DISSEMINATION AND USE

DISSEMINATION OF KNOWLEDGE

We provided the maximum diffusion of the relevant results achieved in the project, except in the case of patentable results. Then, the following activities have been carried out (for more information, see *Appendix 1*):

- Publication of the project objectives and achievement in the technical journals and conferences
- Publication of press releases in international magazines
- Setting up the PROLIGEN website (http://www.proligen.euthat will be continuously updated
- Participation in workshops to present results of the project
- And finally, we have participated in national programmes to take profit of other different resources available as well as programmes in the Commission for media and information activities (http://www.mostra.com/dg_research/smes_catalogue_080318.PDF).

- **SECTION 1 - EXPLOITABLE KNOWLEDGE AND ITS USE**

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
1.New cell therapy to regenerate injured kidneys		pharmaceutical industry medical	2012 2010	A patent is filled	Partner 1
2. Different sets of regenerative genes		pharmaceutical industry medical	2011	Patents are planned, sets are validated, overexpression of the different genes is required fro theraphy sugestion.	P1, P2, P3 and P4 could be owner.
3.- Carbonic anhidrase II in renal regeneration		pharmaceutical industry medical	2012	A patent is filled	Partner 1
4.- 3D cell assays.		pharmaceutical industry	2010	A patent is filled	Partner 4 and 6
5.- Organ protection (renal ischemia).		pharmaceutical industry medical	2012	A patent is filled	Partner 4

- **SECTION 2 – DISSEMINATION OF KNOWLEDGE**

Overview table

Planned/actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
15/November/2006	Press release (press)	General public	**Around the world**		Partner 6
28/February/2008	Press release (press)	General public	**Spain**		Partner 1
2008, 2009, 2010	Conference	Research	**Undetermined**		Partner 1, 2, 3, 4
2008, 2009, 2010	Publications	Scientific public	**Undetermined**		Partner 1, 2, 3, 4
June/2008	Project web-site	General public	**Undetermined**		Partner1
28/February/2008	leaflets	Specialized public	**Europe**		Partner1
March/2009	Exhibition (Parliament magazine 285, 30 March 2009)	General public	**Europe**		Partner 1
March/2009	Exhibition (Parliament magazine 283, 2 March 2009)	General public	**Europe**		Partner 1

Google page gives 261 matches when PROLIGEN and Hotter keywords are introduced. As example, below some links:

www.igerontologico.com/noticias_desarrollo.php?idnoticia=4528 - 12k
www.levante-emv.com/secciones/noticia.jsp?pRef=3828_44_413421__Ciencia-y-Salud-Investigan-regeneracion-ce... - 71k
www.plataformasinc.es/index.php/esl/content/keyword/PROLIGEN
www.jano.es/jano/ctl_servlet?_f=11&iditem=1833&idtabla=1

Publications for scientific public:

Publication of achievements derived from the project in international scientific literature. Specifically, the following work has been published:

A. Weigert, A. Johann, A. von Knethen, H. Schmidt, G. Geisslinger and B. Brüne. Apoptotic cells promote macrophage survival by releasing the anti-apoptotic mediator sphingosine-1-phosphate. Blood 108:1635-1642 2006

B. Brüne and J. Zhou.Hypoxia inducible factor-1Î± (HIF-1Î±) under the control of nitric oxide. Methods in Enzymology 435:463-478 2007

B. Herr, J. Zhou, S. Drase and B. Brüne. The interaction of superoxide with nitric oxide destabilizes of hypoxia inducible factors-1.Cell. & Mol. Life Sci., 64:3295-3305 2007

A. Weigert, N. Tzieply, A. von Knethen, A.M. Johann, H. Schmidt, G. Geisslinger and B. Brüne. Tumor cell apoptosis polarizes macrophages - role of sphingosine-1-phosphate. Mol. Biol. Cell, 18:3810-3819 2007

B. Brüne and J. Zhou. Nitric oxide and superoxide: interference with hypoxic signaling. Cardiovasc. Res. 75:275-282 2007

A.M. Johann, V. Barra, A.M. Kuhn, A. von Knethen, A. Weigert and B. Brüne. Apoptotic cells induce arginase II in macrophages thereby attenuating nitric oxide production. FASEB J. 21: 2704-2712 2007

J. Zhou, A.E. Damdimopoulos, G. Spyrou and B. Brüne.Thioredoxin 1 and thioredoxin 2 have opposed regulatory functions on hypoxia inducible factor-1.J. Biol. Chem. 282:7482-7490 2007

Vinuesa E, Hotter G, Jung M, Herrero-Fresneda I, Torras J, Sola A.Macrophage involvement in the kidney repair phase after ischaemia/reperfusion injury. J Pathol. 2008 Jan; 214(1):104-13

Vinuesa E, Sola A, Jung M, Alfaro V, Hotter G. Lipocalin-2-induced renal regeneration depends on cytokines. Am J Physiol Renal Physiol. 2008 ;295:F1554-62.

J. Zhou, C. Eleni, G. Spyrou and B. Brune. The mitochondrial thioredoxin system regulates nitric oxide-induced HIF-1Î± protein. Free Rad. Biol. & Med. 44:91-98 2008

A.M. Johann, A. Weigert, W. Eberhardt, A-M. Kuhn, V. Barra, A. Weigert, A. von Knethen, J. Pfeilschifter and B. Brüne. Apoptotic cell-derived S1P promotes HuR-dependent COX-2 mRNA stabilization and protein expression. J. Immunology 180:1239-1248 2008

I. Rama, B. Brüne, J. Torras, R. Köhl, J.M. Cruzado, O. Bestard, M. Franquesa, N. Lloberas, A. Weigert, I. Herrero-Fresneda, O. Gulias, J.M. Grinyo: Hypoxia stimulus: An adaptive immune response during dendritic cell maturation Kidney International 73:816-825 2008

C. Jennewein, A-M. Kuhn, M.V. Schmidt, V. Meilladec-Jullig, A. von Knethen, F. Gonzalez and B. Brüne: Sumoylation of PPARγ by apoptotic cells prevents LPS-induced NCoR removal from kB binding sites mediating transrepression of pro-inflammatory cytokines. J. Immunology 181:5646-5652 2008

Weigert and B. Brüne: Nitric oxide, apoptosis and macrophage polarization during tumor progression. Nitric Oxide 19:95-102 2008

S. Schnitzer, A. Weigert, J. Zhou and B. Brüne: Hypoxia enhances SphK2 activity and provokes S1P-mediated chemoresistance in A549 lung cancer cells. Mol. Cancer Res. 7:393-401 2009

N. Weis, A. Weigert, A. von Knethen and B. Brüne: Heme oxygenase-1 contributes to an alternative macrophage activation profile induced by apoptotic cell supernatants. Mol. Biol. Cell 20:1280-1288 2009

J. Zhou, N. Dehne and B. Brüne: Nitric oxide causes migration of macrophages via HIF-1-stimulated small GTPase Cdc42/Rac1. Free Rad. Biol. & Med. 47:741-749 2009

A. Weigert, S. Schiffmann, D. Sekar, S. Ley, H. Menrad, C. Werno, S. Grösch, G. Geisslinger and B. Brüne: Sphingosine kinase 2 deficient tumor exnografts show impaired growth and fail to polarize macrophages towards an anti-inflammatory phenotype. Int. J. Cancer 135:2114-2121 2009

B. Herr, J. Zhou, C. Werno, H. Menrad, D. Namgaladze, A. Weigert, N. Dehne and B. Brüne: Polarization of macrophages by apoptotic cells causes transcriptional activation of HIF-1α via sphingosine-1-phosphate and transforming growth factor-ß. Blood 114:2140-2148 2009

A. Weigert, D. Sekar and B. Brüne: Tumor associated macrophages as targets for tumor immunotherapy. Immunotherapy 1:83-95 2009

N. Dehne and B. Brüne: HIF-1 in the inflammatory microenvironment. Exp. Cell Res. 315:1791-1707 2009

A. Weigert, C. Jennewein and B. Brüne:
The liaison between apoptotic cells and macrophages – the end programs the beginning. Biol. Chem. 390:379-390 2009

Jung M, Hotter G, Viñas JL, Sola A.Cisplatin upregulates mitochondrial nitric oxide synthase and peroxynitrite formation to promote renal injury. Toxicol Appl Pharmacol. 2009 15;2346.

M. Franquesa, M. Riera, I. Herrero-Fresneda, A. Sola, G. Hotter, N. Lloberas, J.M. Cruzado, J. Torras, and J.M. Grinyó Tubular Epithelial Cells Transfected With hHGF Counteracts Monocyte Chemotactic Protein-1 Up-regulation After hpoxia/Reoxygenation Insult Transplantation Proceedings, 41, 2069–2072 (2009) 2069

A. Weigert, N. Weis and B. Brüne: Regulation of macrophage functions by sphingosine-1-phosphate. Immunobiology 214:748-760 2009

E. Igwe, S. Essler, N. Al-Furoukh, N. Dehne and B. Brüne: Hypoxic transcription gene profiles under the modulation of nitric oxide in a nuclear run on-microarray and proteomics. BMC Genomics 10:408 2009 (doi:10.1186/1471-2164-10-408)

Website:

Project website is available since June 2008, the private web is functional since the beginning of the Project.
The web site is the following: http://www.proligen.eu

Leaflets:

Leaflets has been performed and sent to the partners in order to disseminate the project.
Leaflets could be obtained directly from our web http://proligen.eu

Congress:

VESICULAR TRANSPORT AND CANCER, Beatson
Institute, Scotland. SUNDAY 21ST SEPTEMBER 2008. Poster presentation.
Cell Based Assays, London. June 2008. Oral Presentation

Gorden Research Conference
1 - 7 of March, 2009, Il Ciocco, Italy
Talk: Mechanism of nitric oxide regulation of HIF-1α

2009 Villa Vigoni Conference of Redox-Regulation
11 – 14 of March 2009, Menaggio, Italy
Talk: Nitric oxide and HIF-1: Regulation and function

8th Luebeck Conference on EPO
30th of July to 1st of August, 2009, Lübeck, Germany
Talk: Activation of macrophages by apoptotic cells causes transcriptional activation of HIF-1α via sphingosine-1-phosphate and tumor growth factor-ß

IX Worl Congress of the International Society for Adaptive Medicine
2 - 5 August 2009, Taipei, Taiwan
Talk: Macrophage polarization by apoptotic cells

Second international symposium on cancer biology "Cancer: Found in Translation"
2 - 3 of September 2009, Belfast, UK
Poster 1: HIF-1 affects polarization and functional responses of tumor-associated macrophages
Poster 2: Divergent roles of HIF-1α vs. HIF-2α in the survival of multicellular tumor spheroids

European Association for Cancer Research "Inflammation and Cancer"
24 - 25 of September, 2009, Berlin, Germany
Poster 1: Activation of macrophages by apoptotic cells causes transcriptional activation of HIF-1α via sphingosine-1-phosphate and tumor growth factor-ß
Poster 2: High-throughput RNAi screen for inducers of IL-10 production in macrophages

EMDS Meeting (European Macrophage & Dendritic Society)
24 - 29 of September 2009, Regensburg, Germany
Poster 1: Heme Oxygenase-1 contributes to an alternative macrophage activation profile induced by apoptotic cells
Poster 2: Regulation of NADPH-Oxidase during alternative macrophage activation
Poster 3: Role of HIF-1 in polarizing tumor-associated macrophages
Poster 4: Sphingosine kinase-2 deficient tumor xenografts show impaired growth and fail to polarize macrophages towards an anti-inflammatory phenotype
Talk: Polarization of macrophages by apoptotic cell-derived S1P

6th DGKL Conference
7 - 10 of October, 2009, Leipzig, Germany
Talk: S1P of apoptotic cells polarizes macrophages

1st International Symposium on Research in Pharmaceutical Sciences
13 - 17 of October, Sao Paulo, Brazil
Talk: Reprogramming of macrophages by apoptotic cells – implications for the tumor microenvironment

5-9 October 2008, Mallorca, Spain. EMBO Conferences Series: The Molecular and Cellular basis of regeneration and tissue repair
Poster 1: Lipocalin-2 inducing renal regeneration depends on cytokines
Poster 2: Genetically modified bone marrow-derived macrophages overexpressing IL-10 protect from renal ischemia/reperfusion injury and promote regeneration.

20-21 November 2008, Barcelona, Spain. CONGRESO:XV Symposium of the "Reina Sofía" Institute of the Iñigo Álvarez de Toledo Renal Foundation and IV Joint Symposium of the European Renal, Dialysis and Transplantation Association (ERA-EDTA)
Talk: Role of macrophages in epithelial regeneration after renal ischemia

22-25 February 2009, Barcelona, Spain. Décimo congreso societat catalana de Trasplantament.
Poster 1: La reparación tisular es dependiente de macrófagos en la isquemia/repercusión renal.
Poster 2: La regeneración renal inducida por Lipocalina-2 tras el síndrome de isquemia/reperfusión depende de citoquinas.

30 september- 2 october 2009, Granada, Spain. V Congreso de la sociedad española de terapia génica y celular.
Talk: Lcn-2 overexpressing bone marrow- derived macrophages promote renal regeneration.