### KIDNEY CANCER - Molecular mechanisms underlying control of renal epithelial proliferative homeostasis

**Time period:** 2010-12-01 - 2015-11-30  
**Instrument:** Support for Frontier Research (ERC)  
**Call:** ERC-2010-StG_20091118

This research grant has two major aspects. The first seeks to understand the molecular and cellular basis of the evolution of clear cell renal cell carcinoma (ccRCC), the most frequent form of kidney cancer. We will utilise an integrated approach based on mouse genetics, the use of primary kidney epithelial cell culture systems, genetic screening approaches using RNA interference libraries and analysis of the genetic and molecular changes that arise in human kidney tumours. The rationale behind these studies is that by better understanding the molecular causes of ccRCC it will be possible to identify new molecules or signaling pathways that could serve as appropriate therapeutic targets. The second aspect of this grant relates to the development of a flexible experimental platform that will allow the rapid and simultaneous up- and down-regulation of gene expression in the mouse kidney in a manner in which the affected cells are marked by a luminescent marker. This system will be based on the injection of modified lentiviral gene overexpression and gene knockdown vectors, allowing us to exploit recently-developed genome-wide cDNA libraries and RNA interference libraries. This experimental system should be equally applicable to other organ systems and will allow for the first time a systematic approach to the manipulation of gene expression in living mice, additionally bypassing the time limitations associated with conventional mouse genetic approaches. We aim to develop this system within the biological context of this grant and will combine it with live-animal imaging approaches to generate a series of mouse models of ccRCC. These will ultimately serve as invaluable tools for testing novel therapeutic approaches against this currently untreatable disease.

**Principal investigators**

**Scientific co-ordinator:**  
Ian James Frew (UNIVERSITAET ZUEРИCH)

### KIDREGEN - Investigating the ability of embryonic stem cell derivatives to improve renal function in a murine model of kidney disease.

**Time period:** 2010-09-01 - 2012-08-31  
**Instrument:** Marie Curie Actions (MCA)  
**Call:** FP7-PEOPLE-2009-RG

The number of people worldwide with end-stage renal disease (ESRD) is increasing every year. Current treatment options consist of dialysis and transplantation, both of which have significant side effects in terms of quality and quantity of life. Therefore there is an urgent need to develop alternative therapies. My recent work has shown that if mouse embryonic stem cells (mESC) are directed to differentiate to mesodermal cells, they show high potential for integrating into developing nephrons in a mouse kidney rudiment ex vivo. Moreover, the ability of mESC derived mesoderm to generate renal cell types was highly comparable to that of metanephric mesenchyme (MM), which are the cells that give rise to the nephron in the developing kidney. Although these results are encouraging, a key test will be to investigate if the mESC-derived mesoderm cells can generate nephric cell types in a rodent model of kidney disease and if these cells are able to improve renal function. Therefore the aim of this project is to explore the potential for renal replacement therapy from exploitation of the unique properties of mESC. This will be tested by injecting the stem cells into the tail vein of mice with induced kidney injury, following which, the ability of the cells to generate renal cell types and improve renal function will be analysed. The propensity of the stem cells to generate inappropriate cell types or tumours in the animal model will also be tested. A further objective will be to develop an MRI-based tracking system so that the stem cells can be monitored non-invasively following transplantation. The project will form the basis of a long term collaboration between the applicant and the host group at the University of Liverpool.

**Principal investigators**

**Scientific co-ordinator:**  
Patricia Ann Murray (THE UNIVERSITY OF LIVERPOOL)
KIDREGEN - Investigating the ability of embryonic stem cell derivatives to improve renal function in a murine model of kidney disease.

Time period: 2010-09-01 - 2012-08-31
Instrument: Marie Curie Actions (MCA)
Call: FP7-PEOPLE-2009-RG

The number of people world-wide with end-stage renal disease (ESRD) is increasing every year. Current treatment options consist of dialysis and transplantation, both of which have significant side effects in terms of quality and quantity of life. Therefore there is an urgent need to develop alternative therapies. My recent work has shown that if mouse embryonic stem cells (mESC) are directed to differentiate to mesodermal cells, they show high potential for integrating into developing nephrons in a mouse kidney rudiment ex vivo. Moreover, the ability of mESC-derived mesoderm to generate renal cell types was highly comparable to that of metanephric mesenchyme (MM), which are the cells that give rise to the nephron in the developing kidney. Although these results are encouraging, a key test will be to investigate if the mESC-derived mesoderm can generate nephric cell types in a rodent model of kidney disease and if these cells are able to improve renal function. Therefore the aim of this project is to explore the potential for renal replacement therapy from exploitation of the unique properties of mESC. This will be tested by injecting the stem cells into the tail vein of mice with induced kidney injury, following which, the ability of the cells to generate renal cell types and improve renal function will be analysed. The propensity of the stem cells to generate inappropriate cell types or tumours in the animal model will also be tested. A further objective will be to develop an MRI-based tracking system so that the stem cells can be monitored non-invasively following transplantation. The project will form the basis of a long term collaboration between the applicant and the host group at the University of Liverpool.

Principal investigators
Scientific co-ordinator: Patricia Ann Murray (THE UNIVERSITY OF LIVERPOOL)

CAGEKID - Cancer Genomics of the Kidney

Time period: 2010-03-01 - 2014-02-28
Instrument: Collaborative Project
Call: FP7-HEALTH-2009-two-stage

The International Cancer Genome Consortium (ICGC) has the goal of obtaining a comprehensive description of genomic, transcriptomic and epigenomic changes in 50 different tumour types and/or subtypes, with the aim of elucidating the genomic changes present in the many forms of cancers that contribute to the burden of disease throughout the world. We present a proposal for a European contribution to this effort through application of state-of-the-art approaches to the genomics of the most common form of renal cell cancer (RCC). RCC is of particular importance within Europe where the highest global incidence rates are observed. Disease incidence has increased over the last two decades, and it is now the 8th most common cancer in the EU. CAGEKID brings clinical and epidemiological resources that are unique worldwide together with the necessary genetics and genomics expertise required for this effort. In the first phase of the study, we will provide a full genomic characterisation of 100 matched pairs of DNA extracted from the tumour and constitutional samples. DNA will be completely sequenced, and the data brought together with those from whole genome transcript and methylation analyses. Follow-up studies of potential targets will be made in further samples. The results acquired will be relied on targeted protein analyses. The primary data will be made available to the scientific community, and the programme will contribute to establishing norms for the manipulation and storage of biological samples. CAGEKID will provide the first systematic analysis of this tumour site providing new insights into disease aetiology with application for diagnosis and treatment. It addresses a major need to identify new biological markers for renal cell cancer, one of very few tumour types for which there are currently no biological markers in routine clinical use. Renal cancer is not yet supported by any of the members of the ICGC.
**CX43-CRF - Implication of connexin 43 in chronic renal failure**

**Time period:** 2008-12-01 - 2010-11-30

**Instrument:** Marie Curie Actions (MCA)

**Call:** FP7-PEOPLE-2007-2-1-EF

Chronic renal failure (CRF), one of the main causes of disability in western societies, is promoted by a variety of factors including hypertension, diabetes, ischemic, immunological and toxic injury. These factors are linked by their common ability to promote chronic inflammation and fibrosis leading to decline of renal function. Dialysis and transplantation are the only available options that allow survival of patients. Arresting the progression of CRF is one of the major challenges of public health today. Alterations of the expression of the gap junction protein connexin 43 (Cx43) have been associated to the development of inflammation in chronic vascular pathologies. Thus, Cx43 expression was increased during atherogenesis, whereas Cx43 inhibition protected vessels from the development of atherosclerotic plaque. An up-regulation of the Cx43 expression has been also reported in renal inflammation and hypertension suggesting that this connexin may be involved in renal disease. In this project we intend to study the role of Cx43 in CRF and to propose treatments and diagnostic tools targeting this protein to protect against the disease. Thus, in our project: i) we will use the RenTg mice, expressing high steady levels of renin, to study modulation of Cx43 expression during progression of hypertension-induced renal disease; ii) Cx43-specific blockers will be administer to RenTg mice to see whether decreasing Cx43 expression could reverse the decline of renal function. Cx43 expression will be also reduced genetically by interbreeding the RenTg with the Cx43+/- mice; iii) we will attempt to delineate molecular mechanisms involving Cx43 regulation in endothelial cells in vitro under angiotensin II treatment, a peptide known to participate to renal vascular fibrosis. As perspectives, we will transfer knowledge obtained from this project in humans by testing renal biopsies. Thus, we hope to establish a correlation between Cx43 expression and human CRF.

**Principal investigators**

**Scientific co-ordinator:**
Jean-Claude Dussaule (INSERM - Institut national de la sante et de la recherche medicale)

---

**RESCARF - Renal stem cells: possible role in kidney pathologies and as new therapeutic tools**

**Time period:** 2008-10-01 - 2012-09-30

**Instrument:** Support for Frontier Research (ERC)

**Call:** ERC-2007-StG

Chronic Kidney Disease (CKD) affects 11% of the adult population and is considered by the WHO as one of the health
emergencies of the 21st century. Although cell therapy might be beneficial for CKD, human stem cells that might be used to improve kidney function were so far unknown. Recently, we demonstrated the existence of resident stem cells in the urinary pole of the Bowman’s capsule of adult human kidney and therefore named as adult parietal epithelial multipotent progenitors (APEMP). Injection of APEMP in SCID mice affected by acute renal failure, induced regeneration of tubular structures and reduced morphological and functional kidney damage. More recently, we found that APEMP are highly represented in embryonic kidneys and constitute the common progenitor of tubular cells and podocytes. The first aim of this project is to assess the regenerative properties of APEMP in in vivo models of glomerular injury and their potential use as a novel therapeutic tool to prevent the deterioration of kidney function in chronic renal failure. Second, we will try to identify the mechanisms that regulate the growth, survival, differentiation, and migration of APEMP, which is critical to set up cell therapies of renal injury which should be effective and safe. To this end, the role of different molecular pathways such as Sonic hedgehog, Wnt/beta-catenin, Notch, TGF-beta/BMP and of CXCR4, CXCR7 or CXCR3-B chemokine receptors in the regenerative activity of APEMP will be investigated. Third, to assess whether APEMP directly contribute to kidney regeneration after glomerular or tubular damage, transgenic animals in which APEMP are genetically tagged will be generated. Fourth, by using transgenic animals we will try to understand if an alteration of APEMP growth and/or differentiation is implicated in the pathogenesis of some renal disorders that frequently progress towards end stage renal disease.

Principal investigators
Scientific co-ordinator:
Mario Serio (UNIVERSITA DEGLI STUDI DI FIRENZE)

RENALSTEM - Developing a stem cell based therapy to replace nephrons lost through reflux nephropathy

Principal investigators
Scientific co-ordinator:
Patricia Ann Murray (THE UNIVERSITY OF LIVERPOOL)

EUNEFRON - European Network for the Study of Orphan Nephropathies
In this proposal, we have mobilized a critical mass of expertise to investigate, on a Europe-wide scale, the natural history and pathophysiology of rare inherited diseases affecting important structures of the kidney. The project will use and develop multiple models (in vitro and in vivo) with the aim to develop preventive, diagnostic and therapeutic interventions that should alleviate the burden of these diseases, particularly in children. A central part of the proposal is the creation of a European registry and a network of genetic laboratories to foster a tight interaction between physicians and researchers, promote clinical and basic research, and ensure the efficient dissemination of knowledge. By increasing our knowledge of these rare diseases, the EUNEFRON project will also yield new insights into basic processes relevant for the general population (progression of renal disease, blood pressure control, prevention of renal stones, effect of gender and ageing, etc.), the complex relationship between different nephron segments, and the multi-systemic involvement of renal diseases.

Principal investigators

Scientific co-ordinator:
Olivier Devuyst (UNIVERSITE CATHOLIQUE DE LOUVAIN)
Other principal investigators:
Corinne Antignac (INSERM - Institut national de la sante et de la recherche medicale)
Erik Ilse Christensen (AARHUS UNIVERSITET)
Peter Deen (STICHTING KATHOLIEKE UNIVERSITEIT)
Dominik Muller (CHARITE - UNIVERSITAETS MEDIZIN BERLIN)
Carsten Wagner (UNIVERSITAET ZUERICH)
Luca Rumpoldi (FONDAZIONE CENTRO SAN RAFFAELE DEL MONTE TABOR)
William van’t Hoff (UNIVERSITY COLLEGE LONDON)
Elena Levtchenko (KATHOLIEKE UNIVERSITEIT LEUVEN)

CHRONIOUS - An Open, Ubiquitous and Adaptive Chronic Disease Management Platform for COPD and Renal Insufficiency

Time period: 2008-01-31 - 2012-01-31
Instrument: Collaborative Project
Call: FP7-ICT-2007-1

Chronic diseases are those that occur across the whole spectrum of illness, mental health problems and injuries. Management includes medication and/or lifestyle changes such as diet and exercise. At the same time, it should be noted that chronic diseases may get worse, lead to death, be cured, remain dormant or require continual monitoring. CHRONIOUS primary goal is to define a European framework for a generic health status monitoring platform schema addressing people with chronic health conditions. This will be achieved by developing an intelligent, ubiquitous and adaptive chronic disease platform to be used by both patients and healthcare professionals. CHRONIOUS addresses a smart wearable platform, based on multi-parametric sensor data processing, for monitoring people suffering from chronic diseases in long-stay setting. It is constantly monitoring their activity using audio observation methods and activity sensors while at the same time tracking their medical condition via vital signs sensors. Any trait of abnormal health status and possible alerting incidents are detected by CHRONIOUS Intelligence. The system generates alerts in case of invalid medical data or if current activity and behaviour lay outside the well established activity patterns and locomotion behaviour. Furthermore, CHRONIOUS objective is to face Europe’s challenge for delivering quality healthcare to all its citizens by offering a ubiquitous and more personalised care solution that addresses the user needs, personal data security, confidentiality and privacy of information and all that at an affordable cost. Our proposed solution will be applied to the chronic diseases of Chronic Obstructive Pulmonary Disease (COPD) and Chronic Kidney Disease (CKD) and Renal Insufficiency.

Principal investigators

Scientific co-ordinator:
Roberto Rosso (TESAN S.P.A.)
Other principal investigators:
Daniela Matarrese (AZIENDA OSPEDALIERO-UNIVERSITARIA CAREGGI)
Xavier Gutierrez (UNIVERSITAT DE BARCELONA)
Miriam Weißenborn (UNIVERSITATET BREMEN)
Paula Baeta (LINK CONSULTING - TECNOLOGIAS DE INFORMACAO S.A.)
Diseases of the kidney represent a major cause of morbidity and mortality in Europe. The elderly are disproportionately affected, but renal disease is also a condition that severely affects children. An estimated 4.5 Million Europeans suffer from renal disorders. The death rate in patients with renal failure is 20% annually. This disease burden and its challenge for our societies is the focus of this proposal. Elucidation of the human and other genomes heralds a new era in biomedical research offering unprecedented opportunities to understand disease processes and to identify strategies to improve health. We will embrace these opportunities and implement an interdisciplinary research program, the European Renal Genome Project (EuReGene) that integrates European excellence in research relevant to renal development, pathophysiology and genetics. Our goal is to discover genes responsible for renal development and disease, their proteins and their actions. To achieve this goal, we have established a consortium of leading scientists, clinicians and SME partners that will focus on the development of novel technologies and discovery tools in functional genomics and their application to kidney research. We will rely on comparative genomic studies in many systems that provide utilitarian models ranging from zebrafish, to Xenopus, to mice, to rats. Our studies will be performed at different levels including the gene, the cell, the organ and the organism. Ultimately, identification of disease genes will lead to a better understanding of renal disease processes, to improved diagnosis and to new concepts in therapy. Our program will establish a paradigm for an integrated post-genomic approach to analyze renal disease-related developments that may be transferred to other organ systems or disease entities in the future.

Principal investigators

Scientific co-ordinator:
Thomas E. Willnow (MAX DELBRUECK CENTRUM FUER MOLEKULARE MEDIZIN)

Other principal investigators:
Nicholas Hastie (MEDICAL RESEARCH COUNCIL)
Corinne Antignac (INSERM - Institut national de la sante et de la recherche medicale)
Olivier Devayst (UNIVERSITE CATHOLIQUE DE LOUVAIN)
André Werner Brändli (Eidgenossische Technische Hochschule Zuerich)
Giuseppe Remuzzi (ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI")
Heini Murer (UNIVERSITAET ZUERICH)
Gregor Eichele (MAX PLANCK GESELLSCHAFT ZUR FOERDERUNG DER WISSENSCHAFTEN E.V.)
Seppo Juhani Vainio (OULUN YLIOPISTO)
Thomas J. Jentsch (UNIVERSITAET HAMBURG)
Erik Ilse Christensen (AARHUS UNIVERSITET)
Rajesh V. Thakker (THE CHANCELLOR, MASTERS AND SCHOLARS OF THE UNIVERSITY OF OXFORD)
Matthias Kretzler (LUDWIG-MAXIMILIANS-UNIVERSITAET MUENCHEN)
Anders Nykjær (RECEPTICON APS)
ALDOSTERONE-FELLOW - Molecular determinants of sodium transport: role of a new aldosterone induced gene

The epithelial Na+ channel (ENaC) plays a major role in the homeostasis of extracellular Na+ and consequently of blood volume and pressure. Its importance is underlined by its genetic linkage to two renal diseases, pseudohypoaldosteronism type I, and of Liddle’s syndrome, which are both caused by mutations in the genes encoding ENaC. ENaC, which facilitates entry of Na+ into the cell, is the rate-limiting step of Na+ reabsorption. It is highly regulated by a variety of factors, including aldosterone and vasopressin, but the molecular mechanisms of their action are still poorly understood.

Aldosterone induces and/or represses a number of genes, which consequently lead to the stimulation of transepithelial transport. We have identified a novel protein, NDRG2 (N-myc Downstream Regulated Gene 2) whose expression is very early stimulated by aldosterone, both in established cell lines, and in the kidney and colon of rats. NDRG2 belongs to a family of genes of unknown function, which is conserved in plants, invertebrates and mammals, suggesting important functions. Its identity with MESK2, a gene recently identified in Drosophila, suggests that it may be involved in the Ras/MAPK signaling pathway. Our preliminary data suggest that aldosterone does influence Ras activity, and co-expression of NDRG2 with ENaC into Xenopus laevis oocytes elevates ENaC activity as compared to control oocytes. My project will be focussed on the analysis of NDRG2 function, in cell cultures, and in vivo by transgenesis. We will use conditional systems (tet inducible and HoxB7 promotor kidney-targeting in mice, Tamoxifen-sensitive Cre-Lox in cells) to evaluate the consequences of NDRG2 overexpression on renal collecting duct differentiation, polarity and sodium transport capacities.

Constructs have been made, cell transfection is in progress and mouse generation will be initiated in March 2004. We will search for alterations in sodium transport after NDRG2 overexpression.

Principal investigators
Scientific co-ordinator:
Nicolette Farman (INSERM - Institut national de la sante et de la recherche medicale)

ADDNET - Paradigm shift from kidney biopsies to advanced molecular diagnostics from patient urine

BACKGROUND: Proteinuria is a sign of kidney involvement in association with common infectious, inflammatory, immunological or metabolic (diabetes) diseases. When persisting, proteinuria leads to scarring and end-stage kidney disease requiring dialysis or renal transplantation. Both treatments are chronically debilitating, increase risk for severe secondary complications and are extremely expensive. Altogether, kidney complications constitute more than 15% of total health-care costs in most Western countries, mainly due to increasing prevalence of diabetes-associated kidney disease. PROBLEM: Earlier diagnostics is urgently needed to target intensive treatment efforts and to avoid the projected explosive increase in the number of kidney patients in near future. Due to the demographic trends, kidney diseases are a particular problem for Europe. At present, the diagnostics include serum markers (mostly non-sensitive, non-specific) and urine analysis (too late markers) but relies mainly on patient kidney biopsy samples. Although accurate, this procedure is severely inconvenient, invasive and carries a notable risk for complications. SOLUTION: We propose to use the latest molecular information of verified pathogenetic routes, proprietary bioinformatics platforms, well established in vivo models as well as extensive human sample repositories together with the SME activities to establish and validate new diagnostics. This includes the identification of an expanding set of key molecular markers directly from patient urine to yield novel measurables for early and accurate non-invasive diagnostics. With the set of markers accurately reflecting pathophysiologic changes we expect to replace the traditional kidney biopsies with more patient-friendly, accurate and economical diagnostics directly from urine, easily accessible source. Development will also allow construction of distant monitoring diagnostic platforms to prevent permanent kidney #
**Principal investigators**

**Scientific co-ordinator:**
Harry Holthöfer (HELSINGIN YLIOPISTO)

**Other principal investigators:**
Klaus-Robert Müller (FRAUNHOFER GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V.)
Per-Henrik Groop (SAMFUNDET FOLKHAELSAN I SVENSKA FINLAND R.F.)
Jesus Egido (UNIVERSIDAD AUTONOMA DE MADRID)
Kimmo Kaski (HELSINKI UNIVERSITY OF TECHNOLOGY)
Eric Fung (CIPHERGEN BIOSYSTEM A/S)

---

**NMDANOARF - Role of NMDA receptors on experimental renal failure. Relationship with NO**

**Instrument:** Marie Curie Actions (MCA)

**Call:** FP6-2002-MOBILITY-12

N-methyl-D-aspartate receptor (NMDA-R) is an amino acid receptor and membrane calcium channel. NMDA-R is activated by binding of co-agonists, L-glutamine and L-glycine. In the brain, calcium entry via NMDA-R activates type I nitric oxide synthase (NOS I). The kidney also contains NOS I and vasodilates in response to L-glycine. We recently demonstrated that NMDA-R are expressed in kidney cortex, where they exert a tonic vasodilatory influence and may account for the vasodilatory response to glycine infusion. Nitric oxide is a gas that exerts, among others, vasodilatory effects in the kidney. Me and others have demonstrated a role of nitric oxide in several types of acute renal failure, from aminoglicoside-induced, to ischemic in nature. Also, in a recent paper, I suggested a role for nitric oxide in renal mass reduction due to uninephrectomy. Glycine infusion has been proven to be beneficial in several renal conditions, but the mechanism by which this happens is yet to be elucidated. From cyclosporine nephrotoxicity to ischemic renal failure, increases in glycine availability lead to improvements in renal function. Furthermore, use of NMDA antagonists has been proven to reduce ischemic damage in the brain and aminoglicoside otoxicity. Thus, the relationship between NMDA-R and NO in the kidney in normal and pathological conditions is an exciting field that could lead to new therapies in the treatment of renal diseases.

---

**Principal investigators**

**Scientific co-ordinator:**
Elvira Fernandez Giraldez (FUNDACIÓN DR. PIFARRÉ. HOSPITAL UNIVERSITARIO ARNAU DE VILLANOVA)

---

**KIDSTEM - Developing a stem cell based therapy to replace nephrons lost through reflux nephropathy**

**Time period:** 2006-09-01 - 2010-08-31

**Instrument:** Marie Curie Actions (MCA)

**Call:** FP6-2005-MOBILITY-1

The aim of this project is to design a stem cell-based therapy to prevent end-stage renal disease caused by reflux nephropathy in children. The two main reasons to focus on this condition are that it is the major cause of kidney failure in children and young adults and secondly, the disease typically takes several years to reach end stage, allowing time for therapies to repair damage kidneys before they become completely non-functional. Recent advances in stem cell science and tissue engineering present an unprecedented opportunity to design a stem cell therapy for this clinical problem. This project will investigate the properties of several different stem cell types (embryonic stem cells, kidney stem cells, amniotic fluid stem cells and mesenchymal stem cells) in order to determine which is most appropriate for the generation of functional kidney tissue. To do this, novel biomaterials will be designed that will provide a substrate both for the generation of kidney progenitor cells and for their transplantation.
Principal investigators

Scientific co-ordinator:
Patricia Ann Murray (THE UNIVERSITY OF LIVERPOOL)

Other principal investigators:
Giovanni Cannussi (UNIVERSITA DEGLI STUDI DI TORINO)
Carsten Werner (LEIBNIZ-INSTITUT FUER POLYMERFORSCHUNG DRESDEN E.V.)
Markus Hengstschläger (MEDICAL UNIVERSITY OF VIENNA)
Jamie A. Davies (THE UNIVERSITY OF EDINBURGH)
Giuseppe Remuzzi (ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI")