



**RoFAR**  
Foundation for Anemia Research

# *Annual report*

December 2005

RoFAR is an independent foundation run by an international Board of Trustees and funded by an unrestricted grant from Roche. All submitted applications are peer reviewed by an independent Scientific Advisory Board.



# *Mission*

The Roche Foundation for Anemia Research (hereinafter “the RoFAR”) is a registered Medical Research Charity with the mission of “encouraging innovative research that will open new avenues of exploration in the study of anaemia, its mechanisms and outcomes.” Individuals eligible for grants are members of academic staff in universities, dialysis centres and research institutes.

The RoFAR was established by the Roche Group in 2004 under Swiss law and incorporated in Basel, Switzerland. The Roche Group is committed to providing funding of CHF four million annually for at least four years from inception to a total of at least CHF 16 million.

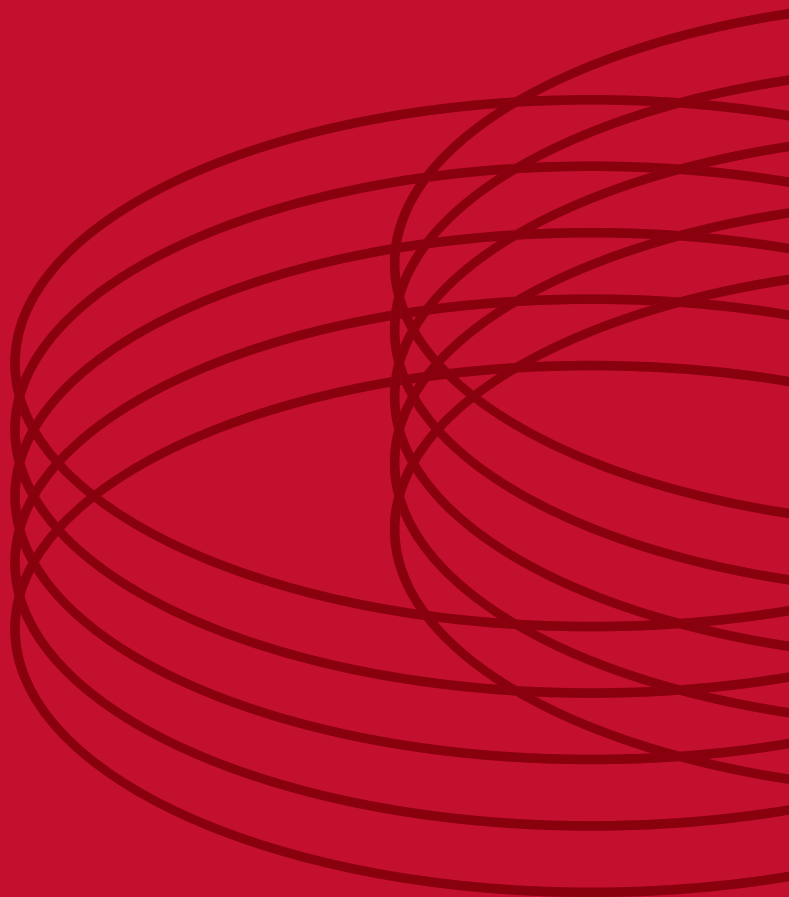
The RoFAR is a non-profit, autonomous and legally independent charitable organisation.

The RoFAR encourages the exploration of new research in areas associated with the study of anaemia, its mechanisms and outcomes. The Board of Trustees will set the focus of research for the specific cycle.

In addition to focusing on anaemia related to kidney disease and oncology, the RoFAR also will encourage research into:

- Anaemia of chronic disease
- Anaemia related to congestive heart failure and stroke
- Effects of erythropoietin and erythropoietin-like substances as protective drugs in various target organs
- Central resistance to erythropoietin
- Biology of anaemia and outcomes

The RoFAR will consider providing limited support for unique initiatives such as projects of general interest to anaemia researchers.



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# 1 *Preface*

We are pleased to announce that in its first two years of activity, RoFAR has awarded grants of over 4.1 million CHF to outstanding research projects dedicated to advancing knowledge in the field of anaemia, its associated complications erythropoietic agents and outcomes. This is a major accomplishment of which all involved can be proud.

Twenty-two applications made during three cycles of competition have been selected to receive RoFAR research grants of up to 200,000 CHF distributed over two years.

There are two cycles of RoFAR awards each year. Timelines for the cycles and the submission deadline for application of an award are published on the Foundation's website.

The first step in the application process is to submit a Letter of Intent (LOI), which once submitted, is reviewed by our Scientific Advisory Board (SAB). Applicants who are considered by the SAB to have submitted the most compelling LOIs are then invited to proceed to the next stage and submit a full application. Full applications are considered in detail by the SAB and final decisions on award winners are confirmed by the Board of Trustees (BT) who undertake to notify applicants of their decision six months after submission of the LOI.

The SAB and the BT have been impressed with the quality of applications received from the first three RoFAR grant award cycles. The Foundation's high expectations for the quality of research projects and applicants have been met.

In addition to regular grants, RoFAR also plans to initiate a special grants programme for a topic of outstanding importance.

A small number of internationally renowned research groups and institutions will be invited to submit applications for the special grant competition. The submission and selection procedure – similar to the one in use for regular grants – will result in an award to a single research group or institution of a grant of up to 2,400,000 CHF.

- advertisements in major scientific journals and on web portals
- distribution of brochures to major cardiology, oncology and nephrology centres
- distribution of leaflets and brochures at national and international scientific congresses
- information booths at selected international congresses
- public announcement of awarded applicants at important international congresses.

In 2006, RoFAR plans to continue advertising its programme both through announcements in major scientific journals and web sites, and by selected activities at a number of scientific congresses.

RoFAR is committed to its mission of fostering innovative anaemia-related research, and RoFAR sincerely hopes to make a major contribution to the scientific community by encouraging scientists to apply their skills and intellect to furthering knowledge and understanding in this field. The Trustees and the Scientific Advisory Board of the RoFAR all join in expressing their gratitude to F. Hoffmann-La Roche Ltd. for its generous gift to the anaemia research community and for Roche's enduring commitment to anaemia and related avenues of research.

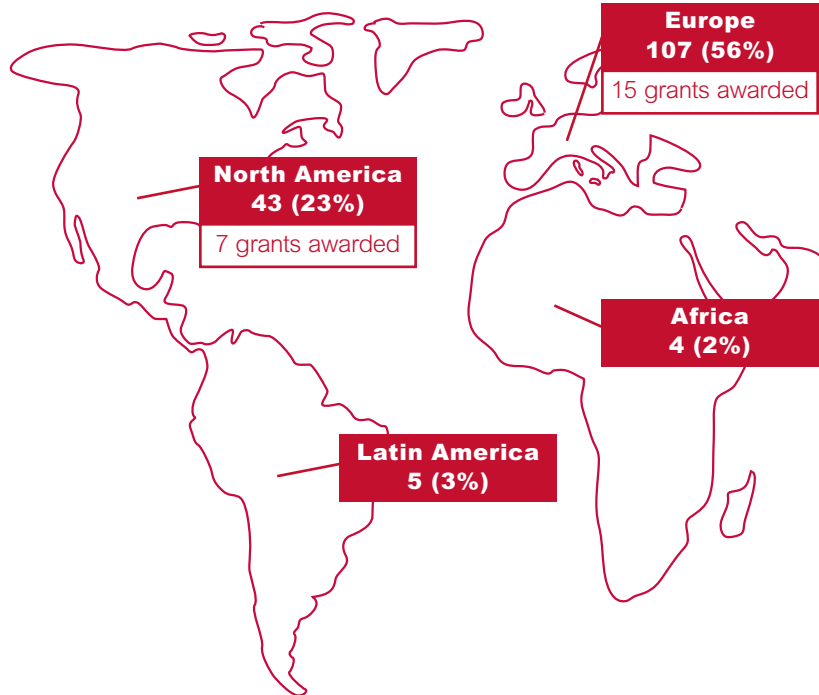
The RoFAR welcomes any feedback or suggestions to assist us in accomplishing its stated mission.

On behalf of the Board of Trustees



Dr. Nathan W. Levin  
Chairman of the Board of Trustees  
Roche Foundation for Anemia Research

## Geographical breakdown of submitted research proposals



Applicants in the first three cycles of competition represent a range of institutions in 38 countries. More than half (56%) of all the LOI applications have been submitted from Europe, primarily from Germany, Italy, Poland, Spain, Switzerland and the UK. About one quarter of the applications (23%) have been submitted from the United States and Canada. About 26% of the applicants are female scientists. The great majority (98%) of applicants work in universities or university-affiliated institutions. Research proposals are distributed among clinical studies (54%), animal trials (31%) and basic science projects. Submitted projects focus on nephrology and diabetology (48%), haematology (41%), oncology (21%) and cardiology (15%) with some overlap between areas.



**Asia and Oceania**  
32 (17%)

Fifteen grants have been assigned to European applicants, seven to North American applicants.

### Submitted research proposals by

#### Study type

Human trials	(54%)
Animal studies	(31%)
Others	(15%)

#### Research focus

Nephrology	(48%)
Haematology	(41%)
Oncology	(21%)
Cardiology	(15%)
Others	(17%)
(multiple allowed)	

#### Gender of main applicant

Males	(74%)
Females	(26%)

#### Institution type

Universities and related	(98%)
Others	(2%)

**Dr Nancy C. Andrews**



### **Children's Hospital Boston, USA**

#### *Hepcidin regulation in the anaemia of chronic disease*

The anaemia of chronic disease (also called the anaemia of chronic inflammation) is an acquired condition that affects patients with a variety of inflammatory disorders including infection, arthritis, inflammatory bowel disease, trauma, organ failure and cancer. It can be severe enough to require blood transfusions. However, even milder anaemia of chronic disease can impair quality of life and general well being. We previously showed that the anaemia of chronic disease results, at least in part, from induction of synthesis of a hormone called hepcidin in response to inflammation. Hepcidin controls how the body uses iron by regulating its absorption through the intestine and its recycling by tissue macrophages. When increased levels of hepcidin are produced both intestinal absorption and iron recycling are blocked, resulting in decreased iron available for erythropoiesis and consequent anaemia. The goal of this project is to understand the normal regulation of hepcidin production and changes in regulation in response to inflammation. We hope that this work will provide information to facilitate the development of drugs that can prevent increased expression of hepcidin in inflammation and ameliorate the anaemia of chronic disease.

**Dr Martin Bergmann (principal applicant)**

*Prof. Reiner Dietz (co-applicant)*



## **Franz Volhard Klinik, Charité Berlin, Germany**

*Effect of 5,000 IU erythropoietin beta once weekly subcutaneously (sc.) administered for three months in patients subjected to percutaneous coronary intervention (PCI) displaying reduced LV-ejection fraction due to regional left ventricular wall motion defects*

Despite increasing success of interventional methods to restore blood supply to ischaemic myocardium, the resultant functional improvement of myocardial contractility is often limited. Erythropoietin (EPO) has been shown to protect cardiomyocytes from apoptosis, enhance the level of circulating progenitor cells possibly contributing to restore functional myocardium in previous ischaemic areas and increase oxygen supply by increasing the haemoglobin levels. All these effects may enhance myocardial function after percutaneous coronary interventions (PCI). Therefore, the study will test the effect of a once weekly dose of EPO beta applied subcutaneously beginning at the time of PCI on the recovery of regional wall motion defects at three month. Cardiac function will be precisely assessed by cardiac magnetic resonance imaging.

**Dr Andrew McKie (principal applicant)**

*Dr Robert J Simpson (co-applicant)*

*Prof. Robert C Hider (co-applicant)*



### **King's College, London, UK**

*Characterisation of a novel intestinal heme transporter*

Iron is an essential nutrient required by the body to make the protein haemoglobin in red blood cells which is essential for delivering oxygen to working muscles and other cells. Too little iron in the diet (iron deficiency) leads to anaemia causing fatigue. On the other hand too much iron is toxic and can damage vital organs like the heart and liver. Red meat is a good source of iron because it contains a lot of heme iron which is efficiently absorbed by the small intestine. We have now identified the protein responsible for the absorption of heme from the diet. In this application we study the proteins involved. One aim will be to develop synthetic dietary supplements based on the structure of heme which would be suitable for treatment of iron deficiency anaemia in vegetarian communities.

**Dr Marco Merlano (principal applicant)**

*Dr Silvana Ungari (co-applicant)*



## **S. Croce General Hospital, Cuneo, Italy**

### *In vitro analysis of tumour response to radiation in oxic and hypoxic conditions*

Despite therapeutic improvements and efforts to develop more efficacious therapies, the majority of Head and Neck Squamous Cell Carcinoma (HNSCC) patients face a poor prognosis. Therefore, the primary goal of current treatment is improvement of quality of life (QOL) and prolongation of survival. Anaemia frequently occurs in HNSCCs and has been associated with decreased QOL, impaired treatment outcomes and shortened survival. Furthermore, anaemia is a causative factor of tumour hypoxia, which compromises the efficacy of radiotherapy. Thus, correction of anaemia seems to have a beneficial effect on QOL and outcome. The human recombinant erythropoietin (rHuEPO) has proven efficacy and safety in correcting anaemia in numerous clinical studies and over a decade of clinical practice. The relationship among anaemia, hypoxia, transfusion and treatment outcome is complex and there certainly remains a lot of room for discussion about the role of hypoxia for tumour development and radiation response. Nevertheless, understanding the biological mechanisms is important to reach the ability of reversing radioresistance, improving QOL in anaemic patients, cancer control and clinical efficacy of radiation. The purpose of the presented study is to establish an experimental model and to provide experimental evidences to examine the relationship between hypoxia, EPO/EPOR and EGFR transcription/expression and their effects on the cellular response to radiation; we will investigate different cellular responses after radiation under oxic and hypoxic conditions and compare these findings to what happens when the cells cultured in hypoxia are reported to oxic conditions and then irradiated. The aim is to better define the biological and molecular bases for the *in vitro* response to hypoxia and to explore its effects on radiotherapy, in order to add knowledge of the mechanisms underlying the negative effects of anaemia on radiotherapy outcome: the identification of the causes of treatment failure may facilitate the development of treatment strategies to improve efficacy and reduce toxicity.

**Dr Peter Mertens**



### **University Hospital Aachen, Germany**

#### *Mechanisms for erythropoietin resistance in transformed and non-transformed cells*

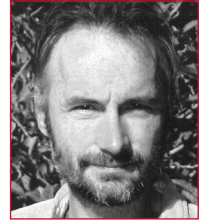
Recent studies indicate that erythropoietin (EPO) fulfils important functions not only in haematopoiesis, but also related to cell survival of non-transformed and transformed cells under hypoxia. Our goal is to unravel causes of cellular EPO resistance with the focus being on an archetypical stress responsive protein, namely Y-box protein-1 (YB-1). This transcription factor is hypothesised to counteract EPO cytoprotective effects in non-transformed cells at several key levels, including EPO gene transcription, EPO signalling and target gene regulation. Our data indicate that YB-1 itself is regulated under hypoxia. After elucidating whether YB-1 has an immediate effect on the hypoxic response, e.g. by binding to specific DNA regulatory elements within the EPO gene, further results deal with the effects of manipulated cellular YB-1 levels (up and down) on EPO signalling and cell survival. For tumour cells an increased nuclear YB-1 content has been described, which is associated with poor outcome due to metastasis formation. An underlying mechanism may be counteracted EPO regulation with the initiation of a hypoxia cell program. Such a response includes the upregulation of target genes relevant for angiogenesis and metastasis formation. Our approach is to test for the EPO response in dependency of YB-1 expression levels that will be manipulated by molecular biology tools.

The in-depth understanding of EPO resistance for mesenchymal cells as well as tumour cells may provide the rationale for specific interventions. These may include targeting of YB-1 under both conditions, to sensitise cells to EPO and thereby increase the survival of mesenchymal cells under hypoxia, and to shut off the “angiogenesis program” of tumour cells responsible for metastasis formation in a wide range of tumours, like breast and lung cancer.

**Dr Chris D Vulpe (principal applicant)**

*Dr Ted Holman (co-applicant)*

*Dr Zhu Zhiwu (co-applicant)*



## **University of California, Berkeley, USA**

### *Characterisation of a family of putative mammalian heme chaperones*

Iron is an essential nutrient that is required for a wide range of biochemical reactions in the body. One of the important roles of iron is in heme which is used by a variety of proteins including haemoglobin, cytochromes important for mitochondrial function and P450s involved in chemical detoxification. Despite the importance of heme and the recent progress in understanding iron metabolism in both yeast and mammals, the distribution of heme from the mitochondria to the organelles and proteins that require it has remained a central intractable enigma of mammalian metal metabolism. We have identified a family of eukaryotic proteins that very likely represent the long elusive heme chaperones which deliver heme to apo-proteins. We propose characterising the mammalian members of this family of proteins and investigating the mechanisms of heme transport and delivery. Understanding of heme metabolism is vital to the understanding iron deficiency, the most common nutritional disorder in the world, and the resulting anaemia. This study will provide insight into the clinical consequences, include impaired psychomotor and cognitive development in children, increased morbidity in anaemic mothers and diminished work capacity in affected adults, and possibly lead to therapeutic interventions.

**Prof Hans-Ulrich Bucher (principal applicant)**

Dr Joachim Riethmüller (co-applicant)



### **University Hospital Zurich, Switzerland and University of Tübingen, Germany**

*Erythropoietin reduces brain, eye and lung damage in very preterm infants:  
proof-of-concept study*

Erythropoietin (EPO) has been shown to be protective against hypoxic-ischaemic and inflammatory injuries in a broad range of tissues and organs promoting red cell formation. Especially protective effects on brain, retina and bowel can be shown in animal models and first human studies.

EPO has been used widely for several weeks in preterm infants to prevent anaemia and is well tolerated. No short- and long-term adverse effects have been documented with EPO treatment in preterm infants.

Because EPO has been shown to influence several mechanisms associated with these short-term and long-term complications of prematurity, and furthermore has been shown to have a positive effect even *post hoc*, i.e. if given within a period of hours after an hypoxic-ischaemic insult, EPO may ameliorate the damage in very premature infants. Very preterm infants may suffer from a variety of short-term complications and long-term sequelae of pre-mature birth. The most critical period is the first days after birth, and inflammatory changes as a consequence of hypoxia-ischaemia or infection seem to have a major impact on short-term as well as permanent damage.

To determine whether early administration of EPO alters the incidence and severity of complications typically associated with preterm birth in infants born between 24 0/7 and 27 6/7 gestational weeks, we want to investigate in a clinical trial for prophylactic therapy with EPO.

**Dr Edward Debnam (principal applicant)**

*Prof Robert J. Unwin (co-applicant)*



## **Royal Free & University College Medical School, London, UK**

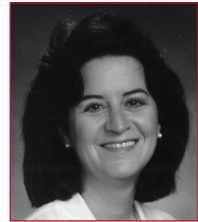
### *Is inflammation an important factor in the anaemia of chronic renal failure?*

Anaemia is a feature of renal failure and it is, in part, due to insufficient production of the hormone erythropoietin (EPO), the action of which is to stimulate the bone marrow to produce mature red blood cells. However, an increased production of red blood cells also requires the absorption of adequate amounts of dietary iron (since iron is a vital component of red blood cells). It is a common clinical observation that many renal failure patients receiving EPO require intravenous iron supplementation, despite the fact that they have adequate dietary iron. This strongly suggests an abnormality of iron uptake by the intestine.

Recent studies show that another hormone, hepcidin, reduces iron absorption from the gut. Interestingly, hepcidin secretion is increased during infection (also a common finding in renal failure) and this raises the possibility that raised hepcidin levels in renal failure interfere with gut iron uptake, i.e. high blood levels of hepcidin may override the effect of administered EPO in renal failure and limit the supply of dietary iron for red blood cell production. Hence the need for intravenous iron treatment. However, this procedure has risks of iron overload and tissue injury.

To date, there is no information available on the relationship between hepcidin secretion, EPO secretion and infection in renal failure. If an association is found between infection and hepcidin expression in renal failure, the process by which hepcidin influences iron transport may prove to be an important target for therapy of this form of anaemia.

**Dr Diana Gilligan**



### **Puget Sound Blood Centre, Seattle, USA**

#### *Regulation of gene expression during erythropoiesis*

The goal of this project is to understand the regulation of appropriate gene expression during red blood cell production. Anaemia, the lack of sufficient numbers of red blood cells, is a common disorder that affects patients with many different illnesses. Specifically, it is a side effect of chemotherapy for cancer and it is a secondary sign of kidney failure. Much has been learned about the production of red blood cells since the discovery of erythropoietin, a hormone produced by the kidney that stimulates haematopoietic stem cells to produce red blood cells. At a molecular level, the binding of erythropoietin to its receptor signals the precursor cells to increase expression of genes that are important to red blood cells, and to decrease expression of genes that are not important to red blood cells. This signalling pathway is not yet well understood and our experiments are designed to gain more information about the molecular steps that are required to increase production of red blood cells. We have been studying a family of genes, the adducins, that are expressed in all cells, but were first discovered as components of red blood cells. We have demonstrated lineage – specific expression of two of the adducin genes. The beta adducin gene is highly expressed in red blood cells, but is not expressed in platelets, while the gamma adducin gene shows the opposite characteristics, highly expressed in platelets, but not expressed in red blood cells. We will study the adducin gene family as a model system for lineage – specific gene expression, with particular significance for red blood cell production. We may identify novel factors that are important for red blood cell gene expression and these results may lead to novel therapies for patients with anaemia.

**Prof Alexander Maxwell (principal applicant)**

Prof Terence Lappin (co-applicant)



## **Queen's University Belfast, UK**

### *Investigation of the role of JUNE-1 in erythropoiesis*

Erythropoietin (EPO) stimulates bone marrow cells to differentiate into mature erythrocytes. Recombinant human erythropoietin therapy is widely used to treat anaemias but there is relatively limited knowledge of how EPO regulates genes in cells and tissues. Investigation of EPO-regulated genes should improve our understanding of how EPO influences erythropoiesis and the function of non-haematopoietic organs which express EPO receptors such as the brain and heart. We investigated transcriptional events occurring downstream of EPO binding to its receptor. We have now identified a novel gene, JUNE-1, encoding 5 exons expressing a 1.2 kb transcript translated into a 44 kDa protein. Its highly conserved DNA sequence has both nuclear targeting and plant homeodomain motifs, thought to mediate protein-protein or protein-DNA interactions. This suggests that JUNE-1 is involved in chromatin remodelling or transcriptional regulation. RT-PCR confirmed JUNE-1 expression in a variety of tissues and tumour cell lines, implicating JUNE-1 in processes beyond erythropoiesis. Functional studies of JUNE-1 are proposed using an *in vitro* model of erythropoiesis (murine erythroleukaemia MEL cell system), where proerythroblasts are cultured and differentiate into mature haemoglobin producing cells. We plan to localise JUNE-1 protein by immunohistochemistry. The consequences of overexpression and underexpression (knockdown via RNA interference) of JUNE-1 protein on erythroid cell proliferation and differentiation will be assessed. MEL cells will be examined for changes in phenotype, such as cell growth rate, apoptosis, and differentiation capacity. Since JUNE-1 may function as a transcription factor, the genes downstream of JUNE-1 will be identified in the MEL cell model using mouse gene microarrays. To identify proteins that interact with JUNE-1 mammalian expression vectors will be used to produce JUNE-1 fused to GST, and the protein then immobilised on a GST column. Cellular extracts will be loaded on the columns, and interacting proteins eluted and identified by SDS-PAGE and mass spectrometry. JUNE-1 specific antibodies will also be used to identify JUNE-1 interacting proteins via co-immunoprecipitations from the cell lysates. The proposed research should enable the functional characterisation of JUNE-1, a novel EPO-regulated gene.

**Dr Jun-ichi Nishimura (principal applicant)**

Dr Marilyn J. Telen (co-applicant)



### **Duke University Medical Center, Durham, USA**

#### *Innovative drug design using RNA aptamers for various anaemias*

Aptamers are small RNA molecules that bind to target proteins similar to antibodies. Through a selection process, termed SELEX, aptamers have been identified against a wide range of therapeutic targets. Dr. Sullenger (Mentor and Consultant) has established the SELEX system, and Dr. Nishimura (PI) has recently successfully identified RNA aptamers for treatment of paroxysmal nocturnal haemoglobinuria, a rare type haemolytic anaemia. Our overall goal is to develop aptamers to treat various anaemias using the SELEX system. In this proposal, we exclusively focus on the development of aptamers that inhibit red cell adhesion to prevent or treat vaso-occlusion in sickle cell disease (SCD).

Vaso-occlusive crises are the major clinical feature of SCD, and the adhesion of sickle erythrocytes (SS-RBC) to vascular endothelium is important to the generation of vaso-occlusion. SS-RBC express many adhesion molecules, such as Lutheran protein (B-CAM) and LW. Adhesive SS-RBC may bind to endothelial cell P-selectin, integrin  $\alpha V\beta 3$ , as well as extracellular matrix proteins, such as laminin. All of these molecules and their ligands are potential targets for reagents to prevent or treat the vaso-occlusive crises of SCD, and we focus 3 molecules, including B-CAM, P-selectin, and integrin  $\alpha V\beta 3$ . Since RNA aptamers for P-selectin have already been identified, we have synthesised one of these known P-selectin-binding aptamers. We have also begun to identify RNA aptamers that bind specifically to B-CAM and integrin  $\alpha V\beta 3$ . Once these aptamers are optimised, we will advance to flow chamber assays, established by Dr. Telen (Co-investigator), to test their ability to inhibit adhesion of SS-RBC to vascular endothelium. Selected high-affinity and high-inhibitory aptamers will be further modified in preparation for *in vivo* studies, using a system established by Dr. Telen. The development of combinatorial blocking aptamers against these 3 adhesion molecules represents a novel potential therapeutic option for patients with SCD.

**Prof Radek Skoda**



## **University Hospital Basel, Switzerland**

### *The role of Smad4-dependent signalling in anaemia*

We observed that mice lacking the signalling protein SMAD4 developed a severe anaemia. SMAD4 is an essential mediator for signals generated by the transforming growth factor beta (TGF $\beta$ ) family of proteins. This signalling pathway is important for proper embryonic development and regulates differentiation and growth in many adult tissues. However, an association of the TGF $\beta$ -signalling pathway with the appearance of anaemia in adults has not been described to date. We used an inducible system to delete the gene for SMAD4 in adult mice and observed anaemia 3–4 weeks after induction. Blood cells lacking SMAD4 developed normally when transplanted into healthy recipients, but anaemia developed when normal blood cells were transplanted into a SMAD4 deficient recipients. Thus, SMAD4 is required to provide the environment for normal red blood cell homeostasis. Our preliminary results suggest that loss of SMAD4 causes alterations in the iron metabolism that could interfere with red blood cell formation. Iron is essential for the formation of haemoglobin and iron deficiency is the most common cause of anaemia. More subtle changes in the availability of iron are thought to play a role in anaemia associated with chronic inflammatory diseases and cancer. We propose elucidating the mechanism of how anaemia develops in these mice and defining a novel role for the TGF $\beta$ -signaling in the regulation of red blood cell formation.

**Dr Carole Soussain**



### **Oregon Health and Sciences University, Portland OR, USA**

#### *Neuroprotective effect of erythropoietin on chemo- and radiotherapy-induced toxicity*

Combined radiotherapy and chemotherapy approaches have provided significant efficacy against brain tumours such as primary central nervous system lymphoma (PCNSL). A significant number of patients experience severe neurotoxicity with these therapeutic approaches, and this is a limiting factor in the management of patients with intracerebral cancer. Erythropoietin (EPO) is an important hormone for the development of foetal brain and homeostasis of the adult brain, and both EPO and EPO receptor (EPO-R) are expressed on astrocytes and neurons in the adult brain. Exogenous EPO has demonstrated a protective effect on neuronal cell cultures after injuries such as nitric oxide or glutamate exposure, as well as in animal models of ischaemia, inflammation, seizures, and subarachnoid haemorrhage. In clinical trials, EPO has been safely and efficiently used to prevent neurological damage after acute stroke. These findings suggest that EPO may be neuroprotective against chemotherapy and radiotherapy-induced neurotoxicity. In this proposal, the potential therapeutic role of erythropoietin in reducing therapy-induced neurotoxicity will be tested in molecular and cellular studies *in vitro*, and in *in vivo* animal models. We will assess the effect of EPO on tumour cell growth and chemotherapy toxicity *in vitro*, and determine the protection provided by EPO in an *in vitro* neuronal cell model. We hypothesise that EPO has no growth and survival activity on lymphomatous cells, but is neuroprotective against chemotherapy and radiotherapy toxicity in cultured neuronal cells. We will search for EPO-induced alteration of the blood-brain barrier permeability in normal rats and rats with intracerebral tumours. We hypothesise that EPO does not prevent the entry of chemotherapy in the brain. Finally, we will determine if there is a neuroprotective effect of EPO in rats exposed to neurotoxic doses of chemotherapy and radiotherapy. We hypothesise that EPO chemoprotection can improve the management of treatment-related CNS toxicities.

**Dr Christina Warnecke (principal applicant)**

*Prof Kai-Uwe Eckardt (co-applicant)*



## **University Erlangen-Nürnberg, Germany**

### *Molecular mechanisms underlying the hypoxic induction of erythropoietin by HIF-2 $\alpha$*

The hormone erythropoietin (EPO), which is predominantly produced in the kidneys, is the primary regulator of red blood cell production. Hypoxia, i.e. insufficient oxygen supply as encountered at high altitudes or after blood loss, leads to a compensatory increase of EPO production due to an increase in EPO gene expression, which is mediated by hypoxia-inducible transcription factors. In disease states that impair kidney function such as diabetes, chronic renal disease or heart failure the production of erythropoietin in the kidney fails to meet the needs and patients suffer from anaemia, which deteriorates their general condition and often aggravates the primary disease. Treatment with human recombinant EPO is highly effective and so far the therapy of choice for erythropoietin-deficiency anaemias. An alternative and very attractive approach, because of the high costs of recombinant EPO, could be the stimulation of endogenous EPO production.

In 2001 a family of transcription factors inducible by hypoxia (Hypoxia-inducible factor = HIF) was identified and their regulation has since then been actively investigated. Interestingly, although HIF was primarily identified as a regulator of EPO expression, it turned out that it also induces many other genes that serve to protect against oxygen deficiency. Although this underscores the importance of HIF, this lack of specificity makes it more difficult to use it as a molecular target to stimulate EPO generation. Only recently, we and others demonstrated that one specific isoform of HIF, called HIF-2 $\alpha$ , is responsible for the regulation of erythropoietin, but not that of most other HIF-dependent genes. By studying the molecular basis of this specificity in EPO-producing cells lines we attempt to increase our understanding of the normal regulation of red cell production, as well as its impairment in different diseases and hope to identify novel targets for medical intervention.

**Prof Max Gassmann**



### **Vetsuisse, University of Zurich, Switzerland**

#### *The impact of erythropoietin on the hypoxic ventilatory response of mouse and man*

For decades, the blood hormone erythropoietin (EPO) has been thought to exert a solely erythropoietic function. Within the last few years, we and others discovered that EPO is expressed by neuronal cells, too, and that it has (neuro)protective effects such as protecting the brain from stroke or the retina from degeneration. However, a physiological role for brain-derived EPO has not been established so far. We very recently demonstrated that EPO directly influences the respiratory centre via central (brainstem) and peripheral (carotid bodies) organs (Soliz et al., in press). This finding proves that EPO has a crucial role in the acclimatisation to reduced environmental oxygen. We propose extending our studies as follows: A) Considering that women and female mammals demonstrated a better capacity to adapt to hypoxia, we are interested in defining whether gender-specific differences occur in the EPO-enhanced ventilatory response in hypoxia. B) We also plan to investigate whether EPO increases the carotid body sensitivity to oxygen changes in blood. C) As the data described above has been obtained in mice only, we plan to determine as a first step whether EPO influences ventilation in man, too. We expect to provide new mechanistical insights in the EPO-mediated ventilatory response to hypoxia that may translate into clinical application involved in ventilatory diseases such as Chronic Mountain Sickness and premature newborn apnoea.

**Peter J Kirkpatrick**



## **University of Cambridge, United Kingdom**

### *Effects of systemic erythropoietin therapy on cerebral autoregulation and the incidence of delayed ischaemic deficits in patients with aneurysmal sub-arachnoid haemorrhage*

Intracranial bleeding from a ruptured cerebral blood vessel (called a subarachnoid haemorrhage) affects 7000 patients each year in the UK and is a source of considerable death and disability, even in young adults. Recent observations indicate that these bleeds can cause narrowing of the brain vessel (vasospasm) leading to reduced blood flow and eventual stroke. In this study we wish to use erythropoietin (EPO), a widely used natural human hormone for treating anaemia, to reduce vasospasm and clinical deterioration from low blood flow. A man-made version has shown promise in improving outcome of general stroke patients, and beneficial effects can be seen within a few days of treatment. In this proposal we would like to treat subarachnoid haemorrhage patients with EPO soon after they are admitted to hospital. We will compare these patients with those treated with a dummy drug (placebo). Safety will be scrutinised, and ultrasound used to examine aspects of cerebral blood flow known to influence patient outcome. We also aim to identify any evidence of reduced episodes of neurological worsening in patients given EPO. Results from this study will help in the design of a larger trial needed to examine clinical outcome. Benefits identified may be helpful in other conditions associated with cerebral haemorrhage.

**Dr Véronique Lefebvre**



### **Cleveland Clinic Foundation, Cleveland OH, USA**

#### *Roles of Sox6 in erythropoiesis*

This project is designed to help us better understand how the gene called Sox6 controls formation and function of red blood cells in the mouse under normal and anaemia conditions. Sox6 codes for a protein (a transcription factor) that helps specific types of cells activate the genes that they need to fulfil their specialised functions. We found recently that red cells are among these cell types. Both the overall production and the quality of red cells are affected in mouse foetuses and pups that lack Sox6. Using cellular and molecular approaches, we have found that Sox6 helps red cell precursors proliferate and develop fast and undergo terminal maturation properly. Sox6 is thereby required for optimal function and long-term survival of red cells in the blood stream. Sox6 is made in red cell precursors in response to erythropoietin and thus contributes to mediating the effect of this essential hormone in boosting red cell formation. Our first aim is to continue our studies in the mouse to determine whether Sox6 is also important in red cell formation in childhood and adulthood under normal conditions and to recover quickly from anaemia. Our second aim is to use cellular and molecular assays to identify the specific genes that Sox6 activates and that have major roles in red cell formation. We will particularly ask whether Sox6 helps red blood cells assemble the specialised protein network (cytoskeleton) that critically helps them mature and acquire their specific shape, and thereby fulfil their functions and survive in the circulation. We anticipate that this study will greatly increase our molecular understanding of red cell formation and the roles of Sox6 in this process and in anaemia, and will thereby suggest genetic causes for some types of anaemia diseases and new treatments for various forms of anaemia diseases.

**Prof Stephen L. Leib**



## **University of Berne, Switzerland**

### *Effect of erythropoietin on brain injury and regeneration in bacterial meningitis*

Bacterial meningitis is associated with a mortality rate of up to 30% and up to half of survivors suffer from permanent neurological sequelae including deafness and learning impairment. The dramatic mortality and morbidity rates have remained unchanged for several decades in spite of advances in antimicrobial and intensive care therapies.

Injury caused by bacterial meningitis predominantly affects the inner ear and two brain structures, namely the cortex and the hippocampus. In the cortex, the damage includes areas of cerebral infarction. A specific form of brain injury, namely apoptosis in the hippocampus has been observed in patients dying from bacterial meningitis. The affected brain structure is responsible for learning and memory functions. Erythropoietin (EPO) has been shown to protect the brain from injury by stroke, a disease with similarities to bacterial meningitis. In addition to this protective effect, EPO has been shown to increase brain repair mechanisms.

We thus hypothesise that adjuvant EPO exerts a beneficial effect in bacterial meningitis by the combined effect of preventing acute brain damage and increasing brain repair mechanisms.

In the proposed project we plan to assess in experimental bacterial meningitis (i) whether therapy with EPO prevents acute brain damage; (ii) whether this beneficial effect is still evident when therapy with EPO is delayed until symptomatic disease and started together with antibiotics at 18 hours after infection (reflecting the clinical situation) (iii) whether EPO attenuates injury to the inner ear and thus prevents hearing loss, the most frequent sequel of bacterial meningitis; (iv) whether EPO increases brain repair mechanisms (e.g. expansion of brain stem cells) in the late phase of the disease and; (v) whether the combined effect of EPO mediated protection and increased brain repair leads to improved outcome assessed by learning and hearing performance in long term survivors of bacterial meningitis.

**Dr Barbara Scheiber-Mojdehkar**



### Medical University of Vienna, Austria

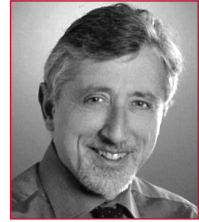
#### *Recombinant human erythropoietin: a new treatment for Friedreich's ataxia*

Friedreich's ataxia (FRDA) is the most common of the inherited ataxias, affecting one in 50,000 people. FRDA is caused by a GAA-trinucleotide expansion in the frataxin gene, resulting in a reduced expression of frataxin, a small mitochondrial protein. The exact physiological function of frataxin is unknown, but it may be involved in mitochondrial iron homeostasis and/or assembly of iron-sulfur (FeS) proteins and heme synthesis. Clinically there is an intramitochondrial iron accumulation in heart, liver, nervous system and spleen of FRDA-patients, as well as a reduction of mitochondrial DNA, the FeS cluster-containing subunits of the mitochondrial electron transport chain (complex I-III) and of the enzyme aconitase.

We found that in addition to its reported neuro- and cardioprotective properties recombinant human erythropoietin (rhuEPO) significantly increases frataxin expression in primary lymphocytes from FRDA-patients. Additionally rhuEPO can increase frataxin expression in many other cell types among them the most affected in FRDA such as neurons and cardiac cells. The potential therapeutic role of rhuEPO for the treatment of FRDA will be directly tested in an open-label, single-dose pilot study in FRDA-patients. For this study we will recruit 13 FRDA patients where 7 out of 13 have been tested for *in vitro* response of their lymphocytes to rhuEPO-treatment. We will test whether the effects on frataxin expression seen *in vitro* can also be seen in patients. Therefore the safety and efficacy of rhuEPO for the treatment of FRDA will be tested in an open-label, single-dose pilot study.

**Prof Jürg Schifferli (principal applicant)**

*Dr Christoph Hess (co-applicant)*



## **University Hospital Basel, Switzerland**

### *Erythropoietin or erythrocyte transfusion for anaemia?*

Over years evidence has accumulated suggesting that blood transfusions may be immunosuppressive, favour infections and diminish the survival of patients with severe disease. The mechanisms responsible for this immunosuppression are not well understood. Red blood cells release small vesicles during storage. These vesicles are budding off from the cell surface of red blood cells. They are found in every red blood cell bag, and apparently do no immediate harm when transfused.

The aim of the present project is to test the hypothesis that these small vesicles released by red blood cells are immunosuppressive.

This hypothesis is based on the similarities between the structure of vesicles released by red blood cells and white blood cells (polymorphonuclear leucocytes); the latter have been shown to have immunosuppressive properties.

To test our hypothesis, we will perform experiments in the laboratory using vesicles released by human and mouse red blood cells, to see whether they inhibit inflammation and immunity in cell culture models and in mice. The work will require 2 years. The next steps would be to see whether such mice are prone to infections. An immunosuppressive activity of red blood cell vesicles would evidently mean that transfusion should be avoided whenever possible, and particularly in patients who are already immunosuppressed. The logical consequences would be to explore further the possibility of replacing red blood cell transfusion by erythropoietin treatment whenever possible, particularly in chronic diseases.

## 5 *Grant awards in Cycle III*

**Dr Marcela Votruba (principal applicant)**

Prof Mike Boulton (co-applicant)

Dr Paul Comes (co-applicant)



### **Cardiff University, United Kingdom**

#### *Erythropoietin neuroprotection in retinal neurodegeneration*

Programmed cell death (apoptosis) is the final common pathway of neural loss in the visual system in a variety of neurodegenerative diseases, some of which are primarily genetic, such as photoreceptor degenerations (retinitis pigmentosa, cone dystrophies and Stargardts macular dystrophy) and retinal ganglion cell (RGC) degenerations (inherited optic neuropathies and glaucoma). We have recently generated a novel model of RGC neurodegeneration, in which the genetic defect is in the murine gene *opa1*, and leads to a primary retinal ganglion cell loss in postnatal life by a mechanism of apoptosis triggered by mitochondrial dysfunction. The human counterpart of this is the primary inherited optic neuropathy, autosomal dominant optic atrophy (ADOA), caused by mutation in the *OPA1* gene. We have also established *in vitro* model systems using primary retinal ganglion cells, with *OPA1* knockdown, in our laboratory.

The identification of the growth and survival factor erythropoietin (EPO) receptor on tissues and cells other than red cell progenitor cells suggests that EPO may have biological roles and functions other than the stimulation of erythropoiesis. Central nervous system (CNS) EPO receptors are expressed on neurons, astrocytes, microglia and myelin sheaths and EPO is thought to have neuroprotective and neurotrophic effects on neuronal cells.

The aim of this study is to explore the neuroprotective effect of EPO in neural retina in a model of primary RGC degeneration, using both a whole organism and cells in culture. We suggest that EPO, which is expressed in neural retina and acts at the mitochondrial membrane to protect nerve cells from apoptosis under adverse cellular conditions, can protect against RGC loss triggered by single gene defects in the neural retina. Our models will be used to investigate the effect of EPO administered systemically and locally on retinal ganglion cell loss and retinal degeneration. The end-point will be assessment of retinal morphology, functional vision and cell survival.



## 6 *RoFAR Board of Trustees*

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**Who is eligible for LOI submission?**

RoFAR funds established members of academic institutions, dialysis units, and research centres. There are no age or geographical restrictions.

**What kind of projects are RoFAR interested in?**

RoFAR supports both clinical and basic science projects focused on anaemia related to kidney disease and oncology, effects of erythropoietin and erythropoietin-like substances as protective drugs in various organs, central resistance to erythropoietin, anaemia of chronic disease, anaemia related to congestive heart failure and stroke, biology of anaemia and outcomes. Especially, RoFAR encourages innovative research that will open new avenues of exploration in the study of anaemia, its mechanisms and outcomes.

**What will I need to provide RoFAR with if my project is funded?**

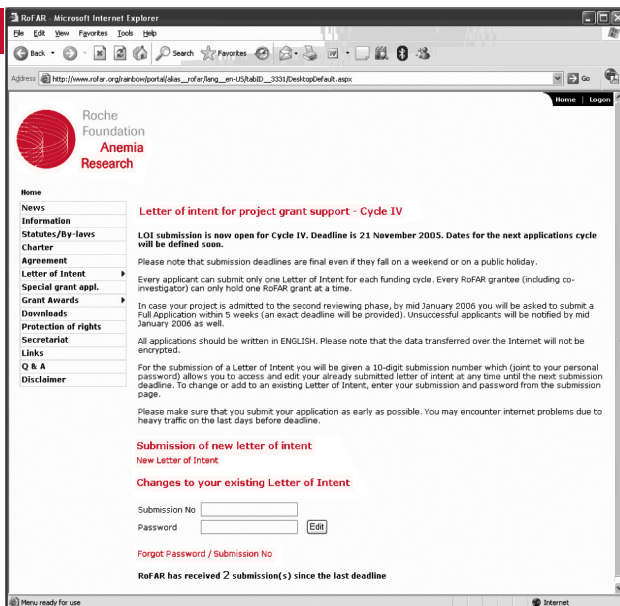
Funds are paid in three instalments over a maximum of 2 years and are dependent on the delivery of an interim and a final report for public use. Additionally, RoFAR must be acknowledged in publications, on posters, etc. Applicants may be asked to attend events organised by RoFAR and present their results.

**Are budget indications approximate or am I committed to them?**

RoFAR assigns funds to awarded projects based on provided budget details. It is not possible to renegotiate the amount after project approval. Indirect costs (institutional overheads, insurance, etc.) are the responsibility of the applicant. A maximum of 10% of the assigned funds can be used for the indirect costs.

**Am I allowed to submit more than one project to RoFAR?**

Applicants are allowed to hold only one grant at a time. Furthermore you may not submit more than one LOI in the same cycle. This rule holds both for main applicants and co-investigators.



## What kind of assistance is RoFAR giving to awarded applicants?

The purpose of RoFAR is to provide awarded applicants with funds for the submitted project and to share outcomes with the scientific community. RoFAR will not provide any administrative assistance or scientific consultancy, nor recommend any preferential channels for the purchase of drugs or machinery necessary for the completion of the study.

## Where can I find relevant information about RoFAR?

The RoFAR website (www.rofar.org) is the main information channel. There you can find important announcements, future deadlines, submission forms, charters and regulations, as well as reports on awards and on funding history. If you have any specific questions, please do not hesitate to contact the secretariat (admin@rofar.org).

### Projects are submitted electronically via our website

#### Projects are submitted as Letters of Intent (LOI)

Submissions twice per year  
(June and November)

You are asked to provide your personal details, indications about the budget, a short description of your experience and of the submitted project (latter two limited to 750 words). No figures, tables or extensive literature list can be submitted at this stage.

#### LOI are evaluated by a Board of Scientific Advisors

6–9 weeks

LOI are thoroughly reviewed by 3 members of the Advisory Board and judged by considering relevance to RoFAR, originality, scientific excellence and feasibility. Applicants are informed of the outcome 6–9 weeks after submission. Declined applications are not provided with any feedback from the reviewers.

#### Top-ranked applicants are invited to submit a full application

4–6 weeks

Based upon the Scientific Advisors' evaluation, top-ranked applicants are invited to submit a full application with an approximate 50% chance of funding. Sample forms and guidelines are available in the Download section of the RoFAR website. Usually, 4–6 weeks are given for submission. Only completed applications are accepted and the stated deadline is final.

#### Full applications are evaluated by a Board of Scientific Advisors

8–10 weeks

Applications are thoroughly reviewed by at least 3 Scientific Advisors and judged by considering relevance to RoFAR, originality, scientific excellence and feasibility. The Board of Trustees selects the projects to be granted based upon the evaluations made by the Scientific Advisors. Applicants are informed about the outcome 8–10 weeks after submission of the full application.





Postfach 226  
6045 Meggen – Switzerland  
Phone: +41 41 377 3669  
Fax: +41 41 377 5334  
E-mail: [admin@rofar.org](mailto:admin@rofar.org)

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