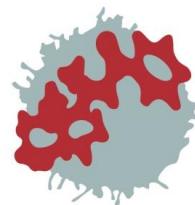


# Research Brochure



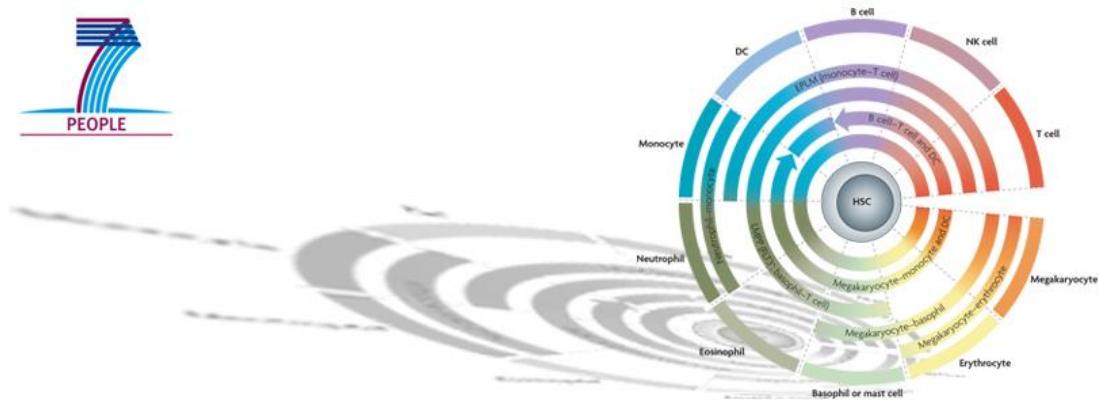
**DECIDE**



DECIDE brings together scientists who have made important advances in the fields of haematopoiesis and differentiation therapy and has both scientific and therapeutic targets. DECIDE partners are from the UK, Switzerland, Poland, Ireland, Israel, Spain, Germany and the USA. The network combines the research efforts of prestigious universities and research institutes, Poland's leading governmental Pharmaceutical Research Institute, and two successful biopharmaceutical companies.

## Theme 1 Haematopoiesis – Lead Geoffrey Brown

DECIDE aims to advance understanding of normal blood cell development and why primitive cells fail to differentiate in acute myeloid leukaemia.



## Theme 2 Differentiation Therapy – Lead Andrzej Kutner

We use the information gained from Theme 1 to develop ways of alleviating the differentiation block in acute myeloid leukaemia and so deliver new agents, including novel vitamins D and retinoid analogues, for use in differentiation therapy. This type of therapy aims to respond to the urgent need to devise milder treatments, especially for older and frailer patients with acute myeloid leukaemia and other cancers.



## Theme 1 Haematopoiesis



UNIVERSITY OF  
BIRMINGHAM



Geoffrey Brown  
Institute of Clinical Sciences  
College of Medical and Dental  
Sciences  
University of Birmingham

### **Research Interests**

For many years the mammalian blood cell system has provided cell biologists and haematologists with one of the best experimental models in which to unravel how one stem cell – the haematopoietic stem cell – gives rise to the many different types of cells of the blood and immune systems. Laboratory work aims to meet the need to revise textbook accounts of the generation of blood cells. We have provided a new, and highly regarded, model for blood cell development. The 30 year old ‘classic’ model of haematopoiesis states there are two families of cells, namely myeloid/erythroid and lymphoid. The model we favour refutes this viewpoint. We have proposed there is a series of pair-wise relationships between all lineage fates – a fate choice continuum. We test this model by examining various primitive haematopoietic progenitor cells to see whether there is a pattern to sub-sets of lineage potentials within single cells that fits with placing cell lineages adjacent in our pair-wise model. Ongoing studies are also looking at the mechanisms that govern the commitment of haematopoietic stem cells to becoming one particular type of cell. Endeavours to understand the controls that drive the maturation of committed cells to functional end cells are focussed on signals that arise from differentiating agents such as all-*trans* retinoic acid and vitamin D<sub>3</sub>. We are also interested in the use of novel retinoid analogs to modulate haematopoiesis and to treat malignancies.

### **Key Publication**

CEREDIG R, ROLINK T and BROWN G (2009) Opinion Models of haematopoiesis: seeing the wood for the trees. *Nature Reviews Immunology* 9, 293-300

## Theme 1 Haematopoiesis



**NUI Galway**  
**OÉ Gaillimh**



**Rhodri Ceredig**  
Regenerative Medicine Institute  
National University of Ireland, Galway

### ***Research Interests***

I am a basic cellular and molecular immunologist whose approach to immunology has been heavily influenced by experimental hematology, that is that lymphocytes are the progeny of hematopoietic stem cells and that their development follows many of the general rules of hematology. Whilst in Strasbourg, my laboratory generated a transgenic mouse over-expressing the cytokine Interleukin-7 (IL-7), a molecule involved both in lymphocyte development and survival. Since that time (1993), I have focused much of my research interest on IL-7. In IL-7 transgenic mice, production of T cells by the thymus does not appear to be altered. However T lymphocyte survival in peripheral lymphoid organs is enhanced with accumulation of large numbers of memory-phenotype T cells. Many mice developed leukemias and with Dr Geoff Brown in Birmingham, we could show that these tumours had a bi-potent B/macrophage phenotype, a cell type not found in the “classic” scheme of haematopoiesis. B lymphocyte production was massively perturbed in the bone marrow and to study this, a close collaboration was established and still exists with the group of Prof. Ton Rolink at the University of Basel, Switzerland. Whilst in Basel, I collaborated with scientists investigating the immunological properties of mesenchymal stem cells (MSC), cells essential in supporting many of the steps in early haematopoiesis. MSC constitute the major research focus of the Regenerative Medicine Institute in Galway. Currently, we are focusing on investigating the biology of MSC particularly is it pertains to the support of haematopoiesis especially in the context of bone marrow transplantation.

## Theme 1 Haematopoiesis



**Antonius Rolink**  
Department of Biomedicine  
University of Basel

### **Research Interests**

The research focus of the “Developmental and Molecular Immunology” group is the unravelling of the molecular and cellular mechanisms underlying the development of the various cells of the hematopoietic system and especially the cells of adaptive immune system i.e. the T and the B cells. In order to do this we have generated different in vitro systems that allow the long-term propagation of early progenitor cells that can be used for reconstitution experiments *in vivo*. Thus we successfully established culture systems that allow the long-term growth of hematopoietic stem cells (HSC's), pro-B cells and pro-T cells. These cells can be very efficiently used for reconstituting the various hematopoietic compartments in sub lethally irradiated immune deficient mice. Moreover, they are ideal tools to study the function of genes *in vivo* since it is relatively easy to introduce retrovirally genes or siRNA constructs in these. In addition our group also uses genetically modified mice in order to study hematopoietic development.

Yet another research focus of the “Developmental and Molecular Immunology” group is how immunological tolerance is established and/or broken. Therefore, our group has generated several mouse models that spontaneously show the development of various autoimmune diseases like lupus and rheumatoid arthritis. Moreover, we are trying to unravel the mechanism(s) underlying the increased frequency of autoimmunity observed in aging individuals.

## Theme 1 Haematopoiesis



**OrbsenTherapeutics**  
Redefining Cell Therapy



**Stephen Elliman**  
**Orbsen Therapeutics Ltd., Galway**

### ***Research Interests***

Here in Europe, recent EMA guidelines indicate that more stringent methods of stromal stem cell (SSC) purification and characterisation are necessary for medicinal use. During the FP7 project, PURSTEM (ended April 2012), Orbsen advanced the state-of-the-art towards meeting these EMA criteria by developing the novel ORB1 antibody-based method for prospectively purifying SSC with class-leading levels of purity. ORB1 prospectively isolates comparable, equivalent ORB1<sup>+ve</sup>SSC from multiple species' (human, horse, mouse and rat) tissues – a first for SSC technologies. Moreover, ORB1 may be used to isolate defined SSC from multiple tissues, including bone marrow, fat and placenta – reducing manufacturing dependence on a single, limiting tissue sources that require surgery to obtain. The FP7-funded discovery of ORB1 represents a novel, class-leading and tissue-independent cell isolation technology that enables the development of ORB1-based cell therapies. No equivalent cell technology is currently available. The therapeutic potential of the ORB1<sup>+ve</sup>SSC is being evaluated in the EU FP7 REDDSTAR project ([www.reddstar.eu](http://www.reddstar.eu)) which will advance the ORB1<sup>+ve</sup>SSC to a Phase 1b clinical trial. To inform the ORB1 route to the clinic we must define the role of ORB1<sup>+ve</sup>stromal cells *in vivo*. To that end we are generating a series of ORB1 transgenic models in collaboration with Prof. Douglas T. Fearon at the University of Cambridge. The lineage-specific deletion of ORB1 protein and ablation of the ORB1<sup>+ve</sup>stromal cells will enable the elucidation of the specific roles of this perivascular stromal cell in the homeostasis and pathologies of haematopoiesis.

## Theme 1 Haematopoiesis



**George Studzinski**  
Pathology and Laboratory Medicine  
New Jersey Medical School

### **Research Interests**

The MicroRNAs miR-223, miR-181, and miR-32 are important to myelopoiesis. miR-223 activation is required for ATRA-induced granulocytic differentiation of human cells in culture, and miR-223 targets Transcription Factors that are important to differentiation: C/EBP $\beta$ , C/EBP $\alpha$ , RelB, and Mef2c. The miR-181 family members target the cell cycle inhibitor p27<sup>KIP1</sup> and the reduction in miR-181a expression accelerates the initial stages of monocytic differentiation, suggesting that miR-181a can stabilize a cell fate decision by enhancing p27<sup>KIP</sup> expression. During monocytic differentiation miR-32 is up-regulated and enhances cell survival by decreasing the level of the pro-apoptotic protein Bim. Thus, anti-miR32 constructs can have a role in the therapy of AML. Studzinski is also expert on 1,25-(OH)<sub>2</sub>D<sub>3</sub> and agents that sensitize AML cells to 1,25-(OH)<sub>2</sub>D<sub>3</sub> and compensatory MAPK pathway activation in cancer therapy, with current focus on ERK5.

### **Key Publications**

1. GOCEK E, WANG X, LIU X, LIU CG, **STUDZINSKI GP** (2011) MicroRNA-32 up-regulation by 1,25-dihydroxyvitamin D3 in human myeloid leukemia cells leads to Bim targeting and inhibition of AraC-induced apoptosis. *Cancer Research* 71,6230-6239.
2. WANG X, GOCEK E, NOVIK V, HARRISON JS, DANILENKO M, **STUDZINSKI GP** (2010) Inhibition of Cot1/Tlp2 oncogene in AML cells reduces ERK5 activation and up-regulates p27Kip1 concomitant with enhancement of differentiation and cell cycle arrest induced by silibinin and 1,25-dihydroxyvitamin D3. *Cell Cycle* 9,4542-4551.

## Theme 1 Haematopoiesis



**Daniela Finke**  
Department of Biomedicine  
University of Basel  
University Children's Hospital, Basel

### ***Research Interests***

The crosstalk between hematopoietic and stromal cells is fundamental for the development of the immune system, but also for generating protective host defense. Research by Daniela Finke is shedding light on how innate lymphoid cells and mesenchymal stromal cells orchestrate the generation and function of secondary lymphoid organs. Daniela Finke and her colleagues at the Department of Biomedicine, Basel University (CH), did pioneer work on identifying a subset of innate lymphoid cells named lymphoid tissue inducer (LTI) cells. Without LTI cells, the body can't develop lymph nodes and Peyer's patches. Daniela Finke's laboratory could identify cytokine-driven pathways involved in the life cycle and differentiation of LTI cells and in their function to control lympho-organogenesis. More recently, other subtypes of innate lymphoid cells in the intestinal mucosa were discovered, but their relationship to LTI cells and their function during immune responses is poorly understood. Daniela Finke, working as full professor at the Basel University since 2010, is now focusing on the origin, development and the immune function of LTI cells and related cell subsets using various genetic models. Moreover, cell dynamics such as growth, differentiation and survival are evaluated comparing fetal and adult LTI cells. The understanding of how the growing family of innate lymphoid cells regulates innate and adaptive immunity will be substantial for the discovery of new biomedical targets of inflammation and autoimmunity.

## Theme 1 Haematopoiesis



**Eustace Johnson**  
**Biological Sciences**  
**University of Chester**

### ***Research Interests***

Research activity is focused on the cell and molecular biology of neural and connective tissue, particularly, in relation to the spine. The aim is to develop cell-based therapies in regenerative medicine. This is currently targeted toward the use of autologous adult stem cells to promote functional recovery following spinal cord injury, and for bone and cartilaginous tissue repair.

The research converges on trying to develop a deeper understanding of how intercellular and cell-matrix interactions regulate stem cells and cell function, but the research is also driven towards clinical intervention. For example, initial in vitro studies conducted on the regulation of nerve growth by extracellular proteoglycans were adapted using co-culture systems to examine how human mesenchymal stem cell isolated from patients with spinal cord injury (SCI) may promote axonal regeneration following transplantation.

Current projects include mesenchymal stem cells in the zebrafish model of development/wound healing, high content/high throughput screening for adult cell therapies in orthopaedics, and comparative bone marrow transplants for osteoarthritis.

## Theme 2 Differentiation Therapy



**Instytut Farmaceutyczny  
Warszawa**



**Andrzej Kutner**  
Chemistry and Analytical Departments  
Pharmaceutical Research Institute,  
Warsaw

### **Research Interests**

One approach to treating acute leukaemia, termed differentiation therapy, is to find ways of alleviating the block in cell differentiation to allow cells to terminally mature. An agent that has to be used to drive differentiation of a wide variety of cell types is the active form of vitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>). However, clinical use of 1,25-(OH)<sub>2</sub>D<sub>3</sub> is limited by its high calcemic potential. We are aiming to design low calcemic analogs that retain cell differentiation potency. The design of new analogs of 1,25-(OH)<sub>2</sub>D<sub>3</sub> is based on the 3D structure of the ligand binding domain of the vitamin D receptor (VDR-LBD), to ensure a specific fit between the new analog and the VDR-LBD amino acid residues. New hybrid analogs, modified in both distinct fragments of the vitamin D molecule, will be obtained by a novel convergent synthetic strategy from the advanced key intermediates. The new compounds are tested against a variety of leukaemia cells and leukaemic cell lines to determine their therapeutic potential. We are also investigating the potential therapeutic benefit of combining new low-calcemic analogs of vitamins D<sub>3</sub> with lowered doses of standard chemotherapeutic agents.

### **Key Publication**

1. **KUTNER A, ZHAO H, FITAK H, WILSON SR** (1995) Synthesis of retiferol RAD<sub>1</sub> and RAD<sub>2</sub>, the lead representatives of new class of des CD analogs of cholecalciferol, *Bioorganic Chemistry* 23, 22-32
2. BAURSKA H, KŁOPOT A, KIEŁBIŃSKI M, CHROBAK A, WIJAS E, **KUTNER A, MARCINKOWSKA E** (2011) Structure-function analysis of vitamin D<sub>2</sub> analogs as potential inducers of leukemia differentiation and inhibitors of prostate cancer proliferation, *Journal of Steroid Biochemistry and Molecular Biology* 126, 46-54.

## Theme 2 Differentiation Therapy



**Louis Chesler**  
**Pediatric Oncology**  
**Institute of Cancer Research, London**

### **Research Interests**

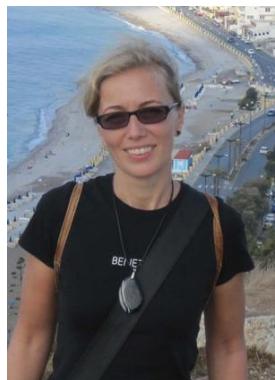
Retinoid-based differentiation therapy is curative in acute promyelocytic leukaemia (APL) with a complete remission rate of 94% and a long-term survival rate greater than 90%. Recently, we demonstrated a critical role for the histone H3 lysine 4 mono/di demethylase LSD1 as a negative regulator of the ATRA-mediated myeloid differentiation pathway. Treatment with LSD1 inhibitors (LSD1i) dramatically potentiated the pro-differentiative effects of ATRA with associated gene-specific increases in histone methylation, and greatly impaired engraftment of primary AML cells from patients in NOD.*SCID* gamma mice, suggesting that ATRA/LSD1i targets cancer-initiating cells. Combinatorial treatment also synergistically down-regulated expression of the MYC oncogene, which AML cells have been shown to be “addicted” to. This work identified LSD1 as a therapeutic target.

Like AML, neuroblastoma (an aggressive neural crest-derived malignancy of infants and young children) can be considered to arise at least in part from a failure to properly implement developmental retinoid differentiation pathways. Another retinoid (13-cis-RA) is used to treat minimal residual disease in high-risk neuroblastomas, which are characterized by amplification of the *MYCN* oncogene. Also in common with AML, neuroblastomas with high levels of *MYCN* are addicted to its expression. A key downstream target of MYC oncproteins in tumourigenesis is ornithine decarboxylase, the rate-limiting enzyme of polyamine biosynthesis and recent work suggest that agents that target the polyamine pathway show efficacy in high-risk, *MYCN*-amplified neuroblastoma. We are investigating the clinical potential of combinatorial use of retinoids with polyamine analogues, dual inhibitors of LSD1 and polyamine biosynthesis, as well as tranylcypromine analogs, in AML and high-risk neuroblastoma.

## Theme 2 Differentiation Therapy



Uniwersytet  
Wrocławski



Ewa Marcinkowska  
Faculty of Biotechnology  
University of Wroclaw

### Research Interests

There is an increasing need to devise milder treatments for older patients with acute myeloid leukaemia (AML), and other cancers. In AML, and other acute leukaemias, differentiation is impaired resulting in the accumulation of immature cells. One approach to treating acute leukaemia, termed differentiation therapy, is to find ways of alleviating the block in cell differentiation to allow cells to terminally mature. The agents that have been used to drive differentiation of a wide variety of cell types are either vitamin D<sub>3</sub> (1,25-(OH)<sub>2</sub>D<sub>3</sub>) or its low calcemic analogs, however many AML blasts remain resistant. Recently we discovered that vitamin D receptor (VDR) gene is repressed in vitamin D resistant AML cell line KG-1, and that this repressed state can be reversed by *all* trans retinoic acid (ATRA). We are investigating why some patients' AML cells respond to vitamin D<sub>3</sub> whilst other patients' cells are unresponsive to the differentiating effects of vitamin D<sub>3</sub>. This will involve examining vitamin D receptor expression, signalling events upstream and downstream of this receptor, transcription factor-mediated events, and epigenetic regulation of gene promoters. We are also examining the use of vitamin D<sub>3</sub>-sensitizing agents to attempt to drive differentiation of resistant patients' cells.

### Key Publication

GOCEK E, MARCHWICKA A, BAURSKA H, CHROBAK A, MARCINKOWSKA E (2012) Opposite regulation of vitamin D receptor by ATRA in AML cells susceptible and resistant to vitamin D-induced differentiation. Journal of Steroid Biochemistry and Molecular Biology 132, 220-226.

## Theme 2 Differentiation Therapy



**Nicholas Barnes**  
**Celentyx, Birmingham**

### ***Research Interests***

Celentyx aims to discover and develop novel chemical entities (NCEs) that have potential to treat leukaemia and other types of cancer.

Professor Barnes joined the University of Birmingham as a lecturer in 1990. He is a neuropharmacologist and has generated an extensive list of published works in international journals including prestigious journals such as *Nature*, *Proceedings of the National Academy of Science USA*, *Journal of Clinical Investigation*, *Blood* and *Gastroenterology*. Professor Barnes is Chairman of the International Union of Basic and Clinical Pharmacology (IUPHAR) Nomenclature Committee for Serotonin Receptors, a Councillor of the International Society for Serotonin Research and a Fellow of the British Pharmacological Society. He is also Editor-in-Chief, Editor and Associate Editor for the international journals *Frontiers in Neuropharmacology*, *Neuropharmacology* and *Frontiers in Neuroscience*, respectively. Professor Barnes is also a member of the UK Government's Working Group on Simultaneous Polysubstance Misuse, which is responsible for writing a White Paper for the Home Secretary and Minister of Health.

In addition to his academic-related positions, Professor Barnes is the Principal Founder of Celentyx Ltd ([www.celentyx.com](http://www.celentyx.com)); a venture capital financed research and development pharmaceutical company (the company is based at the Birmingham Research Park). Research from Professor Barnes' academic laboratory has also generated a number of licensing deals for the University of Birmingham. To further help commercialisation and knowledge transfer activities in the College of Medical and Dental Sciences, Professor Barnes established the External Commercialisation Board ([www.birmingham.ac.uk/mds-ecb](http://www.birmingham.ac.uk/mds-ecb)) that is populated with highly experienced professionals covering a broad range of expertise that provides strategic and operational advice relevant to third stream activities.

## Theme 2 Differentiation Therapy



Ben-Gurion University of the Negev



**Michael Danilenko**  
Department of Clinical Biochemistry  
and Pharmacology  
Ben-Gurion University of the Negev

### **Research Interests**

The major research focus of my group is on the cooperation of various plant polyphenolic antioxidants and differentiation-inducing agents against acute myeloid leukemia (AML) in various preclinical models. We have demonstrated that certain plant polyphenols synergistically potentiate the differentiation and antiproliferative effects induced by physiological concentrations of active forms of vitamin D or retinoic acid on AML cells. Furthermore, the combination treatment with low-calcemic vitamin D analogs and plant polyphenols substantially inhibits the development of leukemic tumors and systemic AML in mice.

The molecular mechanism of this synergy involves cooperative modulation of (a) intracellular antioxidant systems and overall cell redox status; (b) cell cycle machinery; (c) nuclear receptors for vitamin D and retinoic acid, (d) mitogen-activated protein kinase cascades, and (e) redox- and extracellular signal-regulated transcription factors, such as Nrf2, AP-1, and EGR-1.

Recently, we have reported that in the absence of differentiation agents certain polyphenols can strongly synergize with one another in the induction of growth arrest and apoptotic cell death in leukemic cells. These effects are selective towards malignant cells and are not observed in normal cells. The long-term goal of the above research is to develop combinatorial approaches for the treatment of leukemias and, possibly, of other cancers using phytochemicals and differentiation inducers.

### **Key Publications:**

BOBILEV I, NOVIK V, LEVI I, SHPILBERG O, LEVY J, SHARONI Y, STUDZINSKI GP, **DANILENKO M** (2011) The Nrf2 transcription factor is a positive regulator of myeloid differentiation of acute myeloid leukemia cells. *Cancer Biology and Therapy* 11,317-329.

PESAKHOV S, KHANIN M, STUDZINSKI GP, **DANILENKO M** (2010) Distinct combinatorial effects of the plant polyphenols curcumin, carnosic acid and silibinin on proliferation and apoptosis in acute myeloid leukemia cells. *Nutrition and Cancer* 62, 811-824.

## Theme 2 Differentiation Therapy



**Isidro Sanchez-Garcia**  
Centro de Investigacion del Cancer  
University of Salamanca

### **Research Interests**

Leukemia-associated oncogenes induce unscheduled proliferation as well as genomic and chromosomal instability. According to current models, therapeutic strategies that block oncogene activity are likely to selectively target tumor cells. However, recent evidences have revealed that oncogenes are only essential for the proliferation of some specific tumor cell types, but not all. Indeed, the latest studies of the interactions between the oncogene and its target cell have shown that oncogenes contribute to leukemia development not only by inducing proliferation, but also via developmental reprogramming of the HSC cell fate. These findings challenge the current accepted/working model of the role of oncogenes in cancer and provide the first evidence that tumorigenesis can be initiated by stem cell reprogramming. These Sca1 mouse models and cell-based assays are unique tools to address this challenge, and they will be used by our research team as the basis for understanding the molecular mechanisms that govern the development of cancer stem cell as a result of a reprogramming-like mechanism. We hope this investigation will result not only in new concepts in leukemia biology and HSC development, but it will also provide the basis for the development of both a new strategy in cancer therapy and new methods for assessing treatment efficacy.

### **Key Publication**

VICENTE-DUEÑAS C, ROMERO-CAMARERO I, COBALEDA C, SÁNCHEZ-GARCÍA I (2013) Function of oncogenes in cancer development: a changing paradigm. EMBO Journal 32, 1502-13

## Theme 2 Differentiation Therapy



**Antonio Mourão Mosquera**  
Departamento de Química Orgánica  
Universidade de Santiago de  
Compostela

### ***Research Interests***

Vitamin D<sub>3</sub> is not a real vitamin D because it can be produced in the skin by sunlight irradiation of 7-dehydrocholesterol. It is now generally accepted that vitamin D<sub>3</sub>, before eliciting its biological activity, must be hydroxylated to 1a,25-dihydroxyvitamin D<sub>3</sub> (1,25D). This metabolite is considered the hormonally active form of vitamin D<sub>3</sub> and interacts with the vitamin D nuclear receptor (VDR) to control important biological functions such as mineral homeostasis, cell differentiation, cell-antiproliferation, cell growth, apoptosis and the immune system. The fact that VDR has been found in more than 30 target tissues and cell tumors has led to the consideration that 1,25D is involved in a wider array of biological functions including cancer prevention.

Over the past 30 years we have dedicated our efforts to the development of convergent synthetic routes that were used to synthesize the most important vitamin D<sub>3</sub> metabolites and numerous vitamin D<sub>3</sub> analogues functionalized in various parts of the parent vitamin D skeleton. The biological properties of these analogues have served to understand the structure of the bioactive vitamin D conformation. More recently, and on the basis of the crystal structure of 1,25D in complex with VDR, we have designed by docking calculations and synthesized a few vitamin D analogues with higher potency than the natural hormone.

Our interest in the field of vitamin D is now focused on the application of new and flexible synthetic routes to the total synthesis of 1,25D and new vitamin D<sub>3</sub> analogues, which are difficult to obtain by the current synthetic strategies. It is expected that the new analogues will serve to understand the mechanism of action of the natural hormone in particular the non-genomic rapid responses and for the isolation of other VDR, for example membrane-VDR. Particular points of interest are the development of new vitamin D analogues with selective properties and low or negligible calcemic effects for treatment of cancer (colon, breast, lung and prostate cancers), osteoporosis, psoriasis, vitamin D-resistant rickets and Alzheimer. To accomplish our aims we have established external collaborations in the areas of crystal structure and biological activity.

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