**Report to the ANZBMS Council on travel undertaken with funding from the Christine and T. Jack Martin Travel Grant**

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TJ Martin and Vicky Kartsogiannis

I was so very thrilled last year to be awarded the Christine and T. Jack Martin Travel Grant at the 13th Annual Scientific Meeting of the Australian and New Zealand Bone and Mineral Society that was held in Coolum, particularly since that meeting was to honor the achievements of Jack Martin's career.

I am deeply honored to have received this award which is very special to me for several reasons. Firstly, it is awarded in memory of Christine Martin who I had the pleasure of meeting on a number of occasions during the early years of my PhD and I will always remember her. Secondly, the award honors the outstanding and major scientific contributions of one of my mentors Professor Jack Martin to bone and mineral research, but more so his distinguished contributions and interactions to associates and trainees in teaching and research. Thirdly, this award enabled me to visit several prestigious international laboratories to present and discuss various aspects of my post-doctoral studies as well as develop and initiate new experiments and at the same time learn new methods. The award further enabled me to attend for my first time, one of the best International bone cancer meetings, the Frontiers of Skeletal Biology (10th workshop on Cell Biology of Bone and Cartilage in Health and Disease, Davos, Switzerland). At this meeting I presented my most recent research on the OCIL family of Osteoclast Inhibitors and received a Young Investigator's Award.

**Frontiers of Skeletal Biology (10th workshop on Cell Biology of Bone and Cartilage in Health and Disease), Davos, Switzerland, March 24-26, 2004**

The workshop was divided into an instructional part, oral presentations chosen from the abstracts, and poster sessions. The instructional part was devoted to "hot" topics presented in State of the Art Lectures and Invited Presentations. The poster sessions remained indeed the fundamental pillar of the meeting and covered all the fields of skeletal biology. Some of the posters were selected for 10 minutes presentations. The additional workshop "**NEW AND EMERGING TREATMENTS FOR SKELETAL DISEASES**" had been incorporated into the Workshop "**FRONTIERS OF SKELETAL BIOLOGY**".

My personal highlight of this meeting is the fact that it is a small meeting sufficiently intimate to enable extensive interactions with delegates; something that is not achieved by the larger meetings such as ASBMR and IBMS. I can honestly say that I met more scientists in this meeting than I have in all the meetings that I have attended in the past 5 years. The focus of this meeting was to highlight and discuss latest "hot" topics that cover the rapidly advancing field of skeletal biology, both in health and disease.

I was invited to give an oral presentation describing the latest insights on OCILs – a family of new osteoclast inhibitors, for which I was awarded a Young Investigator award.

Highlights of the "Frontiers of Skeletal Biology" workshop were:

- Elegant presentations (Keynote address) on:
    - "VEGF in cartilage and bone" by B. Olsen

- "Current knowledge of osteoblast differentiation" by Gerard Karsenty
- "Regulators of TGF-β/BMP signaling" by S. Piccolo
- "The role of NFATc1 as a Fos target gene during osteoclast formation" by K. Matsuo
- "How basic studies in chronic inflammation are useful in targeting tissue destruction" by J.M. Dayer
- "Back to the beginning: Pyrophosphate revisited" by Graham Russell
- "Strategies for the discovery of new bone anabolics" by Roland Baron
- "Development of RANKL-directed therapeutics: General strategies, challenges, surprises, and successes" by David Lacey
- "SERM's; where can they go?" by H.U. Bryant

- Some equally stimulating presentations (Hot topics) on:
  - "Aggregan knock-in mice resistant to MMP and ADAMTS mediated aggregan catabolism. Effects on growth and development" by Amanda Fosang
  - "Phosphaturic factors and disorders of phosphate metabolism" by S. Fukumoto
  - "Should thyrotropin (TSH) be named as a bone hormone?" by M. Zaidi
  - "Anabolic agents and the BMP2 pathway" by Ross Garrett
  - "Novel genes regulated by parathyroid hormone and their possible roles in its anabolic effects" by Nicola Partridge
  - "ClC-7 as target for drug discovery – Inhibition of bone resorption without inhibition of bone formation?" By Mortan Karsdahl
  - "Inhibitors of alpha v beta 3 integrin – The *in vitro* and *in vivo* evaluation of a potent alpha v beta 3 antagonist for the prevention of osteoporosis" by L.T. Duong

**Laboratory visits**

**Principal Laboratories**

**Bone Biology Group, Division of Clinical Sciences (South), G Floor, University of Sheffield, Medical School, Beech Hill Road, Sheffield, U.K.**

The Head of the Bone Biology Group, **Professor Peter Croucher,** has a long standing interest in Multiple Myeloma having established (with collaborators from Belgium) a useful experimental mouse model of the disease that closely resembles the human disease. The principal interests of the group are in three main areas. These include studies of the regulation of osteoclast formation and survival, and bone resorption. There is a particular emphasis on the role of members of the tumour necrosis factor family (Peter Croucher & Peter Grabowski). Secondly, there is a major focus into understanding the cellular and molecular mechanisms of tumour-induced bone disease. Thirdly, there is strong interest in osteoblast biology and bone formation with a particular interest in the role of peroxisome-proliferator activated receptor (PPAR) family (Karen Still). In addition to specific research projects, the bone biology group have also established a bone analysis laboratory. This laboratory has been created to provide members of the Musculoskeletal Theme and academics throughout the School with access to contemporary approaches to analysing bone. These include access to experienced histologists, bone histomorphometry expertise, x-ray equipment, and access to bone densitometry. The laboratory is currently looking into providing 'state of the art' imaging facilities including micro CT analysis. The activities of this laboratory are managed on a day to day basis by Orla Gallagher.

The primary reason for my visit to Professor Croucher's group in Sheffield was to give me the opportunity to initiate ongoing collaborative studies between the two groups that will ultimately determine the role of Osteoclast Inhibitory Lectin (OCIL) family members in multiple myeloma. As the current interest of my postdoctoral studies in the St Vincent's Institute group (Melbourne, Australia) focuses upon determining the role of OCIL in normal bone remodeling, arthritis and cancer-induced otseolysis, another appropriate disease in which OCIL may have actions is in the progression of multiple myeloma. Professor Croucher's laboratory was ideally suited in developing my research interests with OCIL and osteolytic bone diseases.

Like other diseases of bone, the development of osteolytic bone disease is one of the major clinical features in multiple myeloma. The increase in bone destruction is attributed to uncontrolled osteoclastic bone resorption, and although recent studies have implicated a number of factors such as RANKL and macrophage protein-1 alpha, the major culprits responsible for mediating the increase in osteoclast formation in myeloma, remain unclear. Croucher and colleagues (Shipman *et al*., 2003) have recently demonstrated the ability of recombinant osteoprotegerin (Fc-OPG), a soluble decoy receptor for RANKL, as well as ibandronate and zoledronate, to inhibit the development of myeloma bone disease in the 5T2MM murine model of multiple myeloma (Croucher *et al*., 2003). They showed that Fc-OPG prevented the development of osteolytic bone lesions in 5T2MM bearing animals. These changes were associated with a preservation of the cancellous bone loss induced by myeloma cells and an inhibition of osteoclast formation. The RANKL/RANK/OPG system may indeed play a critical role in the development of osteolytic bone disease in multiple myeloma, as it does in a number of other pathological systems. Like OPG, OCIL also appears to be effective to block RANKL-induced osteoclast formation, but unlike OPG, OCIL can also block TNFα-induced and TGFβ-primed osteoclast formation. Thus the 5T2MM experimental model of multiple myeloma is an appropriate model to evaluate the efficacy of OCIL to limit the progression of this disease. More recently, Professor Croucher has focused on the role of bone marrow endothelial cells in the development of MM bone disease as well as the formation of new vessels as an important feature in the development of myeloma. The MM models are not established in any other laboratory within Australia, and my experience with this model would be invaluable for its transfer to Australian laboratories.

Presenting my current research to the Bone Biology group as well as invited members from other Research groups (Urology and Molecular Medicine) was very effective in setting up an active interaction within the Bone Biology Group.

There were four main research groups that I interacted with: 1) the histology laboratory run by Orla Gallagher who helped me tremendously in my efforts to establish the *in situ* hybridization and immunohistochemistry techniques in Professor Croucher's laboratory. I was very pleased in the end that both techniques were established quite successfully for looking at the expression of OCIL; OPG; RANKL and PTHrP mRNA and protein in paraffin sections from the 5T2MM mouse model (with myeloma disease) and 5T33 model (no myeloma disease); 2) the Molecular Biology Laboratory where I interacted with Dr Clive Buckle, Evy De Leenheer and Jennifer Phillips in undertaking RT-PCR analysis and sequencing, as well as setting up REAL-TIME PCR (SYBRgreen) for examining the expression of OCIL family members in human and mouse MM cell lines; 3) the tissue culture and osteoclast assay laboratory for tissue culture work using non-adherent cell lines (5T33; OPM-2; JJN-3; NCIH929; RPMI-8226; XG1) and the adherent cell lines STR10, STR12 (endothelial cell lines); 4) Dr Angela Rogers (Professor Croucher's group) and Sue Clarke (Cancer Studies) who were instrumental in FACS experiments using OCIL antibodies on 5T33, STR10, and STR12 cell lines.

**Nuffield Department of Orthopaedic Surgery, University of Oxford, Oxford, U.K. The Botnar Research Centre, University of Oxford, Institute of Musculoskeletal Sciences**

The Botnar Research Centre is a purpose built research facility housing the research arm of the Nuffield Department of Orthopaedic Surgery. The research endeavors cover a wide range of musculoskeletal disorders ranging from joint problems such as osteoarthritis and rheumatoid arthritis, to bone diseases such as osteoporosis, as well as assessment and design of joint replacements. The research centre can accommodate 120 scientists. Major interests of the centre's researchers include genetics of osteoarthritis, osteoporosis and inflammatory arthritis, osteoclast and osteoblast biology, and biomedical engineering.

**Professor Graham Russell** has played a central role in studying the biological effects of bisphosphonates, and in their evaluation for the treatment of bone disorders. Bisphosphonates are now the most widely-used drugs for the treatment of bone diseases throughout the world. His other research interests include bone cell biology - work which is directly concerned with the improvement of treatment of osteoporosis, Paget's disease and malignant disease of bone. His research team is now based in the new institute, where they play a key role in the investigation of the cell biology and biochemistry of common bone diseases, especially osteoporosis and malignant disease of bone, including trials of new treatment.

I was invited to give a seminar on my recent work to several research groups at the Botnar Research Centre which generated a number of questions and significant interest for future experiments. I spent three days meeting members of the Botnar Institute, including: Professor Nick Athanasou who studies pathogenetic mechanisms in bone and joint disease (possibilities of examining OCIL/OPG/RANKL/PTHrP expression by in situ hybridization and immunohistochemistry in human patient samples of multiple myeloma, rheumatoid arthritis, Giant Cell Tumour and Pajet's disease of bone were discussed); Dr Claire Shipman who studies bone disease associated with cancer and myeloma (Claire provided some helpful insights into future experiments involving OCIL and was directly involved in determining OCIL expression by FACS analysis); Dr Afsie Sabokbar who studies osteoclast biology (Afsie provided stimulating discussions on possibilities of using OCIL in osteoclast-type assays); Dr Philippa Hulley who studies bone cell biology and intracellular signaling (a number of interesting topics were discussed); James Edwards who's a PhD student (James was very helpful in organizing for me to do a trial run of in situ hybridization and immunohistochemistry on a selection of various human samples of multiple myeloma; rheumatoid arthritis; Giant Cell Tumour; and Pajet's disease of bone); Isabelle Gennero who is finishing her post-doctoral studies at the Botnar Centre (it was great to have a chat about something outside OCIL!!).

**Overall Outcomes**

Overall, I was very pleased to achieve the aims that were outlined in the travel grant. Whilst being provided with the opportunity to attend and present my work at the Frontiers of Skeletal Biology ($10^{th}$ workshop on Cell Biology of Bone and Cartilage in Health and Disease, Davos, Switzerland) and The Botnar Research Centre [University of Oxford, Institute of Musculoskeletal Sciences, Oxford, U.K.], my predominant time overseas was spent working in the laboratory of Professor Croucher [Bone Biology Group, Division of Clinical Sciences (South), University of Sheffield, Medical School, Sheffield, U.K]. The ten weeks were used for laboratory-based research whereby I established the *in situ* hybridization and immunohistochemistry techniques

quite successfully in Professor Croucher's laboratory and gained a lot of experience and insights into the models and cell lines of multiple myeloma disease which are applicable to my research interests. I determined the expression of OCIL in various models of multiple myeloma disease using a number of different techniques such as RT-PCR and REAL-TIME PCR; FACS; immunohistochemistry and *in situ* hybridization. My time there has consolidated a collaborative project which will be further developed based on exciting new discoveries from the expression/localisation data achieved. Professor Croucher provided constant guidance and stimulating discussions towards future investigations of OCIL and multiple myeloma. One avenue that we wish to actively pursue in the near future is the lentivirus approach of recombinant protein production which will be established in Professor Croucher's laboratory or alternatively, we might consider using murine Fc-OCIL protein constructs for long term treatment (12 weeks) in the MM mouse models. Another avenue to follow up on will be the potential use of an effective neutralizing OCIL antibody for use in *in vitro* osteoclast assays. This will form the basis of an active ongoing collaboration and one that will hopefully yield a few insights into the potential actions of OCIL in MM disease.

I would finally like to thank the Australian and New Zealand Bone and Mineral Society and the sponsors Merck, Sharpe and Dohme for providing me with the funds which enabled me to attend the "Frontiers of Skeletal Biology" meeting in Davos, Switzerland, and work with Professor Croucher's group in Sheffield. I would also like to extend a special THANK YOU to Professor Peter Croucher and his group for having me. It was an absolute pleasure to work with them and I feel that I have gained so much from this experience. I feel very privileged to have received this award.

Thank you,

*Vicky Kartsogiannis, PhD*