Report from the 2005 recipient Christine and TJ Martin Award

Susan Allison

I would first of all like to sincerely thank the ANZBMS committee and Professor Jack Martin for awarding me this travel grant, allowing me to visit overseas laboratories to present my research, learn new techniques, and to establish new collaborative research; a unique opportunity which I feel very honoured and privileged to have had. This award also enabled me to attend the Frontiers of Skeletal Biology workshop, in Davos, Switzerland for the first time, at which I presented recent findings from my PhD research.

Department of Oral Cell Biology, Faculty of Medicine, Umea University, Sweden

My first laboratory visit was to the Department of Oral Cell Biology, in Umea, Sweden, where I was hosted by Professor Ulf Lerner. Professor Lerner's laboratory has a strong interest in the regulation of bone cell activity by neuronal factors, and has several publications investigating the role of various neuronal factors, including vasoactive intestinal peptide (VIP), and calcitonin gene-related peptide (CGRP), in the control of bone cell activity. Although previously our laboratories have not collaborated, in 2005 our laboratory hosted a post doctoral research fellow from the Department of Oral Cell Biology; Dr Pernilla Lundberg, and who I had worked quite closely with throughout her duration in Sydney.

In addition to a strong background in the regulation of bone by neuronal factors, Professor Lerner's laboratory also has extensive experience in culturing murine osteoclasts, and has previously used cultures of osteoclasts derived from spleen and bone marrow macrophages (BMMs) to investigate the effects of cysteine protease inhibitors and calcitonin on osteoclast formation and differentiation. The purpose of my visit was to learn new techniques for culturing osteoclasts, and to use these techniques to investigate the osteoclast phenotype of the neuropeptide Y (NPY) Y1 receptor knockout mice, developed in our laboratory. The Y1 receptor is one of 5 receptor subtypes which mediates the actions of NPY, a neuropeptide involved in the regulation of multiple and variable functions, including the regulation of energy homeostasis, circadian rhythms, anxiety, and blood pressure. The Y1 receptor is widely distributed, in both the central nervous system and in the periphery, and regulates some of the downstream effects of NPY, including vasoconstriction of blood vessels, and the regulation of adipose deposition. Histomorphometric studies in our laboratory have revealed distal femur from germline Y1 knockout mice to have elevated parameters of bone resorption compared with wild type controls. Before I left for Sweden, Y1 knockout and wild type mice were shipped from our laboratory to Umea. During my time in Umea, I firstly learnt new techniques for culturing osteoclasts from bone marrow macrophages, spleen, and whole bone marrow preparations, and then applied these techniques to our Y1 knockout mice, to investigate the osteoclast phenotype of this model *in vitro*. Unexpectedly, while

parameters of bone resorption were elevated in Y1 knockout mice *in vivo*, the development of osteoclasts from bone marrow macrophage or spleen cells was actually reduced *in vitro*. These and other interesting findings from these experiments has resulted in the development of a larger project, which will continue as an ongoing collaboration between our two laboratories, with which we hope to further understand why these differences *in vivo* and *in vitro* exist.

During my time in Umea I was also invited to present some of my research at the "Cancellous Bone Network", a group of basic scientists, orthopaedic surgeons, and endocrinologists who meet regularly to discuss various current topics. As this was a small group it provided an excellent opportunity to discuss the findings from some of our recent research, with clinicians and scientists who were not particularly familiar with my research area, investigating the control of bone formation by the neuropeptide Y receptors. There was particular interest in areas of my project which may have potential future therapeutic applications for the treatment of osteoporosis, and the advice and insight I received from these discussions has proven useful for planning the next steps of these projects.

The five weeks I spent in Umea was also an excellent opportunity for me to finalise parts of the research which was performed together with Pernilla Lundberg as part of her fellowship within our laboratory in 2005. It was also great to be able to discuss our findings with different scientists with differing areas of expertise to obtain alternative viewpoints on how to interpret our findings and future directions for this project, which will remain as an ongoing collaboration between our two laboratories.

Overall during my time at Umea University, I was amazed at how much was achieved within my short time there, with new exciting findings resulting in the initiation of a new collaboration. This wouldn't have been possible without the commitment from other members of the laboratory; in particular the research assistants who taught me new techniques and worked alongside me to help me achieve my aims as efficiently as possible, and of course Ulf Lerner and Pernilla Lundberg, who provided valuable insight, helped me interpret the data from these studies, and were excited enough by the findings from these studies to initiate the beginnings of a new project.

Frontiers of Skeletal Biology, 11th and Valedictory Workshop on Cell Biology of Bone and Cartilage in Health and Disease, Davos, Switzerland

This was my first opportunity to attend the Frontiers of Skeletal Biology Workshop on Cell Biology of Cartilage in Health and Disease, which was held in Davos, Switzerland (March 18th-22nd, 2006). There were several outstanding presentations by keynote speakers. In particular, I was interested in Professor Gerard Karsenty's talk on the Central Control of Bone Mass, discussing the findings from the leptin-deficient ob/ob mouse model, and other models related to the central control of bone mass by factors which within the brain are generally associated with feeding behaviour and the

regulation of energy homeostasis, as this directly relates to work in our laboratory, investigating the control of bone remodelling by the neuropeptide Y receptors. Another presentation which I found particularly interesting was a 'Hot Topic' presentation given by Paul Frenette, who presented some interesting new findings investigating the regulation of haematopoietic stem cell egress from the bone marrow by the sympathetic nervous system.

I also presented some findings from my PhD investigating the anabolic activity of the hypothalamic Y2 receptor pathways in models of sex hormone deficient bone loss, which was well received.

Cardiovascular and Metabolic Diseases (Osteoporosis) Department, Pfizer Global Research and Development Inc, Groton, Connecticut

Some of the research projects conducted by our laboratory over the past 2 years have been performed in collaboration with the Osteoporosis Research Laboratory at Pfizer Inc., Connecticut. A major portion of this was performed as part of my PhD project, investigating the response of the Y2-receptor mediated anabolic pathway in models of osteoporosis. The first part of these studies investigated the response of pre-existing or conditional activation of the Y2-anabolic pathway to bone loss induced by sex hormone deficiency; data which was presented at the Frontiers of Skeletal Biology Workshop, in Davos. A second component of this collaboration was to investigate the response of conditional activation of the Y2-anabolic pathway to bone loss induced by aging, to investigate whether activation of this centrally-mediated pathway can reverse or repair aging-induced changes in bone microarchitecture. These studies were performed in Sydney, and the bone phenotype of these mice was characterised using histomorphometry. However, the histomorphometric analyses revealed some unusual changes occurring within both the cortical and trabecular bone indicating that further characterisation of these bones was necessary to better understand the changes that were occurring. The purpose of my visit to Pfizer therefore, was to discuss the findings from this project so far, and to perform further analyses to obtain a complete picture of these phenotypes.

During my time at Pfizer, I was able to perform micro-CT on isolated femurs from mice from this study allowing me to further assess the trabecular bone phenotype of these models and to visualise the changes in bone microarchitecture that had occurred in response to aging and to conditional deletion of hypothalamic Y2 receptors. I was also able to perform peripheral quantitative computed tomography (pQCT) to further assess changes in the cortical and trabecular bone, which were subsequently compared to my histomorphometric and micro-CT data. This was a great opportunity for me to learn these new techniques in the vicinity of scientists who regularly apply qQCT and micro-CT to similar samples. The expertise of these scientists provided invaluable insight as to the changes we observed allowing a better understanding of the mechanisms involved. These studies also provided the opportunity to obtain a

comprehensive dataset for this study, including histomorphometric data, and now micro- and pQCT to gain a better understanding of the microarchitectural and density changes that are occurring in these models.

During my time at Pfizer I also presented findings from the studies which were performed over the past 2 years as part of the Pfizer collaboration. This was an excellent opportunity for the scientists within these laboratories to see what we have achieved together over the past 2 years. We also discussed what these findings mean for potential therapeutics, and the next step these projects will take.

In addition to performing micro- and pQCT on my samples from Sydney, I was also able to observe and discuss some new techniques for the isolation of RNA and analysis of gene expression which are currently under development. RNA isolation procedures used at Pfizer are varied, with modifications of standard procedures according to tissue type. The aim is to establish procedures for subfractionation of bone tissue, to obtain consistent samples from subcompartments of bone for gene expression analysis. The importance of performing such analyses within bone is becoming increasingly recognised, due to the heterogeneity of cell types and the specific localisations of signals and responses within bone tissue, which is lost when whole bone samples are ground and extracted. Unfortunately, during my visit, they were experiencing some equipment problems and we were not actually able to perform any analyses. I did however have the opportunity to discuss the development of these techniques and the process of improving them for consistency, which is something that will hopefully be implemented for future analysis of gene expression of bones from our models.

Overall, I am pleased that I have achieved the proposed aims of this award. It was an excellent opportunity to extend specific aspects of my own projects with the knowledge, expertise, and resources of overseas laboratories. The opportunity and the experiences I have gained from interacting with the scientists from these laboratories on a day-to-day basis, planning and implementing novel experiments and techniques, and discussing the findings from these studies was an invaluable and unique opportunity from which the benefits are enormous. The exciting preliminary findings which were obtained during my time in Sweden have developed into an ongoing research project, while the experiments that were performed in Connecticut enabled me to obtain data to complete this portion of my study, and which are currently part of a manuscript in preparation. I would like to again thank Merck, Sharpe and Dohme for the sponsorship of this travel award. I would also like to thank the ANZBMS committee and Professor Jack Martin for providing me with this opportunity; I feel very privileged to have received this award. And of course I would like to thank Professor Ulf Lerner and Dr Lydia Pan, my hosts at Umea University and Connecticut, respectively. It was an absolute pleasure to have worked with you and your teams; the experiences I have gained from my time with you are invaluable.