



ANNUAL RESEARCH REPORT 2006



The Cancer Research Advisory Committee (CRAC) once again worked hard to allocate funding for all of the external research initiatives of The Cancer Council South Australia (TCCSA), including project grants, research fellowships and senior research fellowships, travel grants, vacation scholarships and distinguished visitor travel grants.

With a budget of approximately \$2M we were able to fund 14 new projects in 2006, as well as 6 projects that were continued from 2005. Once again there was an increase in the number of applications for grant funding in the previously defined priority areas. We easily met our target of 40% funding in these priority areas, and we continue to shift the balance further in favour of these TCCSA priorities. The Committee was pleased to see some changes introduced to the NHMRC application process for next year, following our comments on the process this year, and we look forward to seeing the effects of these changes.

TCCSA continued funding of six Research Fellowships and supported the Freemasons and Peter Nelson Leukaemia Research Fellowships. Other initiatives underway include increased funding for the Chair in Cancer Care at the University of Adelaide. Data managers continue to receive TCCSA funding to support clinical trials into cancer therapies.

We funded 13 vacation scholars awarded scholarships for research work in the summer period of 2006-2007. Eighteen travel grants were given for scientists to present their work at conferences of their peers around the world. Two of our travel scholars won awards at the Multinational Association for Supportive Care in Cancer annual meeting in Toronto in June 2006. We had one distinguished visitor in 2006 who successfully showcased their work to South Australia.

Research 2006

RESEARCH PROJECT GRANTS

Name	Subject	Amount
Dr Chris Hahn , A/Professor Jennifer Gamble Vascular Biology Laboratory Hanson Institute	Identification of the role of a novel angiogenic gene, VasGAP, in development and cancer Other	\$76,000

Lay Summary

Angiogenesis (formation of new blood vessels from pre-existing vessels) is well recognised as an essential process for the promotion and expansion of cancers. We have used a zebrafish model to study the importance of a novel gene, VasGAP, which we previously identified in a screen for angiogenic genes. We have identified a role in cell movement or migration which ultimately leads to disruption of the appropriate formation of blood vessels in regions of the fish from which new blood vessel lining cells and blood cells themselves are derived. This exciting finding may have significant implications for a role in tumours and their progression, which involves continual movement and remodelling of blood vessels.



Scientific Summary

Angiogenesis (formation of new blood vessels from pre-existing vessels) is well recognised as an essential process for the promotion and expansion of cancers. We have investigated the role of a novel protein, VasGAP, that we originally identified in an screen for angiogenic genes, in a zebrafish model of vasculogenesis and angiogenesis. This model closely resembles that of blood vessel formation in humans.

VasGAP knockdown perturbs formation of the vasculature in zebrafish

We have cloned the full length zebrafish VasGAP cDNA orthologous to human VasGAP. During early development of the zebrafish, VasGAP mRNA is expressed in mesodermal-derived cell types and in regions characterised by migrating cells. Morpholino antisense oligonucleotide (MO) knockdown of VasGAP protein expression caused a marked "morphant" phenotype characterised by disruption of the vascular circuit within the "intermediate cell mass" (ICM). This resulted in ICM enlargement and a severe reduction in blood flow through this region and ultimately the entire fish. Interestingly, it is in and through the ICM in the early developing fish that haemangioblasts proliferate and migrate out to generate the developing vasculature and blood cells. We also used MO for knockdown of VasGAP in Fli1-GFP transgenic zebrafish, in which GFP is expressed specifically in endothelial cells (EC). This further demonstrated a disrupted vasculature in the ICM region and an accumulation of GFP-expressing cells. In addition, in situ RNA hybridisation for Fli1, a marker of EC of the early vasculature, demonstrated accumulation of Fli1 positive cells in the ICM and proximal tail region and disruption of the dorsal aorta and axial vein. GATA1 expressing cells (ie. marker for haematopoietic cells during embryogenesis) also clustered in the ICM. This result suggests the exciting possibility that VasGAP is involved in progenitor cell movement and ultimately differentiation. We propose that the knockdown of VasGAP in these early mesodermal cells inhibits their migration by disrupting Convergent Extension movement, resulting in inhibition of zebrafish elongation and the consequent inappropriate development of the vascular circuit.

From these and other studies, it is clear that VasGAP plays a crucial role in the migration of cells, most probably via its regulation of the actin cytoskeleton. In addition, in other studies, we have also identified a role in cell-cell interactions in EC.

Thus, these studies have confirmed our hypothesised role of VasGAP in cell migration in an in vivo model and, interestingly, implicated this gene in the development of certain vascular networks or vascular cell differentiation. This may have significant implications for a role in tumours and their progression which involves continual movement

and remodelling of blood vessels.

Investigation of VasGAP expression during mouse development, in human tumours and in a mouse tumour model

We have put considerable effort into in situ RNA hybridisation staining for VasGAP mRNA with little success. This is most probably due to VasGAP being low abundance and hence difficult to detect. We are, however, able to detect endogenous VasGAP expression in whole mount staining in the zebrafish and have therefore concentrated more of our efforts in understanding the role of VasGAP in this model (see above).

While we can detect VasGAP protein in western blots, we have had trouble detecting it in tissue sections using 2 different antibodies we have generated. However, we have further purified and concentrated these antibodies and are now in a better position to perform tissue microarrays.

The mouse tumour model work relies somewhat on the outcome of the expression work above. We already have a number of the adenoviruses generated and are gearing up to for experiments to inhibit tumour growth with adenoviral-mediated VasGAP knockdown.

A/Professor Paul Vasey, Dr Michael Quinn, Professor John Simes, A/Professor Michael Friedlander, Dr Martin Buck, Dr Bogda Koczwara Department of Medical Oncology Royal Brisbane and Women's Hospital	Carboplatin Flat Dosing versus Inpatient Dose Escalation in First Line Chemotherapy of Ovarian Cancer Ovarian	\$6,000
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Lay Summary

Ovarian cancer is a deadly disease, being rarely curable when advanced - a situation which occurs at diagnosis in over two thirds of patients. The treatment of advanced ovarian cancer following surgery is with chemotherapy. The backbone of any treatment regimen is a platinum compound e.g. Carboplatin. Unfortunately, despite many years of usage, the optimal dosing of this important drug is still not clear. Previous studies have suggested that increasing the dose of platinum in an individualised way (ie patient by patient) may improve its effect. In this study, patients are randomised to receive a flat or unvarying dose of carboplatin for six courses (the standard treatment) or to receive carboplatin at increasing doses - if possible - depending on their toxicity. Over 1000 patients are being recruited worldwide including Australia.

Scientific Summary

24 centres active in Australia and New Zealand, 27 patients recruited in Australia and New Zealand.

Dr Stuart Pitson Human Immunology Institute of Medical and Veterinary Science	The cellular regulation of sphingosine kinase by eEF1A and its role in tumorigenesis Breast, bowel, lung, prostate, ovarian	\$70,500
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Lay Summary

The growth and survival of cells is a tightly controlled process that when disrupted can lead to cancer. One mechanism that cells use to control their growth and survival involves the enzyme sphingosine kinase. Recent studies have shown that if sphingosine kinase levels are too high in the cell it can either lead directly to cancer, or increase the likelihood of cancer resistance to chemotherapeutics. Therefore, understanding the way cells regulate sphingosine kinase is likely to lead to identification of new targets for anti-cancer therapeutics. We have recently identified that regulation of sphingosine kinase can be achieved through another protein called EF1A. Notably, EF1A has been previously suggested to be involved in inducing the formation of some solid tumours, but the mechanism was unknown. Our findings suggest that EF1A may cause cancer by increasing sphingosine kinase levels. The main aim of this study is to understand the regulation of sphingosine kinase by EF1A so that anti-cancer therapeutics may be developed to control this regulation.



Scientific Summary

Sphingosine kinases catalyse the formation of sphingosine 1-phosphate, a bioactive lipid that regulates a diverse range of cellular processes. Elevated sphingosine kinase activity in cells prevents apoptosis, enhances cell proliferation, and leads to cell transformation and tumorigenesis. This indicates an oncogenic role for sphingosine

kinase, which is further supported by studies showing elevated sphingosine kinase in a variety of human solid tumours and inhibition of tumour growth *in vivo* by sphingosine kinase inhibitors. We have discovered that sphingosine kinase can be activated by interaction with translation elongation factor 1A (EF1A). EF1A contains a Ras-like GTPase domain and, notably, we have found that it only enhances sphingosine kinase activity when in the GDP-bound form, but not when in the GTP-bound form. Disregulation of EF1A occurs in many tumours, with evidence indicating this can drive tumorigenesis in some cancers by currently unknown mechanisms. In particular, tumorigenesis of some cells appear driven by natural expression of (i) PTI-1 (prostate tumour inducer-1), a truncated version of EF1A that lacks critical residues required for GTP binding, and (ii) TCTP (translationally controlled tumour protein), a guanine nucleotide dissociation inhibitor of EF1A, which is thought to enhance the cellular abundance of EF1A in the GDP-bound form. Since activated sphingosine kinase is oncogenic, and both of these tumour promoters enhance the cellular abundance of forms of EF1A that can activate sphingosine kinase, we hypothesised that tumorigenesis driven by this EF1A disregulation is mediated via sphingosine kinase. Thus, the main aim of this proposal was to examine the regulation of sphingosine kinase by EF1A in both normal and oncogenic signalling, and in particular, elucidate the role of sphingosine kinase in tumorigenesis associated with cellular defects in EF1A.

We have now established that sphingosine kinase activation is an obligatory step in the oncogenesis of PTI-1 since inhibition of sphingosine kinase by either chemical inhibitors or a expression of a dominant-negative sphingosine kinase blocked the oncogenic effects of PTI-1. These *in vitro* findings identify sphingosine kinase as a therapeutic target for human tumours where PTI-1 is expressed. Further studies are now ongoing to examine if these findings are consistent within an *in vivo* setting.

We have also established that TCTP expression enhances cellular sphingosine kinase activity We are currently determining if, like the situation for PTI-1, this sphingosine kinase activation plays a critical role in oncogenesis induced by TCTP. Successful outcomes in these studies may also identify sphingosine kinase as a therapeutic target for human tumours where TCTP expression is elevated.

<p>A/Professor Robert Richards School of Molecular and Biomedical Sciences Molecular Life Sciences The University of Adelaide</p>	<p>Function of the FOR/WWOX gene and its contribution to cancer cell biology Other</p>	<p>\$80,025</p>
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Lay Summary

The main aim of this research is to understand the role that a specific type of DNA instability plays in cancer. This DNA instability has been found to occur in cancer cells at specific places on human chromosomes, known as common chromosomal fragile sites. These common chromosomal fragile sites are found in all individuals. The most readily broken of these sites are spanned by large genes however the function(s) of these genes are not understood. Since the fragile site-associated DNA instability is most likely to affect the function of these genes the primary focus of this research is to understand the normal function of these genes so that the manner in which their perturbed gene expression contributes to cancer cell biology can be determined.

While it is clear that DNA instability at chromosomal fragile sites occurs in cancer the contribution that this makes to cancer cell biology is unclear. Determination of the normal function of the genes spanning the most sensitive of the human fragile sites will enable an understanding of the manner in which this instability contributes to neoplasia and how this knowledge might best be utilised.

Scientific Summary

Using genetic and protein biochemical (proteomic) approaches we have embarked on a functional analysis of the *WWOX* gene that spans the *FRA16D* fragile by analysis of the gene in human cancer cells and of its orthologous gene in the highly manipulable genetic model *Drosophila*. Our laboratory has produced a variety of human (HEK293) cell lines depleted or over-expressing *WWOX* for use in assessing the function of this gene. We have also constructed mutant lines of *Drosophila* that are deficient or over-express *Wwox*. We plan to continue the genetic and biochemical analysis of these human cells and *Drosophila* lines (validating findings in one with experiments in the other). Using this approach we have evidence in *Drosophila* of a role for *WWOX* in relation to reactive oxygen species because of perturbations in superoxide dismutase (SOD1) protein isoforms and mRNA levels. We will confirm these findings in *Drosophila* with experiments in the HEK cells.

Recently, a *Wwox* gene knock-out mouse was found to have distinct phenotypes (early death, high tumour incidence) which was surprising in view of the lack of any observable phenotype in the *Drosophila* mutants we have analysed. The nature of the mouse *Wwox* gene knock-out is such that the N-terminal fragment of the *Wwox* gene is still expressed and this encodes a peptide with high homology to the PIN1 peptide. PIN1 has previously been implicated in tumour formation. We will therefore explore the hypothesis that this naturally occurring splice form (in humans) is responsible for the phenotype seen in the *Wwox* gene knock-out mice.

Outcomes and Significance

While it is clear that DNA instability at chromosomal fragile sites occurs in cancer the contribution that this makes to cancer cell biology is unclear. Determination of the normal function of the genes spanning the most sensitive of the human fragile sites will enable an understanding of the manner in which this instability contributes to neoplasia and how this knowledge might best be utilised.

A/Professor Geoffrey Lindeman, Dr Gillian Mitchell, Dr Alan Stapleton Familial Cancer Centre Royal Melbourne Hospital	Identification of Men with a genetic predisposition to Prostate cancer and their Clinical Treatment - The IMPACT Study Prostate	\$47,700
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Lay Summary

This study forms part of an international effort to study prostate cancer risk and behaviour in men who carry BRCA mutations, by comparing various factors with male siblings who have tested negative for BRCA mutations. IMPACT could thus provide useful information on the role of prostate screening in men who are at particular risk for prostate cancer. As part of the research effort, blood and tissue will be collected in an effort to identify new prostate cancer markers. The discovery of new 'biomarkers' could potentially be applied more broadly to all men.

IMPACT will recruit male subjects from Familial Cancer Centres throughout Australia. Ethical approval has now been obtained from a number of sites and enrolment will commence in March 2007.

Thus far Human Research Ethics Committee (HREC) submissions have been made at Melbourne Health (approved), Peter MacCallum Cancer Centre (approved), Children's Youth and Women's Health Service SA (approved), Repatriation General Hospital SA (approved), Westmead Hospital NSW (approved), Prince of Wales Hospital NSW (approved), Hunter New England NSW (in final phase and approval anticipated), King Edward Memorial Hospital WA (in final phase and approval anticipated) Queensland and Tasmanian state-wide HREC's, electronic HREC submission complete and response awaited. A permanent project officer will be employed in Melbourne (principally located at the Peter MacCallum Cancer Centre) with additional project support provided at the Royal Melbourne Hospital and the Familial Cancer Unit in Adelaide.

As we now have HREC approval at almost all sites and we will be appointing a project officer imminently, we expect that a rapid recruitment will occur in the next few months. Invitation letters have been posted in SA and we will be posting the first invitation letters in Victoria within the next two weeks.

Dr Mitchell (VIC) attended the IMPACT researchers meeting in Europe in December 2006 (funded by the central group study group) and it is apparent that the only country to have started recruitment is the UK (the originators of the study). Our presentation about our experience in setting up the study in Australia so far, showed that we are the country most advanced in preparations for the study and so we have not lost our opportunity to recruit a significant number of patients to the study. We remain in the best position (along with the UK and Israel) to recruit an important proportion of patients to this international study.

Professor David Currow, Dr Amy Abernethy, Ms Debra Rowett, Ms Tania Shelby-James, Ms Belinda Fazekas, Dr Peter Allcroft Palliative and Supportive Services Flinders University	A pilot study of the effectiveness of academic detailing on dyspnoea in cancer patients in a palliative care setting Prostate	\$65,263
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Lay Summary

This project is investigating an educational program to support GPs in clinical decision making about breathlessness in people with advanced cancer. Improving evidence is constantly evolving and would not have been available to current doctors when they were training. Management of breathlessness is a challenge in the care of people and their carers. Effective strategies to translate known evidence into practice are needed. Most comprehensive educational programs are not useful in the real world because they are too expensive, time consuming and complicated. This educational program (Academic Detailing) is brief, easy to understand, based on the best evidence available and is acceptable to GPs. Academic Detailing has a proven track record for improving patient based outcomes by improving physician practice and patient personal care.

This study involves a Palliative Care Specialist visiting GPs in one group who are looking after patients with cancer who are referred to Southern Adelaide Palliative Services. The palliative care specialist will give them evidence based information on treating patients who have cancer and have developed symptoms of breathlessness. The GPs in the other group will not receive Academic Detailing but their patients will receive the best-evidence based treatment from the Southern Adelaide Palliative Service for their symptoms.

This study aims to further our understanding of how Academic Detailing will work for the GPs looking after cancer patients at the end of life.

We will be giving the GPs a questionnaire to see if this benefitted their practice and changed any of their methods of treatment and how usefull they found the process. Patient and their carers will be visited at 6 and 8 weeks following treatment from their GPs to measure their breathlessness.

Evidence shows that Academic Detailing works in palliative care. The aim of this pilot is to test whether it works in improving the management of breathlessness. The aim of the pilot is to see if there is any measureable benefit to see if this method should be pursued.

The ultimate goal of this pilot is to improve the cancer patients breathlessness and to determine the best method of initiating change in the primary care setting. Based on our findings, an implementation and training program could be developed which could be implemented nationally. Better management of breathlessness has the potential to improve quality of life, reduce hospital admissions and reduce total cost.

Scientific Summary

The primary aim is to explore the potential effectiveness of Academic Detailing on dyspnoea in cancer patients within a palliative care setting.

The secondary aims are:

- 1) Developing and evaluating detailing materials for dyspnoea.
- 2) Determining optimum methodology (including sample size and power calculations) for a larger randomised controlled study.

HYPOTHESIS

A brief educational package is acceptable to GP's and can help them better manage the breathlessness of their terminally ill cancer patients.

ANALYSIS

Paired t-test examining dyspnoea scores before (at consent) and after (four weeks post) education session and changes in knowledge and attidtudes dyspnoea. Results will not be statistically different in a pilot study but will be used to inform a larger randomised controlled trial.

METHODS

Unblinded randomised controlled trial of Academic Detailing for GPs versus routine specialised palliative care (no detailing). We will enroll 40 patients and their GPs referred to Southern Adelaide Palliative Services care with cancer with breathlessness over a 12 month period.

Outcome 1)

We will use the Numeric Rating Scale to measure levels of breathlessness.the Australian Modified Karnofsky performance scale and DES and MRC to measure baseline performance status and the McGill Quality of life questionnaire to measure baseline quality of life. Participants GPs in the intervention group will receive two academic detailing visits 2 and 4 weeks after the patients enrollment. We will follow up with the patients at 6 and 8 weeks.

Outcome 2) GPs will be visited at 6 weeks post enrollment, to assess the impact of the educational package.

E/Professor Alexander Morley, Dr Michael
Brisco, A/Professor Pamela Sykes, Dr Bryone
Kuss
School of Medicine
Flinders University

Improving the measurement of minimal
residual disease in acute leukaemia
Leukaemia

\$76,000

Lay Summary

Sensitive measurement of the number of leukaemic cells in the bone marrow enables the effect of treatment to be assessed and can be used to guide treatment decisions. A "marker" which enables a leukaemic cell to be identified is necessary for measurement and the present study aims to improve the method of identifying a suitable marker and to test whether improved marker identification results in improvement of treatment decisions. In the first year of the grant we have evaluated a new approach to marker identification, both in our laboratory and in collaboration with a group of Sydney researchers. Preliminary results suggest that this approach identifies a wider range of markers than the approach currently used.

Scientific Summary

Improving the measurement of minimal residual disease in acute leukaemia

The overall aim of this research project is to improve the measurement of residual disease by fully defining the

repertoire of leukemic clones in acute lymphoblastic leukaemia, to develop a method using multiplex PCR in order to determine the chemoresistance of multiple clones, and to finally determine whether the presence of chemoresistant minor clones at diagnosis can be of use in predicting relapse.

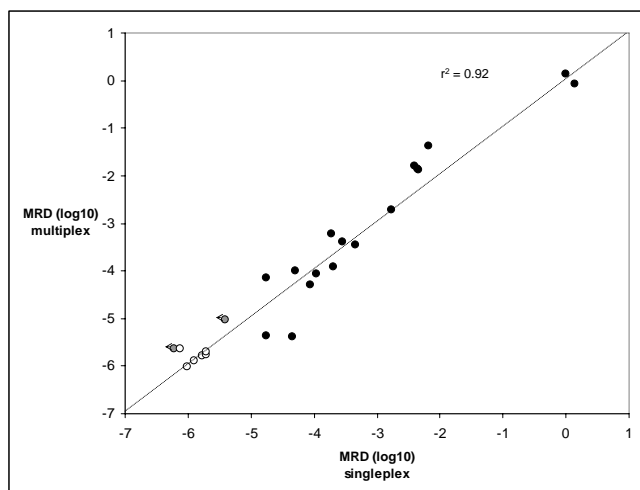
Repertoire analysis in 25 children and 18 adults, shown in the table below, enabled detection of two or more of rearrangements in the great majority of children, whereas only one rearrangement was detected in the majority of adults.

Number of rearrangements	No rearrangements	VDJ rearrangements					DJ rearrangements	
	0	1	2	3	4	5	1	2
Childhood ALL	1	3	9	8	0	1	2	1
Adult ALL	1	11	5	0	0	0	0	1

Extended repertoire analysis using preamplification was performed in 10 children and 10 adults. This analysis resulted in detection of an additional number of rearrangements marking minor clones. A direct comparison with the conventional BFM technique was performed. Repertoire analysis appeared to detect more rearrangements, particularly those marking small clones.

A collaborative study with the Sydney group is directed towards further evaluation of repertoire analysis. The aim is to study 50 patients who have previously been studied by the conventional technique and to determine whether or not repertoire analysis is superior. Thirty-four patients have been studied to date and preliminary results suggest that repertoire analysis is indeed able to detect more immunoglobulin gene rearrangements.

A technique for multiplexed PCR quantification of IgH rearrangements has been developed. In 10 patients quantification by a multiplexed PCR of two or three rearrangements was compared to individual quantification of each rearrangement. The results correlated well and are shown in figure below.



In the second year of the grant it is proposed to complete the repertoire comparison with the Sydney group and to commence investigation of patients who have relapsed, with appropriate controls.

Professor Martin Tattersall, Dr Michael Jefford,
Dr Sid Selva
Department of Cancer Medicine
University of Sydney

Enhancing cancer patient participation when
discussing clinical trial enrolment: evaluation
of a question prompt list

\$36,300

Lay Summary

Surveys of the general public have found widespread support for the concept of clinical trials as an important and ethical means of developing improved medical care. Randomised clinical trials are the gold standard for treatment evaluation, yet trivial proportions of patients enter clinical trials in many institutions that promote clinical trial participation. Patient refusal explains about a quarter of nontrial participation. Even when patients agree to participate

in a clinical trial, they frequently do not understand the rationale for the trial. Patients who actively participate in medical consultations by asking questions are able to change the focus of discussion and control the amount of information provided. We have investigated the provision of a question prompt list to cancer patients before their initial consultation with an oncologist. In four separate studies, we have found that provision of the question prompt list increased patients' question asking. When the oncologist endorsed the use of the prompt list, patients' recall of and satisfaction with the consultation was enhanced, and their anxiety level was significantly reduced. We have prepared a question prompt list for patients to facilitate their participation in clinical trial consent discussions. This project aims to investigate the effects of promoting question asking when cancer patients are asked to consider entering a randomised clinical trial. The proposed study will determine whether providing cancer patients with a clinical trial question prompt list when considering clinical trial participation enhances their understanding of the cancer clinical trial and increases their satisfaction with the informed consent and treatment decision making process. This simple intervention may help patients participate more fully in discussions about clinical trial enrolment, and lower their psychological distress.

Aims

Clinical trials are an essential part of developing better treatments for people with cancer. We have prepared a question prompt sheet to help patients to learn more about clinical trials. By using this, we hope that patients will have a better understanding of trials and will be more satisfied with the informed consent and treatment decision making process

<p>Dr Michael Brown, A/Professor Guy Toner, Professor Villis Marshall, A/Professor Michael Boyer, A/Professor Paul Maruff RAH Cancer Centre Royal Adelaide Hospital</p>	<p>The Effects of Chemotherapy on Cognitive Function in Patients with Testicular Cancer</p>	<p>\$53,500</p>
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Lay Summary

Outcomes of testing with cancer patients suggest chemotherapy may lead to cognitive impairment such as learning, memory, and attention deficits. However, several limitations appear evident in the research to date. First, studies of chemotherapy and cognitive impairment have mainly focused on patients with breast cancer, limiting the generalisability of results beyond this group. Second, most studies have not taken pre-chemotherapy baseline measurements for comparison or studied patients over time, limiting knowledge about long-term effects. Third, other possible factors that may affect cognitive function have not been taken into account including the type of chemotherapy treatment, self-perceived cognitive changes, and psychological factors known to affect cognitive function including depression, anxiety, and fatigue. Finally, inappropriate control groups or no control groups have been used for comparison. The purpose of the current study is to implement a national long-term study of cognitive impairment with patients undergoing chemotherapy, other than breast cancer patients, taking into account a range of factors. Patients diagnosed with testicular cancer appear especially appropriate as most patients undergo surgery, yet only some continue on with chemotherapy. The patients who do not receive chemotherapy therefore provide an appropriate control group for comparison. Many tests used to assess cognitive function are not suitable for ongoing assessment because of the possibility of learning the test over time. Importantly, valid and reliable computerised assessments of cognitive function (CogHealth) taking as little as 10 minutes are now available to allow repeated measurements over time without burdening patients attempting to deal with chronic illness. Research on the extent of cognitive impairment after chemotherapy has implications for the way future trials are investigated, especially with cancer survivors living longer due to advances in medical care

Aims

This project will assess whether chemotherapy used to treat testicular cancer is associated with changes in advanced brain functions such as attention, memory and learning. This is an important issue in testicular cancer as those affected are very young men who are often cured by the chemotherapy and expected to live a normal life after treatment

Professor Wayne Tilley, Dr Lisa Butler, Professor David Roder, Dr Gelareh Farshid
Dame Roma Mitchell Cancer Research Laboratories, Department of Medicine
University of Adelaide

Androgen receptor status as a determinant of breast cancer risk **Breast**

\$76,000

Lay Summary

Current hormonal therapies for the treatment of breast cancer target the cellular mediator of the female sex hormone estrogen, the estrogen receptor (ER), since breast cells rely on estrogen for growth and survival. Unfortunately, not all women respond to antiestrogen treatments, and many that initially respond will relapse with advanced disease that is no longer responsive to hormonal therapies. More recently, the potentially important role of other sex hormones in breast development, such as the commonly perceived male sex hormone androgen, and its mediator the androgen receptor (AR) has been recognised. The AR is expressed more frequently in breast cancers than ER, and is expressed in 25% of metastatic lesions which are negative for ER. There is emerging evidence that the AR has an important role in normal breast development, breast cancer progression and aggressiveness, and that AR signalling can be disrupted by synthetic progestins, such as those used in hormone replacement therapy or oral contraceptives. The objective of our study is to determine whether alterations in AR are associated with increased risk of developing breast cancer and subsequent progression of the disease. To date, we have examined the levels of AR in nearly 200 primary breast cancers. We have found that women with tumours that were negative for AR were 4.8 times more likely to die from breast cancer compared to women with AR positive tumours. Potential outcomes of this study are that AR measures could be developed as an indicator of risk of developing breast cancer, and of the hormone responsiveness of established breast tumours. Ultimately, the AR signalling pathway could be used as a therapeutic target, especially in cancers that are ER negative but AR positive.

Scientific Summary

AIMS:

1. To determine whether a reduction in androgen receptor signalling is associated with more aggressive disease at diagnosis.
2. To determine whether women whose breast tumours express a less active allele of the androgen receptor have more aggressive disease at diagnosis.

Although there have been many advances in the detection of clinically organ confined breast cancer, many women develop metastatic disease, or progress following local therapy. For these women, additional systemic (e.g. hormonal) therapy is required. Current hormonal therapies predominantly act to block either the synthesis of estrogens, or the action of estrogens at the level of its cellular target, the estrogen receptor (ER α). However, as a significant percentage of these women either do not respond to these therapies, or progress with therapy-resistant disease, there is an urgent need for alternative treatment strategies. By understanding how other hormones in women contribute to breast cancer growth, we will be better placed to implement a more rational hormonal regimen in breast cancer sufferers and to design new medications.

One such group of hormones is the androgens. All women have a significant level of androgens in their body and it appears that they act in concert with estrogens and progestins to regulate the development of the normal breast and also breast cancer cell growth. Our group and others have shown that androgens inhibit breast cancer cell growth, and have been historically used as hormonal therapy for advanced breast cancer, demonstrating an efficacy comparable to that of tamoxifen. The androgen receptor (AR), which mediates the actions of androgens, is expressed in 70-90% of primary breast tumours, a higher frequency than either estrogen (70-80%) or progesterone (50-70%) receptors, and in 75% of metastatic breast cancers. This observation suggests that therapeutic strategies that target androgen signalling pathways, but limit androgenic side-effects, may provide an effective alternative to current treatments.

Aim 1. To determine whether a reduction in androgen receptor signalling is associated with more aggressive disease at diagnosis.

To assess the relationship between AR expression and clinical parameters in breast cancer, we measured AR levels in a cohort of 194 unselected primary breast cancers, with the requisite clinical data including survival, from the Garvan Institute of Medical Research (Sydney, Australia). This study was approved by the Human Research Ethics Committees of the University of Adelaide and St. Vincent's Hospital. The cohort of tissues was microarrayed, allowing us to perform the immunohistochemical analyses on all tumours simultaneously. The breast tumours were immunohistochemically stained for AR using the Amy U407 antibody (Figure 1), and manually scored for AR expression using a 4-point scale. Overall, 73% of the tumours stained positively for AR, and AR positivity was significantly associated with lower histological grade and smaller tumour size (Table 1).

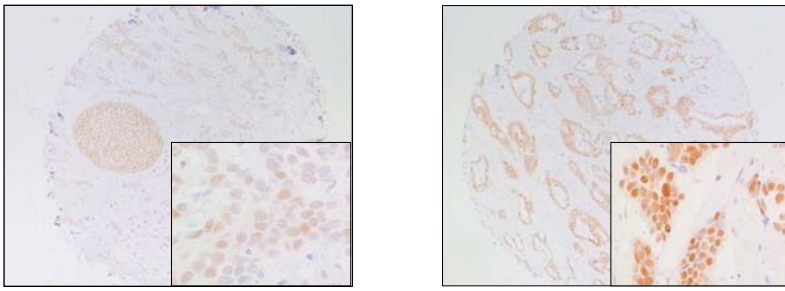


Figure 1. Immunohistochemical staining of primary breast cancer tissue cores. Larger images of the tissue core were taken at 4x magnification and smaller images of breast cancer cells were taken at 40x magnification. Tissues were stained with 1/300 rabbit anti-AR U407. Left panel is an example of a core with weak nuclear AR staining while the panel on the right is an examples of a

Variable	AR immunostaining	
	negative n (%)	positive n (%)
Age		
≤55	23 (31%)	52 (69%)
>55	15 (23%)	49 (77%)
	P=0.34	
Tumour size		
≤20	17 (19%)	71 (81%)
>20	21 (41%)	30 (59%)
	P=0.0053*	
Histological grade		
well or moderate	9 (13%)	61 (87%)
poor	29 (42%)	40 (58%)
	P=0.0001*	
Nodal status		
negative	16 (24%)	52 (76%)
positive	22 (31%)	49 (69%)
	P=0.32	
ER status		
negative	24 (60%)	16 (40%)
positive	14 (14%)	83 (86%)
	P<0.0001*	
PR status		
negative	26 (58%)	19 (42%)
positive	12 (13%)	80 (87%)
	P<0.0001*	

Table 1. Association of AR expression with clinicopathological parameters. *=statistically significant at P<0.05.

Kaplan-Meier survival analyses determined that negative AR immunostaining was associated with a 2.7 fold ($P=0.011$) increased risk of relapse and a 4.8 fold ($P=0.0002$) increased risk of cancer-related death (Figure 2) in the cohort of breast cancer patients. Univariate Cox regression analysis in the node-positive patients demonstrated that AR positivity in breast tumours was a significant predictor of overall and relapse-free survival. However, multivariate analysis indicated that AR was not an independent predictor of relapse or survival. Therefore, we have stained an additional 85 patient samples, again provided as tissue microarrays, to provide greater statistical power for determination of the relationship between AR expression and patient outcome. The size of the cohort is now 279 and the manual AR staining has been scored for all samples by two independent observers. The statistical analyses to determine the relationship between AR expression with patient outcome and survival, in this newly expanded cohort of patients, is currently underway at the Garvan Institute of Medical Research.

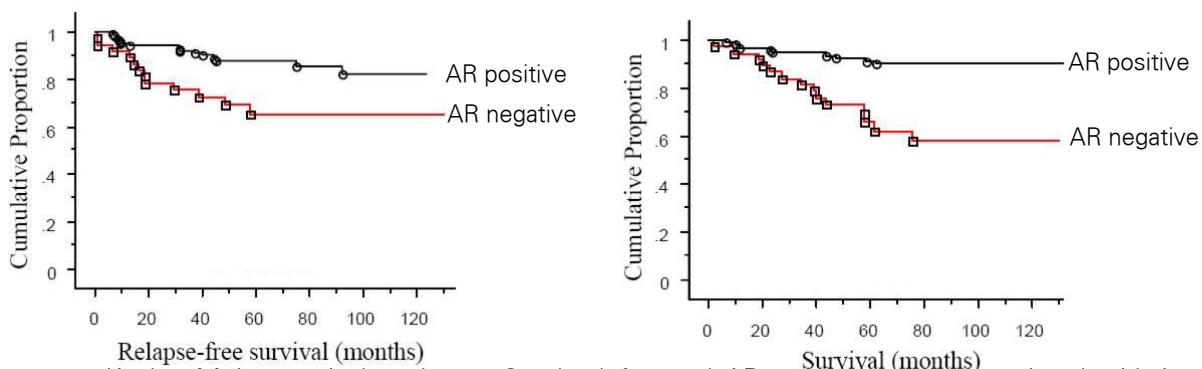


Figure 2. Kaplan Meier survival analyses. On the left panel AR negativity was associated with increased risk of

relapse while on the right panel AR negativity was associated with increased risk of cancer related death.

Aim 2. To determine whether women whose breast tumours express a less active allele of the androgen receptor have more aggressive disease at diagnosis.

For this Aim we are using samples from the Australian Breast Cancer Family Study, which is a well characterised cohort of patients diagnosed with breast cancer under the age of 40. Importantly, the AR-CAG repeat length status, required for the X inactivation studies, is known for the cohort. We have obtained 65 samples from patients which are heterozygous for AR-CAG repeat length. The length of this repeat is related to AR function, with longer repeat lengths having less activity. This aim will determine whether there is preferential inactivation of less active, longer CAG repeat AR alleles, and if this is associated with more aggressive disease at diagnosis and poorer outcome. All the sections have been immunohistochemically stained for AR, and 2-3 AR positive areas captured per slide, by laser capture microdissection (Figure 3). DNA has been prepared from all the samples, and the X inactivation PCR is currently being optimised on test samples before processing the patient samples.

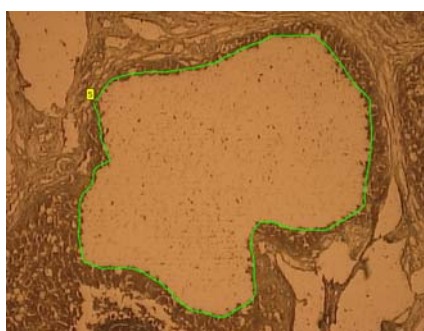
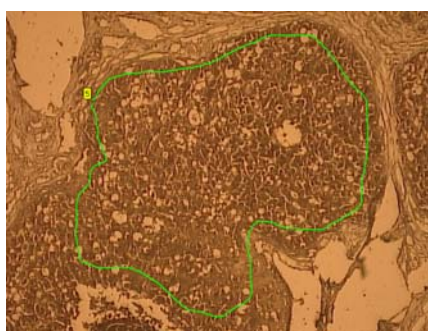


Figure 3. Laser capture microdissection of AR positive cells. LHS, AR positive area of a breast cancer patient sample is highlighted. RHS, After laser capture microdissection, the AR positive nuclei have been catapulted into a tube and then undergone DNA extraction.

CONCLUSION, IMPLICATIONS AND FUTURE DIRECTIONS:

Our studies indicate that expression of AR in breast tumours is associated with longer tumour-free survival and overall survival of patients, indicating that AR is a key determinant of the aggressiveness of breast cancer at diagnosis and of subsequent disease progression. This important finding provides compelling evidence that AR plays a protective role in breast cancer and that loss of AR or disruption of AR signalling may predispose to more aggressive disease or increase the rate of disease progression. The data that we have obtained through funding of this application by the Cancer Council has been presented as an oral presentation at the Endocrine Society of Australia (ESA) and Society for Reproductive Biology (SRB) Annual Scientific Meeting, Queensland, Australia, August 2006 and as a poster at the San Antonio Breast Cancer Symposium, Texas, USA, December 2006. We have also prepared a manuscript, based on the data demonstrating increased survival in patients with AR-positive breast tumours, to be submitted early 2007.

A/Professor Dorothy Keefe
Department of Medical Oncology
RAH Cancer Centre

Chemotherapy-induced diarrhoea:
characterisation of mechanism **Other**

\$70,500

Lay Summary

Cancer chemotherapy causes many undesirable toxicities in patients, with diarrhoea being a common example. Diarrhoea is part of an overall gut toxicity known as mucositis. Diarrhoea has received very little attention in the past with most of the research focusing on the mouth and small intestine. In fact there have been no major advances in this prevention or reduction of this common side effect. In this project we have begun to understand how the normal gut intestinal flora changes over time following chemotherapy. We have also begun to understand how these changes lead to diarrhoea. These new findings are very important and are now allowing us to target more appropriate treatments for mucositis.

Scientific Summary

Mucositis affects the entire gastrointestinal tract and causes pain and ulceration in the mouth and small and large intestines, as well as causing abdominal bloating, vomiting and diarrhoea. Diarrhoea has received very little attention in the past with most of the research focusing on the mouth and small intestine. In the present study we have begun to address this lack of knowledge by characterising the mechanism of development of diarrhoea in our animal model of mucositis, following administration of two cytotoxics (irinotecan and 5-fluorouracil) known to cause diarrhoea in the clinical setting. Two separate studies were conducted, each using 81 rats. Rats were administered with a single dose of the cytotoxic agent and were then killed 30, 60, 90 mins, 2, 6, 12, 24, 48 and 72 h following administration. Faecal samples and stomach, jejunum and colon samples were collected. Standard microbiological culture techniques were used to grow and isolate the flora. Biochemical techniques were used to identify the bacteria.

Irinotecan

Early diarrhoea was observed in rats from 2-6 h after treatment, after which time the diarrhoea resolved. Late onset diarrhoea was apparent 72 h after treatment. Changes were seen in the flora of the stomach, jejunum, colon and faeces. In the stomach, Enterococcus spp. levels peaked at 2-6 h, and Peptostreptococcus spp. levels peaked 30-60 mins after treatment. Serratia spp. levels decreased initially, then peaked at 2 h, and Staphylococcus spp. fluctuated over the time points, with a peak at 12 h. In the jejunum, Clostridium spp., Enterococcus spp., Serratia spp. and Lactobacillus spp. all peaked at 2 h. In the colon, Escherichia spp. increased between 6-24 h, and is a known producer of bacterial B-glucuronidase, which converts the non-toxic metabolite of irinotecan, SN-38 glucuronide (SN-38G) back to the toxic form, SN-38. Clostridium spp. increased at 2 h, Enterococcus spp. increased at 6 h and Serratia spp. increased from 60 min-24h. Staphylococcus spp. peaked at 60 min, and 48 h, and Lactobacillus spp. increased gradually over time. In the faeces, Proteus spp. increased at 24-72 h, Clostridium spp. peaked at 2 h, and Escherichia spp. fluctuated over time. In conclusion, irinotecan treatment causes changes in the flora of the stomach, jejunum, colon and faeces of rats and is associated with the development of diarrhoea. These changes in flora may have systemic effects and in particular may contribute to the development of chemotherapy-induced mucositis.

5-fluorouracil

Changes to the gastrointestinal microflora were seen following treatment with 5-FU. In the stomach, Escherichia spp. decreased at 24-48 h and Staphylococcus spp. decreased significantly at 6 h. In the jejunum, Clostridium spp. and Lactobacillus spp. decreased at 6 h, Streptococcus spp. decreased at 2 h and Escherichia spp. increased at 48-72 h. In the colon, Enterococcus sp. was decreased from 1-2 h and 6-12 h, Lactobacillus spp. was decreased from 1-2 h and 12-24 h, and Streptococcus was decreased from 1-2 h, 12-24 h and 72 h. The faecal flora changes also with Clostridium spp. decreased at 2 h, and Escherichia sp. decreased from 1-48 h. Proteus spp. decreased from 60min-12 h, and Streptococcus spp. decreased at 72 h. In conclusion, 5-FU treatment causes changes in the flora of the stomach, jejunum, colon and faeces of rats, and may be associated with the development of mucositis. The changes in microflora are quite different between 5-FU and irinotecan, suggesting different mechanisms may be involved in the development of diarrhoea and mucositis.

Professor Sharad Kumar
Department of Haematology
Hanson Institute, IMVS

Caspase-2 function in apoptosis and disease
Other

\$87,250

Lay Summary

Most anti-cancer drugs work by inducing cells to die; a process that requires the activation of killer enzymes called 'caspases' that dismantle the cell. One of the earliest caspases to become activated when cells die is caspase-2. Recent studies have shown that caspase-2 levels in some cancer cells may govern the response to anti-cancer drugs. This is important for two main reasons. Firstly, if cancer cells lose the ability to activate caspase-2 they may become resistant to drugs making them difficult to kill. Secondly, the caspase-2 gene is known to be located in a region that is often deleted in many blood cancers, meaning that absence of caspase-2 may be linked to the development of these cancers. The main aim of this project is to understand the mechanism of caspase-2 activation in response to specific chemotherapeutic agents, and its function in tumour development.

Scientific Summary

Defects in apoptosis that result in the inappropriate survival of cells can lead to the development of cancer. Apoptosis is a naturally occurring process of programmed cell death that plays a crucial role in the development, homeostasis and defence of metazoans by eliminating unwanted cells. Central to this process is the activation of a conserved family of cysteine proteases known as caspases. One of the first caspases to be activated during stress-induced apoptosis is caspase-2. Studies have recently shown that caspase-2 is essential for the death of various cancer cells when treated with DNA-damaging agents including anti-cancer drugs and UV irradiation. Given its fundamental role in mediating the death of cancer cells, the aim of this project was to identify and understand how caspase-2 activation occurs during cell death.

This project was funded for one year (2006) by the cancer council of SA. During this period we found that loss of caspase-2 enhances cell survival and that caspase-2 act downstream in a cell death signalling pathway involving cytoskeletal modification. We observed that embryonic fibroblasts (MEFs) from caspase-2 null mice, normally sensitive to a number of chemotherapeutic drugs showed significant resistance to apoptosis when treated with agents that disrupt cellular architecture (cytoskeleton) such as cytochalasin D, paclitaxel, vincristine and zoledronic acid compared to wild type cells. To further extend these findings we propose a model for caspase-2 activation in response to cytoskeletal disruption. This model predicts that following drug treatment, cytoskeletal disruption

activates caspase-2 via either PIDDosome formation or procaspase-2 dephosphorylation. Specific experiments are being performed to determine the mechanism(s) by which caspase-2 is activated. We are also testing whether loss of caspase-2 enhances the ability of cells to transform more efficiently and resist apoptosis. In addition we have set up an E μ -Myc transgenic mouse tumour model to test whether loss of caspase-2 increases the potential of tumorigenesis. E μ -Myc mice develop pre-B or B cell lymphomas and in previous models using this strain have shown that loss of p53 or BH3- only proteins (Bim) accelerate E μ -Myc – induced tumours. Caspase-2 -/- E μ -Myc mice will be monitored for tumour development. Bim -/- E μ -Myc mice are used as positive controls. Tumours and organs will be collected for classification of tumour type and histological examination. Rate and incidence of tumours in mice will be analysed using a statistic software and compare to corresponding littermates and positive controls. This extension of this work have now been funded by an NHMRC grant (2007-2009).

Dr Yeeseim Khew-Goodall
Division of Human Immunology
Institute of Medical and Veterinary Science

A potential novel signalling pathway regulating epithelial-mesenchymal transition **Breast and Bowel** \$70,500

Lay Summary

The progression of solid tumours from primary tumours to malignant disease, characterised by the invasion of surrounding tissues and metastasis to secondary organs, is the major cause of morbidity and mortality in cancer patients. Defining the molecular mechanisms underlying the initial phenotypic change toward malignancy will enhance the accuracy of predicting the risk of metastatic disease. This, in turn, will translate into greater confidence for identification of patients at a high risk of developing metastatic disease to be targeted for more intensive therapy post-removal of the primary tumour or more rigorous screening for metastasis. Identifying the molecular mechanism for the transition may ultimately also reveal novel therapeutic targets for the prevention or treatment of metastatic disease. This proposal addresses some of the fundamental mechanisms that bring forth the transition from a non-invasive, non-migratory to an invasive, migratory phenotype.

Scientific Summary

Epithelial morphogenesis, involving proliferation, migration, invasion, extracellular matrix degradation and replacement, is essential for embryonic development. During development epithelial-mesenchymal transition (EMT), the transient loss of epithelial phenotype and acquisition of mesenchymal characteristics (motility, invasiveness and increased proliferative capacity), enables cells to migrate away and proliferate to form a new tissue. This process is necessary for large-scale tissue reorganisation to occur during development and is tightly regulated. Cancers of epithelial origin make up >80% of all cancers. During their progression, the most aggressive and lethal tumour cells can be viewed as having undergone an EMT and taken on a motile and invasive phenotype akin to that observed during EMT in development.

We have recently found that the protein tyrosine phosphatase (PTP) Pez elicits an EMT in normal kidney epithelial cells. Its expression is elevated in advanced breast carcinomas and in breast cancer cell lines with metastatic potential but low in differentiated epithelial breast cancer cell lines. It is therefore important to elucidate the mechanisms by which its expression is induced to bring forth an EMT. We have recently found that the oncogene Src which is activated in a number of cancers including breast can increase Pez expression.

The main aims of this study (for 1 year funding) were:

- 1: To establish that Src is an upstream effector in the signalling pathway of Pez-induced EMT
- 2: To determine the effect of mutations in Pez identified in colon cancers on the half-life, localisation and activity of the Pez protein

Results:

We found that overexpression of vSrc or constitutive active cSrc but not the kinase-dead Src when introduced into breast cancer cell lines led to a marked (~10-fold) increase in endogenous and exogenous Pez expression. We also found that Pez is tyrosine phosphorylated in the presence of vSrc or constitutively active cSrc. These observations suggest that phosphorylation of Pez by Src increases its expression. The increase in expression of exogenously introduced Pez suggest that one mechanism of increasing Pez expression is by stabilisation of the protein. We have identified 2 Nedd4 (an E3 ubiquitin ligase) binding sites in Pez and found that one of these is phosphorylated by Src. Published literature indicate that phosphorylation of Nedd4-binding sites leads to inhibition of Ned4 binding, further suggesting that phosphorylation of Nedd4 sites in Pez by Src may be one way to prevent its degradation and increase expression. Interestingly, we have preliminary data showing that some growth factors that activate Src also increase Pez expression. Many of these growth factors also induce EMT in certain contexts.

We have also generated a panel of mutations in Pez that were observed in colorectal cancers and begun analysing the effect of these mutations on the level of Pez expression. Preliminary data indicate that 2 of the mutations in Pez may increase its expression. We are continuing with studies to elucidate the effect of these mutations of other Pez

functions.

A/Professor Paul Reynolds , A/Professor Mark Holmes Department Thoracic Medicine Royal Adelaide Hospital Chest Clinic	Gene Delivery of Tissue Inhibitors of Matrix Metalloproteinases for Pulmonary Metastases Lung + Other	\$76,000
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Lay Summary

For cancers to grow and spread they must break down the normal tissues and stimulate the growth of new blood vessels to supply nutrients to the growing tumour. Tissue Inhibitor of Matrix Metalloproteinase 3 (TIMP3) is a naturally occurring molecule that helps to prevent the breakdown of certain components of normal tissues, prevents the growth of new blood vessels and also directly kills cancer cells. We have been working on a gene therapy approach which will allow for sustained local production of TIMP3 in tumours and also in the blood vessels of the lungs. We have found gene delivery for TIMP-3 using a modified form of the cold virus was able to significantly inhibit tumour growth in an animal model. Further, we have now engineered the virus so that it binds to a different receptor and is now much more efficient at infecting lung cancer and mesothelioma cells. This new virus shows greater killing effects against the cancer cells. We have also combined this virus with another virus which is designed to grow in cancer cells. The combination of the two viruses works better for inhibiting tumour growth than either virus alone.



Scientific Summary

This project focuses on the use of gene delivery for TIMP-3 as a strategy for cancer therapy. There was a slight delay initially due to some contractual issues, but that was resolved. Aspects of the project involve direct gene delivery to tumour nodules (both lung cancer and mesothelioma) and delivery to the pulmonary vasculature as a strategy against pulmonary metastases. Initial work involved completion for publication acceptance of a comparison of TIMPs 1, 2, 3 in subcutaneous lung cancer nodules, including evaluation of tumour apoptosis and anti-angiogenic effects. These studies clearly showed the advantage of TIMP-3 over TIMPs 1 and 2. On balance though, we were disappointed at the rather limited number of cells lines that were showing susceptibility to our initial viral construct. To improve efficacy we constructed a new virus having tropism for the Ad3 receptor CD46 (as opposed to the Ad5 receptor CAR). The rationale was based on our flow cytometry assessment showing CD46 was generally more highly expressed in lung cancer and mesothelioma lines than is CAR. Similar results had been found by others in other contexts such as ovarian cancer. We in fact made two new viruses, both an Ad3 version and a new Ad5 version with a matched improved expression cassette for comparison. Viruses were validated by sequence and by analysis of transduced cells by immunohistochemistry, western blot and for functional TIMP-3 by reverse zymography. We have found that both new viruses work better than our original construct, with Ad3 tropism having particularly greater efficacy of cell killing for both lung cancer and mesothelioma cells. We then combined the TIMP-3 Ad3 vector with an Ad3 replicative virus (in which replication is controlled by the mesothelin promoter) and found greater efficacy in vivo than for either virus alone. The evaluation of the new agents in the context of pulmonary vascular delivery and osteosarcoma model is ongoing – to facilitate the evaluation we developed micro-CT analysis of the pulmonary tumours in live rats and are also developing a rat osteosarcoma line stably transfected with luciferase for Xenogen imaging. The improved vectors and the new imaging approaches are greatly improving the evaluation of TIMP-3 efficacy in vivo. Detailed efficacy evaluation is ongoing.

A/Professor Geoffrey Lindeman, Dr David Amor, A/Professor Judy Kirk, Dr Graeme Suthers, Professor Jack Goldblatt, Dr Mike Gattas RMH Familial Cancer Centre/VBCRC Laboratory Royal Melbourne Hospital	kConFab – A Consortium for Research on Familial Breast Cancer	\$60,255
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Lay Summary

Breast cancer is the most common disease in women. In families with an inherited form of breast cancer, nearly half the women in every generation can develop the disease. The aim of this Australasian-wide study, which has been running since 1997, is to complete collection of clinical, epidemiological and genetic data on 1,600 of these severely affected families. The national resource is, and will continue to be of great value for researchers who want to identify and characterise the genetic and life style factors that affect onset and progression of the disease

Scientific Summary

Through the Family Cancer Clinics in each Mainland State, kConFab recruits families that fit the selection criteria. In South Australia, kConFab employs 2 research nurses' who are based at the Women's and Children's Hospital, North Adelaide. To date kConFab has recruited 1,000 families (on average, 10 individuals per family) from within Australia and New Zealand. From these consented family members, kConFab has collected blood, normal and tumour tissue and life style data. We verify most reported cases of cancer within each family. In the last 12 months the kConFab resource has reached a sufficient size to provide researchers with enough power to carryout meaningful research. We currently provide biospecimens and/or data to 68 national and international research projects. So far 46 papers have been published using kConFab material.

The most exciting kConFab publication to date has just been published in the prestigious scientific journal Nature, and describes the identification of 5 new breast cancer genes associated with sporadic and familial breast cancer. kConFab contributed approximately 400 families to this analysis. Also, the publication by our clinical follow up group "Risk-Reducing Surgery, Screening and Chemoprevention Practices Of *BRCA1* And *BRCA2* Mutation Carriers" gave an important insight into the how the female kConFab participants were managing their personal cancer risk assessment and cancer prevention trials.

A/Professor Timothy Hughes

Division of Haematology
Institute of Medical and Veterinary Science

Causes and significance of persistent
leukaemia in CML patients treated with ABL
kinase inhibitors

\$68,527

Lay Summary

The new targeted drug imatinib has been very successful in the treatment of Chronic Myeloid Leukaemia, however in the majority of patients a small number of leukaemic cells remain after years of treatment. These cells can result in relapse if therapy ceases or if resistant mutations occur in the cells. The aim of our project was to examine why this persistence occurs, and if individuals responding less well to therapy can be identified to better tailor treatment.

Scientific Summary

A. Overall background

CML is characterised by the presence of the Philadelphia (Ph) chromosome. At the molecular level the formation of the Ph chromosome results in the fusion of the BCR-ABL fusion protein. This protein is a constitutively active tyrosine kinase. The efficacy of imatinib can be assessed both in-vivo and in-vitro (predictive) by analysing the degree of phosphorylation of Crkl, the major downstream partner of BCR-ABL

B. In vitro IC50 imatinib predicts patient response to imatinib therapy

Our studies on newly diagnosed patients (150 in total and 65 where we have molecular response data in excess of 2 years) demonstrate that a significantly greater proportion of patients with high intrinsic sensitivity to imatinib induced kinase inhibition (low IC50) achieve good molecular responses to 2 years, when compared to patients with low intrinsic sensitivity (high iC50) to (imatinib induced) kinase inhibition. This indicates for the first time that CML patient's cells have intrinsic differences in sensitivity to tyrosine kinase activity, which is not due to acquired resistance, and which dictates their overall long term response to imatinib therapy [1] [2]

C. In vivo inhibition of BCR/ABL kinase activity early in imatinib therapy is a useful prognostic indicator

In addition to in vitro tests of kinase inhibitor response, we have directly assayed the level of inhibition of BCR/ABL kinase activity in vivo, by assessing the decrease in phosphorylation of Crkl over 7, 14 and 28 days of imatinib therapy, compared to levels prior to commencement of therapy. These assays have now been performed on over 100 patients. Results from these assays have demonstrated a very strong correlation between the decrease in phosphorylated Crkl seen over the first 28 days of imatinib therapy and the achievement of a major molecular response (MMR) [3] [4]

D. Differences in drug influx/efflux in CML cells may determine intrinsic sensitivity to tyrosine kinase inhibitors.

The above results led to the examination of the transport mechanisms utilised by CML cells to influx and efflux imatinib and other second generation kinase inhibitors (nilotinib and dasatinib), Utilising ¹⁴C labelled drug we have demonstrated a strong correlation between IC50 imatinib and the intracellular uptake and retention (IUR) of imatinib. Furthermore, using inhibitors we have demonstrated that the major influx transporter for imatinib is the human organic transporter -1 (OCT-1) [5], Interestingly, despite the structural similarity between imatinib and nilotinib we have shown that the transport mechanisms for nilotinib are different, and that OCT-1 is not involved in the transport of this kinase inhibitor. We have also shown that both drugs are effluxed to varying degrees by ABCB1 (MDR-1 pGP), [6] In addition it is the efflux mediated by this pump which results in interesting changes in intracellular drug



concentrations when the drugs are used in combination [6] . Similar studies with Dasatinib are currently ongoing. E. Combination studies – imatinib and nilotinib with commonly used concomitant medications.

Many CML patients require concomitant proton pump inhibitor (PPI) therapy, for gastric disturbance, while on imatinib. The requirement for this therapy for patients treated with nilotinib is yet to be determined. We have demonstrated that commonly used PPI's, such as Pantoprazole and Esomeprazole affect the IUR of both imatinib and nilotinib. We have demonstrated a decrease in the IUR for IM in the presence of both PPI, and in contrast an increase in the IUR for nilotinib, suggesting the mode of interaction may be different for both drugs. We report significant, but distinct interactions between PPI and both IM and NIL. Both responses may result in clinically significant changes with concomitant administration, and therefore warrant further investigation [7]

F. The functional activity of OCT-1 is a critical determinant of intracellular imatinib transport

Using ¹⁴C labelled imatinib and Prazosin, a potent inhibitor of OCT-1 we have demonstrated that the degree of inhibition of OCT-1 (which we have termed the OCT-1 Activity) is a critical determinant of the IUR for imatinib. Patients with low OCT-1 activity generally have high IC50 and achieve low in-vivo kinase inhibition over the first 28 days of inhibitor therapy. Further, we have also demonstrated that low OCT-1 Activity can, in many patients, be overcome with increased dose. Thus screening of patients for OCT-1 Activity pre therapy provides a further determinant to predict long term outcome, and allows for identification of patients who may have better outcomes on increased imatinib dose, or on one of the second generation inhibitors not reliant on OCT-1 for transport. [8]

G. ABCB1 Overexpression May Predispose Imatinib Treated CML Patients to the Development of Abl Kinase Domain Mutations, and May Be an Important Contributor to Acquired Resistance.

Using RQ-PCR for ABCB1 expression relative to the control gene BCR, flow cytometric analysis and IUR we have assessed 32 imatinib treated chronic phase CML patients pre therapy. 29/32(90%) patients had expression of ABCB1 mRNA less than 65% of control (median 49% range 21–65%). The three patients with higher expression of ABCB1 (73%, 130% and 105%) all subsequently developed kinase domain mutations and disease progression. Only 1/29 patients with low mRNA (<65%) expression subsequently developed a mutation. We conclude that patients with high expression of ABCB1 at diagnosis may be predisposed to mutation development. Furthermore increasing expression of ABCB1 over time may be a valuable contributor to acquired resistance. ABCB1 activation may be an important prognostic marker, and potential target for pharmacological manipulation [9]

H. Cytokines protect CML progenitor cells from inhibition by imatinib.

BCR/ABL and cytokines of the IL-3 family share some common signalling pathways. We have shown that GM-CSF, a member of this cytokine family, is able to abrogate the inhibition of CML cell proliferation and killing by imatinib. Furthermore, we have found that a subset of CML cells produce GM-CSF that can protect the CML CD34+ progenitors from imatinib. These studies suggest that if cytokine levels in vivo are sufficiently high, leukaemic cells may escape killing by imatinib and potentially be a source of cells responsible for relapse.

Professor R John Simes, Professor John R Zalcberg, A/Professor Paul Waring, A/Professor G Bruce Mann, A/Professor B Mark Smithers, **Dr Dusan Kotasek**, Dr Guy Van Hazel
NHMRC Clinical Trial Centre
University of Sydney

Intermediate & high risk, resected gastrointestinal stromal tumours expressing kit:RCT of adjuvant imatinib mesylate **Other**

\$10,834

Lay summary

Gastro-Intestinal Stromal Tumours (GIST) are rare cancers effecting about 100 Australians annually. Until recently surgery to removed GIST was the only effective treatment. In 50% of people having an operation the disease returns within 5 years. Imatinib mesylate (IM) is a drug targeting a part of the biochemical pathway that influences tumour growth. The drug prolongs life in people whose GIST is no longer operable. This study compares the impact of imatinib mesylate given after surgery to surgery alone on length of life in people at high risk of the GIST returning.



If the trial demonstrates that adjuvant treatment with imatinib in patients with intermediate and high risk GIST improves survival, is safe and tolerable it may become standard care. If treatment with imatinib mesylate is better left until after recurrence when patients have unresectable advanced disease, the unwarranted burden on individual patients and the health care system will be avoided. This trial will also contribute to the available clinical and scientific information about this important drug.

Scientific Summary

The adjuvant GIST trial was activated in Australasia in October 2005 and since then fifteen clinical trial sites have

started the trial. We currently have 1 South Australian site, Ashford Cancer Centre (ACC), actively participating in the trial. A second South Australian site, Flinders Medical Centre (FMC), will be activated to participate in the trial in the near future (site ethics approval is currently pending). Royal Hobart Hospital is also working towards participating in the trial.

Overall the trial is going very well. Locally a total of 35 patients have been recruited to participate in the trial, including 3 patients from South Australia. With the addition of another SA site, this should help boost the recruitment within the state. Recruitment in each region is consistent with estimated targets per year. The totals per region are NSW-9 patients, VIC- 10 patients, QLD- 8 patients and SA- 3 patients.

International recruitment for this trial is also going very well, with the original sample size of 400 patients being achieved in September 2006, 3 years earlier than predicted. The AGITG investigators took an active role in leading the decision that was made by the EORTC in the last quarter of 2006 to expand the sample size to 750 patients, due to an excess of "intermediate-risk" patients enrolled so far. The increase in the sample size will increase the power of the study to detect differences in the "high-risk" patient group as well.

Recruitment is expected to continue for at least another year, during which time we expect Australasian sites to contribute an additional 20-30 patients, 10% of whom will probably come from South Australia. In our original application, we estimated the AGITG would be able to contribute to 20 patients per year. We have exceeded this projection by an additional 10 patients within the first year.

Patients recruited to participate in the trial are randomly allocated to either the treatment arm (400mg of IM per day) or the observation arm (no further antitumour therapy). Following recruitment, patients are assessed at least every 3 months during the first 2 years, every 4 months during the next 3 years and at least yearly thereafter. The data collected during these assessments will be used to assess whether IM, used after complete surgery, is able to improve the prognosis of patients with GIST who have an intermediate or high risk of relapse.

Treatment (and observation) of all participating patients is ongoing; of the 35 patients enrolled we currently have 21 patients on the treatment arm and 13 on the observation arm. One patient who was originally assigned to the observation arm has consented to passive collection of follow up data. The patient is willing to be contacted every 3 months for follow up via telephone.

There is a strong commitment to this trial as it is addressing a key question in the management of GIST. It will provide definitive data on the role of imatinib mesylate in a controlled study.

Between September and December 2006 recruitment to the trial was temporarily suspended, while the sample size amendment was being prepared and approved. Recruitment at most sites has now recommenced, using Protocol V2, dated 16.10.06.

A/Professor Murray Whitelaw School of Molecular and Biomedical Science University of Adelaide	Investigating the role of Sim2 in pancreatic cancer Other	\$64,500
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Lay Summary

Single Minded 2 (Sim2) is a gene found to be highly expressed in pancreatic cancers, yet absent from normal pancreatic tissue. In addition, repression of the Sim2 gene in pancreatic cancer cell lines led to inhibition of cell proliferation and eventual cell death. This project aimed to explore mechanisms by which aberrant Sim2 expression may stimulate development of pancreatic cancer. The Sim2 gene produces a protein that belongs to a family of a gene regulatory factors, although the exact genes Sim2 regulates and their possible relationship to cancer remain unknown. We have addressed this question and identified some Sim2 target genes which have previously been related to cancer. We now have a working model for how Sim2 may be involved in pancreatic cancer

Scientific Summary

The aims of this project were to explore our hypotheses that the transcription factor Sim2 affects pancreatic tumourigenesis by either

- a) inducing the Sonic Hedgehog (Shh) pathway
 - b) inhibiting Hypoxia Inducible Factor (HIF-1a) induced apoptosis,
- or perhaps by a combination of both a) and b). We also sought to discover novel Sim2 target genes that may be involved in the cancer process.

Several years ago it was reported that ectopic expression of Sim2 in the brains of transgenic mice led to induction of Shh. More recently, transgenic expression of Shh in the pancreas was reported to induce pancreatic intraepithelial neoplasias. We there hypothesised that aberrant expression of Sim2 in the pancreas may lead to induction of Shh, initiating or exacerbating cancer development. We have tested this hypothesis by manipulating Sim2 levels in the human pancreatic cancer cell lines HPAC, CAPAN and PANC-1. As Sim2 is also expressed in aberrant high levels in prostate cancer tissue, we included the prostate cancer cell lines DU145, LNCaP and PC3. From these parent lines, we developed several pools of cells which stably express increased levels of Sim2. Encouragingly, in some of these cell pools we see a corresponding increase in Shh protein. However, our prediction that this would be due to increased transcription of the Shh gene was not verified, as Shh mRNA levels were not changed. Treatment with siRNA targeted to Sim2 was able to deplete Sim2 protein levels and correspondingly decrease Shh levels, providing further evidence that expression of Sim2 and Shh are related. The increase in Sonic Hedgehog was not consistent across all cell pools, however, so we need further research to ascertain if the phenomenon is a genuine contributor to pancreatic cancer development.

We have conducted microarray analyses of our Sim2 overexpressing pancreatic and prostate lines. We have found Sim2 dependent regulation of a number of genes which are known to be involved in cancer. One lead candidate is the proapoptotic gene BNIP3, which we find repressed by Sim2. In this case the Sim2 dependent repression is consistent across a number of cell pools and is reversed by upon treatment with siRNA directed against Sim2. BNIP3 is well known to be induced by HIF-1a. Thus we now have evidence supporting our second hypothesis, that Sim2 may aid cancer development by opposing the proapoptotic effects of HIF-1a. In a related project we have discovered the first direct target gene of Sim2 and through promoter analyses, have discovered a distinct DNA sequence bound by Sim2. This sequence exists in the BNIP3 control region, so our next experiments will be to perform ChIP analyses to determine if this is a true regulatory site in vivo, together with pyrosequencing to determine if promoter methylation is a means of Sim2 dependent repression.

Finally, throughout these studies we have made the unexpected discovery that Sim2 induces its essential partner factor, Arnt. Sim2 must dimerise with Arnt to function as a transcription factor, so it was previously not clear why increased expression of Sim2 alone should lead to significant increases in active Sim2/Arnt dimers. This new understanding makes possible design of transgenic mice to express Sim2 in the pancreas or prostate in order to analyse those tissues for neoplasias.

Dr Mark Guthridge
Human Immunology
Hanson Institute, IMVS

The role of a novel GM-CSF signalling pathway in regulating cell survival in myeloid **leukemia**

\$78,205

Lay Summary

As per Fellowship report

Dr Andrew Zannettino, Dr Stan Gronthos
Division of Haematology
IMVS

Does Stromal Derived Factor 1a (SDF - 1a) Play a Role in Osteolytic Bone Disease and Increased Bone Marrow Microvessel Density in Multiple Myeloma? **Ovarian**

\$75,838

Lay Summary

Multiple myeloma (MM) is a bone marrow cancer that affects approximately 1 in 10, 000 Australians each year. Like many other cancers, collections of tumour cells in the BM are associated with a large network of small capillaries (microvessels) which stem from the vasculature and surround the cancer, providing it with an ample supply of nutrients and oxygen. Cancer cells produce many factors which stimulate the process of capillary formation, also known as angiogenesis. In more than 80% of cases, MM is associated with destructive lesions of the skeleton caused by the cancer cells recruiting large numbers of osteoclasts (cells which normally function to remove unwanted bone) from the peripheral blood into the bone marrow. Our studies show that the MM cancer cells express an abundance of the protein SDF-1, which serves to stimulate both angiogenesis and osteoclast-mediated bone destruction.



Scientific Summary

The destructive osteolytic lesions typically seen in the hematological malignancy multiple myeloma (MM) are characterised by a marked increase in both osteoclast (OC) formation and activity, with a reduced or absent coupled

osteoblast (OB)-mediated osteogenic response. In addition, active myeloma disease is also associated with increased bone marrow (BM) angiogenesis, as evidenced by an increase in microvessel density (MVD). Whilst increased MVD in MM correlates with poor prognosis and survival, the mechanisms underlying the elevated angiogenesis requires further elucidation.

Studies from our laboratory highlight a significant role for the chemokine, stromal-derived factor-1 (SDF-1) in both the osteolytic bone disease and increased MVD seen in patients with MM. Specifically, we have shown that MM patients exhibit higher levels of circulating SDF-1 when compared with age-matched normal donors. In the context of the osteolytic bone disease, the level of SDF-1 was found to positively correlate with the presence of multiple radiological bone lesions in individuals with MM, suggesting a role for SDF-1 in OC precursor recruitment and activation.

To confirm the OC-activating potential of MM plasma cell (PC)-derived SDF-1 *in vivo*, we have established a novel model of MM-mediated osteolysis where the MM PC line 8226-RPMI was directly injected into the intra-tibial space of nude mice, mimicking MM PC infiltration. Implanting parental RPMI-8226 into the tibiae resulted in a 5% loss in bone volume (BV) when compared with the PBS injected control and gave rise to osteolytic lesions at the injection site. Importantly, implantation of RPMI-8226 over-expressing SDF-1 resulted in a 13% decrease in BV and an increase in serum collagen I breakdown products (RatLaps™). These findings confirm our previous hypothesis that in addition to recruiting OC to the BM, MM PC derived-SDF-1 stimulates their bone-resorbing activity, thereby contributing to the development of osteolytic lesions observed in MM.

In the context of angiogenesis, the level of SDF-1 was found to positively correlate with the degree of angiogenesis as evidenced by an increase in MVD of the BM in areas of tumour infiltrate, suggesting a potential role for SDF-1 in endothelial chemotaxis and cell branching morphogenesis. The role of SDF-1 in the angiogenic process was further investigated by culturing HUVEC in an *in vitro* angiogenesis assay in the presence of plasma cell-line (RPMI-8226) derived conditioned media. The contribution of SDF-1 in this process was confirmed with the use of the highly specific, small-molecule CXCR4-specific inhibitor, 4F-Benzoyl-TE14011 (T140), which significantly blocked tube formation stimulated by the myeloma cell line RPMI-8226.

On the basis of these findings, we believe that the synthesis of high levels of SDF-1 by MM PC may not only serve to promote pathological angiogenesis, but also serves to recruit OC precursors to local sites within the BM and enhance their motility and bone resorbing activity. We therefore propose that inhibition of the CXCR4-SDF-1 axis may be an effective strategy for the treatment of MM-induced osteolysis.

Total Research Grants

\$1,249,697

OTHER PROGRAMS FUNDED IN 2006

SENIOR FELLOWSHIPS

Dr Carmella Ricciardelli
University of Adelaide

Role of versican in the development of
metastatic **breast** cancer

\$86,091

Lay Summary

Spread of cancer cells to other sites is the cause of relapse and death in breast cancer patients. This process requires cancer cells to detach from the breast tissue, travel into the blood and attach to other tissues in the body. Understanding of the mechanism whereby cancer cells move and spread to other tissues is limited. We have identified a protein, versican which is present in the tissue surrounding the cancer cells, to be associated with disease relapse in early stage breast cancer. Versican is a molecule known to regulate cellular movement at critical periods in the developing embryo, and during normal tissue repair. Our recent studies suggest that breast cancer cells utilize versican to aid their movement and spread into surrounding tissues. Future studies will investigate whether a group of enzymes (ADAMTS) which can digest versican into smaller fragments can activate versican function and control movement of breast cancer cells. A greater understanding of the way versican controls cell movement may lead to the development of novel drugs to inhibit spread of cancer cells.

Scientific Summary

There is increasing evidence that ADAMTS (adamalysin-thrombospondin) proteases contribute to the development of metastatic breast cancer. ADAMTS are a family of metalloproteinases which are primarily involved in extracellular matrix processing. The predominant extracellular matrix substrate for ADAMTS-1 in breast cancer is the proteoglycan versican. Versican is an 'aggregating' proteoglycan and participates in three-dimensional matrix assembly via hyaluronan binding. Versican has recognised anti-cell adhesive properties, and elevated levels of versican have been correlated with poor outcome in many different cancers including breast cancer. Versican cleavage by ADAMTS-1 action in vivo has an important role in ovarian function and fertility and in vascular smooth muscle cells and has been suggested to contribute to the development of atherosclerosis. It is likely that a local accumulation and processing of versican may promote breast cancer cell mortality and metastasis. This concept is supported by studies associating elevated versican levels with poor breast cancer outcome and an up-regulation of ADAMTS-1 with elevated breast cancer metastatic activity.

The assembly of pericellular matrix, rich in HA, and versican is a prerequisite for proliferation and migration of mesenchymal cells and may also regulate proliferation and migration of mesenchymal cells and may also regulate proliferation and motility of prostate cancer cells. A particle exclusion assay was used to determine whether human breast cancer cells (MDA-MB231) and prostate cancer cell (PC3) were capable of assembling a PCM following treatment with recombinant full length versican and ADAMTS-1 treated versican. A 24 h treatment with full length recombinant versican resulted in prominent PCM formation by PC3, but not by MDA-MB231 cancer cells. Immunocytochemistry demonstrated detectable expression of ADAMTS-1 and ADAMTS-4 in PC3 but not in MDA-MB231 cells. ADAMTS-1 treated versican but not full length versican was able to induce PCM formation by MDA-MB231

Dr Yeesim Khew-Goodall
Hanson Institute

The role of Tyrosine Phosphatase Pez in Cancer
Progression **Bowel-colorectal**

\$44,982

Lay Summary

The progression of solid tumours from primary tumours to malignant disease, characterised by the invasion of surrounding tissues and metastasis to secondary organs, is the major cause of morbidity and mortality in cancer patients. Defining the molecular mechanisms underlying the initial phenotypic change toward malignancy will enhance the accuracy of predicting the risk of metastatic disease. This, in turn, will translate into greater confidence for identification of patients at a high risk of developing metastatic disease to be targeted for more intensive therapy post-removal of the primary tumour or more rigorous screening for metastasis. Identifying the molecular mechanism for the transition may ultimately also reveal novel therapeutic targets for the prevention or treatment of metastatic disease. This proposal addresses some of the fundamental mechanisms that bring forth the transition from a non-invasive, non-migratory to an invasive, migratory phenotype.

Scientific Summary

Epithelial morphogenesis, involving proliferation, migration, invasion, extracellular matrix degradation and replacement, is essential for embryonic development. During development epithelial-mesenchymal transition (EMT), the transient loss of epithelial phenotype and acquisition of mesenchymal characteristics (motility, invasiveness and increased proliferative capacity), enables cells to migrate away and proliferate to form a new tissue. This process is necessary for large-scale tissue reorganisation to occur during development and is tightly regulated. Cancers of epithelial origin make up >80% of all cancers. During their progression, the most aggressive and lethal tumour cells can be viewed as having undergone an EMT and taken on a motile and invasive phenotype akin to that observed during EMT in development.

We have recently found that the protein tyrosine phosphatase (PTP) Pez elicits an EMT in normal kidney epithelial cells. Its expression is elevated in advanced breast carcinomas and in breast cancer cell lines with metastatic potential but low in differentiated epithelial breast cancer cell lines. It is therefore important to elucidate the mechanisms by which its expression is induced to bring forth an EMT. We have recently found that the oncogene Src which is activated in a number of cancers including breast can increase Pez expression.

The main aims of this study (for 1 year funding) were:

- 1: To establish that Src is an upstream effector in the signalling pathway of Pez-induced EMT
- 2: To determine the effect of mutations in Pez identified in colon cancers on the half-life, localisation and activity of the Pez protein

Results:

We found that overexpression of vSrc or constitutive active cSrc but not the kinase-dead Src when introduced into breast cancer cell lines led to a marked (~10-fold) increase in endogenous and exogenous Pez expression. We also found that Pez is tyrosine phosphorylated in the presence of vSrc or constitutively active cSrc. These observations suggest that phosphorylation of Pez by Src increases its expression. The increase in expression of exogenously introduced Pez suggest that one mechanism of increasing Pez expression is by stabilisation of the protein. We have identified 2 Nedd4 (an E3 ubiquitin ligase) binding sites in Pez and found that one of these is phosphorylated by Src. Published literature indicate that phosphorylation of Nedd4-binding sites leads to inhibition of Ned4 binding, further suggesting that phosphorylation of Nedd4 sites in Pez by Src may be one way to prevent its degradation and increase expression. Interestingly, we have preliminary data showing that some growth factors that activate Src also increase Pez expression. Many of these growth factors also induce EMT in certain contexts.

We have also generated a panel of mutations in Pez that were observed in colorectal cancers and begun analysing the effect of these mutations on the level of Pez expression. Preliminary data indicate that 2 of the mutations in Pez may increase its expression. We are continuing with studies to elucidate the effect of these mutations on other Pez functions.

FELLOWSHIPS

A/Professor Gordon Howarth

University of Adelaide

Novel bioactive factors for treatment of chemotherapy-induced intestinal mucositis and colitis **Bowel / Other**

\$64,738

Lay Summary

Cancer sufferers undergoing chemotherapy frequently suffer damage to the intestine as a side-effect. This is known as 'intestinal mucositis'. This is a serious condition that can limit the effectiveness of chemotherapy.

Recently, a number of naturally-sourced agents and compounds known as 'bioactives' have been described with the potential to decrease inflammation. Dr Howarth is testing these factors for their potential to decrease the severity of mucositis using experimental systems.

Dr Howarth's research group has defined a new probiotic bacteria (known as *Streptococcus thermophilus* TH-4) demonstrating the ability to decrease the severity of mucositis. This finding is being pursued further in an attempt to determine the factors responsible for protecting the intestine from mucositis. A second new probiotic has also been identified which can alleviate some of the features of 'colitis' (inflammation of the colon). This probiotic (*Lactobacillus fermentum* BR11) has the potential to reduce the likelihood of colitis progressing to the development of colon cancer.

Dr Howarth is now extending his study of bioactives to include new naturally-sourced bioactives such as Lyprinol (a mollusc oil) and Emu Oil to determine if these compounds may be effective against mucositis.



Scientific Summary

Project 1: New treatment strategies for intestinal mucositis

My key strategy has been to utilize proven animal model systems of chemotherapy-induced intestinal mucositis for the efficacy-testing of newly-developed bioactive factors. These factors have included probiotics, marine extracts, trace elements and growth factor preparations. Prospectively, my intention is to extend mechanistic studies into probiotic action in intestinal mucositis whilst continuing to investigate novel bioactive approaches such as the herbal preparation, Iberogast, and a newly-developed molluscan extract.

Project 1a: *Streptococcus thermophilus* and treatment of intestinal mucositis.

University of Adelaide PhD student, Katie Tooley has completed her experimental studies into the pre-clinical development of the newly-described probiotic *Streptococcus thermophilus* as a new treatment strategy for mucositis. Katie employed the novel sucrose breath test (SBT), developed in our laboratory (1). Katie has been able to demonstrate an improvement in features of mucositis in an animal model of methotrexate-induced mucositis. This work was published in the highly-respected journal *Cancer Biology and Therapy* which has an impact factor of 3.2 (2).

Project 1b: *Lactobacillus* and bifidobacteria species and intestinal mucositis.

Honours students Rasha Kamil (University of Adelaide) and Chad Mauger (University of South Australia) have made the interesting discovery that not all probiotic species may necessarily be effective against intestinal injury (3,4). This work is now being extended by 2006 University of Adelaide Honours student, Cassie Smith, who is investigating the newly-described probiotic, *Lactobacillus fermentum* BR11 (Manuscript in preparation).

Project 1c: Capability for the sucrose breath test to detect intestinal mucositis induced by different classes of chemotherapy drugs.

This project (funded by a \$500,000 BioInnovation Fund grant) was able to demonstrate that the non-invasive sucrose breath test could be applied to intestinal damage induced by a broad cross-section of chemotherapy drugs acting through different mechanisms (5).

Project 1d: Dietary zinc supplementation to reduce symptoms of intestinal mucositis.

This is a new initiative. Swagatha Sundar has commenced her mid-year 2006 Honours studies into this project in the School of Agriculture, Food and Wine at the University of Adelaide. Work is in progress.

Project 1e: New bioactive strategies to combat intestinal mucositis.

2006 Honours student, Diana Torres (University of Adelaide) has discovered that low doses of the marine extract, Lyprinol (New Zealand Green-Lipped Mussel) are able to decrease some features of intestinal mucositis in rats. This work is currently being prepared for submission to the journal *Cancer Biology and Therapy*. We have been able to generate some funding from industry to investigate the potential efficacy of other factors including the growth factor KGF, Emu Oil and a compound known as Spinosad. These studies are in progress and will be prepared for publication in 2007. In 2007, these studies may be extended to include other potential bioactive compounds such as the herbal extract, Iberogast, a newly-developed molluscan extract, and even inhibitors of the enzyme, dipeptidyl peptidase IV.

Project 2: New treatment strategies for inflammatory bowel disease

Consistent with Project 1, my primary strategy has been to utilize our proven animal model systems of inflammatory bowel disease for the efficacy-testing of the newly-developed bioactive factors described under Project 1. These factors have included probiotics, marine extracts, trace elements and growth factor preparations. Prospectively, my intention is to extend mechanistic studies into probiotic action in intestinal mucositis whilst continuing to investigate novel bioactive approaches such as the herbal preparation, Iberogast, and a newly-developed molluscan extract.

Project 2a: *Lactobacillus* and bifidobacteria species and intestinal mucositis.

Final-year PhD student, Mark Geier, has described therapeutic potential for the newly-described probiotic, *Lactobacillus fermentum* BR11 to combat features of inflammation in an animal model of ulcerative colitis, a condition known to predispose to the development of colon cancer (7). We are aiming to pursue the mechanism of action of this 'antioxidant' probiotic through a collaboration with researchers at the Queensland University of Technology who are able to provide us with specifically-modified mutants of *L. fermentum* BR11. This year, Mark and I have published reviews of probiotic action in colorectal cancer (13) and inflammatory bowel disease (14) in the highly-respected journals, *Cancer Biology and Therapy* and the *International Journal of Food Microbiology*, respectively. I have also published a review in this area as part of the Falk Foundation conference proceeding series. Mark has also been investigating the effects of 'prebiotics' in IBD (9).

Project 2b: Effects of fatty acids on gastric and colonic cancer cells

Final-year University of Adelaide PhD student, Geoff Matthews, has described therapeutic potential for the short-chain fatty acids, butyrate and propionate to induce apoptosis in both gastric and colonic cancer cells in vitro. Geoff has a paper In Press (11) and has recently published a review in this area (12). New PhD student, Jane Fauser (commenced July 2006), will be extending Geoff's findings to include other fatty acids in addition to probiotics capable of synthesizing and releasing fatty acids.

Project 2c: Inhibition of dipeptidyl peptidase IV (DPIV) and alleviation of colitis

Flinders University PhD student, Roger Yazbeck, has been working on the role of the enzyme DPIV in inflammation of the colon. Roger has found that inhibition of this enzyme decreases colonic inflammation in a mouse model of IBD, and has submitted this work for publication. Roger next intends to investigate the potential for DPIV inhibitors to decrease symptoms of mucositis.

Project 2d: Dietary zinc supplementation to reduce symptoms of colitis.

2005 university of Adelaide Honours student, Joanne Ball has found that certain zinc supplements were able to decrease features of colitis in a rodent model of IBD. This work has been accepted for publication (10) and is now being extended to the potential treatment of mucositis.

Dr Anna Brown

Child Health Research Institute

Identification of genes with roles in Myeloid

Leukaemias **Ovarian**

\$22,196

Lay Summary

Dr Anna Brown, a Cancer Council Research Fellow based in the Leukaemia Laboratory (headed by A/Prof Richard D'Andrea) at the Child Health Research Institute, has several projects based on finding and analysing genes affected in Acute Myeloid Leukaemia (AML). This year we have made significant progress on several fronts. Excitingly we published a large study in the Journal of Leukocyte Biology on gene expression changes in myeloid cells as they grow and mature and how these changes relate to patient AML samples. Results from this study are being followed up to look at how changes in expression of several individual genes may directly contribute to leukaemic cell growth. This includes studying gene expression changes in response to a mutant form of a growth factor receptor called FLT3, which is the most frequent known gene mutation in AML. As AML patients with FLT3 mutations generally have an unfavourable prognosis, our research is of importance as it could lead to the development of new and better therapies. The Leukaemia Laboratory has recently been successful in obtaining funding from the National Health and Medical Research Council for the next 3 years to fund more researchers on this important project.



For up to 50% of people with AML, the genes that are mutated and cause disease have not been identified, and this means these patients do not have the opportunity to have specifically targeted therapies. It is therefore a major aim of Dr Brown's Fellowship, in collaboration with students and researchers in the Leukaemia Laboratory, to use new methods to identify undiscovered oncogenes (cancer causing genes) from patient samples. We have been using a sophisticated technique called 'retroviral expression cloning' to achieve this aim and have been working very hard this year to get the technique 'up and running' in the laboratory. We have made excellent progress and have now got to the stage where we are using our first patient sample. The year ahead looks to be very exciting for the Leukaemia Laboratory and we are looking forward to identifying several new oncogenes from patients with the hope that they will become targets for new and better therapies.

Scientific Summary

Project 1: Functional identification of Klf5, a potential myeloid tumour suppressor gene. We have continued to accumulate preliminary data on this project with the aim of recruiting a student and applying for project grant. We have confirmed that expression of Klf5 interrupts the growth of myeloid cells and have generated an inducible system to examine this in more detail. We have also shown that Klf5 is upregulated during normal human and mouse myeloid differentiation. This data is consistent with our hypothesis of Klf5 as a myeloid tumour suppressor. We have successfully recruited an Honours student in 2007 to work on this project and have submitted a project grant to the Association for International Cancer Research, the NHMRC and the Cancer Council.

Project 2: Identification of common gene expression changes between leukaemia data sets. We have successfully completed the analysis of our microarray genesets and published an article in the Journal of Leukocyte biology. Our analysis has lead us to focus on the role of Gadd45a as a gene regulated by the leukaemia-associated FLT3-ITD

mutation. We have successfully obtained NHMRC funding for this project commencing this year and also have a PhD student. We have confirmed that Gadd45a is regulated by FLT3-ITD and are currently performing functional studies in myeloid cell lines. Our preliminary data suggests that forced expression of Gadd45a can interfere with the ability of the FLT3-ITD mutant to confer factor-independent or 'leukaemic' cell growth in myeloid cells. We expect to publish some of our findings this year.

Project 3: A new approach to identify novel oncogenes - retroviral expression cloning. Significant progress has been made on this project in the last 12 months with the recruitment of a PhD student and ongoing NHMRC project funding. We have successfully optimised all of the library construction and screening procedures and have successfully made 3 expression libraries from normal and leukaemic myeloid cell lines and well as bone marrow from a patient with the myeloproliferative disorder Polycythemia Vera. Two of these libraries have been put into a functional screen and we are currently examining positive clones to identify the genes they contain. We expect this year to characterise functional clones from the current screens as well screening our other completed libraries. We will also construct libraries from several primary patient AML samples. We expect to write a publication this year describing optimisation of the library construction process.

Dr Rachel Gibson

Royal Adelaide Hospital

Chemotherapy induced mucositis:
mechanisms of damage, time course of
events and possible preventative strategies
Other

\$47,351

**Lay Summary**

Mucositis is a major oncological problem, caused by the cytotoxic effect of cancer chemotherapy and radiotherapy. The condition affects the entire gastrointestinal tract and causes pain and ulceration in the mouth, oesophagus and small and large intestines. In addition it causes abdominal bloating, vomiting diarrhoea and constipation. Mucositis increases the sickness of patients undergoing cancer treatment, prolongs their hospital stay, increases hospital readmission rates, and is occasionally fatal. Currently we do not properly understand why these side effects occur and we have no effective treatment for all of them. However, over the last 12 months we have begun to understand further the mechanisms underlying the development of alimentary mucositis.

Scientific Summary

Mucositis is a serious complication of cancer chemotherapy and radiotherapy. Over the last few years, major advances have been made into understanding the pathogenesis of gastrointestinal mucositis. During 2006, our laboratory has been investigating four key research areas.

Chemotherapy-induced diarrhoea

One of the key areas which has been under-researched in recent times is chemotherapy-induced diarrhoea, with the majority of the information in the published literature based on clinical observations only. Research conducted in our laboratory during 2006 has shown that the microflora of the gastrointestinal tract play a key role in the development of diarrhoea. Chemotherapy alters the normal luminal environment and our research has shown that this altered environment may allow different genera of bacteria to proliferate. In particular we have shown that bacteria which produce B-glucuronidase increase significantly following chemotherapy treatment. Furthermore, we have demonstrated that absorptive functions in the intestines decrease following chemotherapy, this leading to increasing fecal sodium and electrolyte levels. This in combination with the altered luminal environment may contribute to the development of chemotherapy-induced diarrhoea.

Role of Transcription Factors

Changes in expression of transcription factors have been implicated in the development of mucositis, although how this interacts with other biological compounds is as yet unclear. Recent studies conducted in our laboratory have further highlighted the importance of NFkB by identifying an increase in expression in the mouth in patients following chemotherapy and in regions of large intestinal mucositis following radiation and combined chemoradiation in colorectal cancer patients. We have also identified that the p53 and p21 proteins are key players in the development of mucositis, although again the exact mechanism by which they contribute to mucosal injury remain uncertain.

Changes in Gene Expression Following Chemotherapy

Chemotherapy is known to cause dynamic interactive molecular and cellular events throughout the mucosa. Over the last 12 months we have utilised microarray technology to examine these gene changes. We have demonstrated significant changes in expression of genes involved in cell proliferation, differentiation and apoptosis. As early as 1 hour following chemotherapy, there was a significant response involving mitogen-activated protein kinase pathway,

cell cycle regulation and a number of transcription factors. At later time points, changes to the complement cascade became prominent. Our data also indicate likely changes in the keratinocyte differentiation pathway providing a possibility to track markers such as transglutaminase, cystatin and involucrin. As many hundreds of genes appear to be regulated, numerous other potential markers can be identified and evaluated. Furthermore, elucidating the relationship between genes and the network of pathways which are involved in the pathobiology of mucositis will aid in targeting treatment. It will also potentially allow discovery of new and appropriate drugs for the prevention of mucositis, possibly currently being used to treat other diseases.

Radiation Mucositis

Radiation-induced mucositis is a common and serious side effect of radiotherapy. Molecular mechanisms of mucosal injury however, are still poorly understood. During 2006 we developed an animal model using fractionated radiotherapy to investigate the development and occurrence of acute and chronic mucosal injury in the gastrointestinal tract. Briefly, 24 DA rats were randomly assigned to receive either fractionated radiotherapy or no radiotherapy. The irradiated rats received a fractionated course of abdominal radiotherapy at a dose fraction of 2.50Gy 3x/week for 6 weeks. After each week of radiation, a group of irradiated rats was killed. Histomorphology and mucin distribution in the gastrointestinal tract was investigated. The TUNEL assay was utilised to examine apoptosis in the colon and jejunum and intestinal morphometry was used to assess villus length, crypt length, and mitotic crypt count. Immunohistochemistry of p53, NF- κ B, COX-1, and COX-2 was also performed. The six week fractionated radiotherapy course induced mucosal injury throughout the gastrointestinal tract from week 1, with more severe injury seen in the small intestine. The hallmark appearance of apoptosis was present in the crypts of the small and large intestine. In the small and large intestine, goblet cell disorganisation and degeneration was obvious and colonic and jejunal crypt mitotic counts were severely depleted throughout treatment. Expression of p53, NF- κ B, COX-1, and COX-2 were increased in the irradiated intestinal sections. Mucosal injury arising in the alimentary tract as a result of fractionated radiotherapy has been effectively documented in the DA rat and can now be used to further study the molecular mechanisms underlying radiation-induced mucositis with a view to targeted therapy to alleviate its effects.

W BRUCE HALL CANCER RESEARCH FELLOWSHIP

\$81,795

Dr Andrew Sakko
University of Adelaide

Characterisation of the androgen receptor
signalling axis using the novel AR-E231G
transgenic mouse model of **prostate** cancer

Lay Summary

For men with non-localised prostate cancer the only currently effective treatment is androgen ablation. While effective initially, this treatment eventually fails, after which there are no viable alternatives and many patients die from distant metastases within 2-5 years. The basis of treatment with androgen ablation is to inhibit the synthesis of testicular hormones (androgens) and/or block their action in prostate cancer cells. Androgens act on the prostate by binding to a protein known as the androgen receptor (AR). We have recently found that in prostate cancer the AR continues to work even after androgen ablation therapy. This occurs because the AR accumulates genetic alterations and/or other changes that short-circuit its requirement for physiological levels of androgen to be active. If we are to develop better treatments we need to understand how these mutations and changes occur and how to stop their effects. In collaboration with a laboratory from the Fred Hutchinson Cancer Research Center in Seattle in the USA, we recently developed a new experimental model of prostate cancer. In this model, introduction of an AR with a particular genetic change results in prostate tumours and lung metastases in 100% of animals by about 50 weeks of age. We are using this in order to define the involvement of androgens and AR in the pathway to advanced cancer. This offers hope that we can devise more effective and long-lasting ways of blocking the action of androgen, the AR and the growth of non-localised prostate cancer.

Scientific Summary

Owing to the paucity of appropriate experimental systems, it has been difficult to prove a direct and/or causal relationship between AR expression, activation or function, and the initiation or progression of prostate cancer. In collaboration with Professor Norman Greenberg (Fred Hutchinson Cancer Research Center, Seattle, USA) we recently developed a new transgenic model of prostate cancer which provided for the first time evidence that mutant AR can contribute to the development and progression of prostate cancer. AR-E231G mice, which carry a mutation in the AR that is known to influence interactions with cellular co-regulators, develop metastatic prostate cancer in 100% of mice at about 52 weeks of age. Importantly, unlike previous animal models of prostate cancer, such as TRAMP, the AR-E231G model does not rely on overexpression of viral oncoproteins or administration of supraphysiological levels of steroids for tumourigenesis. This finding therefore highlights the oncogenic potential of

AR. This new experimental model opens up new possibilities for novel diagnostics, interventions and therapies.

This project aims to characterise the AR signalling axis in the development and progression of prostate cancer in the AR-E231G model.

To identify key AR co-regulators mediating differential gene regulation by AR-E231G compared to wtAR that contribute to development of prostate cancer, we have performed in collaboration with Prof Norman Greenberg from the Fred Hutchinson Cancer Research Center (Seattle, USA) Affymetrix microarray analysis using pooled prostates of nontransgenic littermates, wtAR and AR-E231G mice at 12 weeks of age. We identified 120 genes up-regulated (>2-fold) in AR-E231G versus wtAR overexpressing mice and 260 genes down-regulated. Differential gene expression was confirmed in independent samples by quantitative real-time PCR. Two genes specifically upregulated in AR-E231G mice are Cbp/p300-interacting transactivator with a Glu/Asp rich carboxy-terminal domain (CITED1/MSG-1) and adrenomedullin (ADM). MSG-1 is a co-activator that enhances the interaction of CBP/p300 with Smad transcription factors, and is an important regulator of the differentiation of mesenchyme into luminal-epithelium during puberty via oestrogen and TGF β signalling pathways. MSG-1 expression is down-regulated after differentiation, and its re-expression appears to be a key factor in the progression of certain tumours. ADM is an androgen-regulated short peptide hormone secreted by normal and malignant prostate epithelial cells that acts in an autocrine and paracrine manner to regulate cell growth and protect cells from apoptosis, and has been implicated in mediating prostate tumourigenesis. Increased levels of MSG-1 and/or ADM could either contribute to, or act as a marker of, tumourigenesis.

Due to the long latency of prostate cancer in the AR-E231G model, in parallel studies we are using immunohistochemistry to determine levels of expression of the AR and AR co-factors and other proteins implicated in aberrant AR signalling in prostate cancer. This includes analysis of SGT (a small glutamine-rich tetratricopeptide repeat containing protein that we have determined interacts with the AR to promote cytoplasmic compartmentalisation and transcriptional silencing of the AR) and the acetylated histones H3 K9 and H3 K19.

We have also obtained antibodies to MSG-1 and ADM and are optimising these for use in mouse and human prostatic tissues. These antibodies will be used to analyse AR-E231G tumours to confirm the microarray and quantitative real-time PCR data. Furthermore these antibodies will be used to analyse human prostate cancer progression and outcome tissue microarrays (n = 263) to establish if MSG-1 and ADM are candidate genes important in clinical prostate cancer. This strategy will inform selection of genes to be further investigated in the laboratory to establish their role in prostate tumourigenesis. Selected genes will be over-expressed or knocked out as appropriate in xenograft and transgenic models of prostate cancer.

Furthermore, protein will be extracted from the AR-E231G tumours for analysis by a combination of immunoblotting and immunoprecipitation in order to further characterise the AR signalling axis in prostate cancer. In addition to the proteins referred to above, p160 co-regulators, such as TIF2 and SRC1, and various post-translational modifications to the AR, including phosphorylation, will be studied in these analyses. These studies will be informative in the identification of new markers and targets that can be utilised in future pre-clinical and clinical trials.

PETER NELSON LEUKAEMIA RESEARCH FELLOWSHIP

\$89,436

Dr Mark Guthridge
IMVS

The role of a novel GM-CSF signalling pathway in regulating cell survival in myeloid **leukemia**

Lay and Scientific Summary



Leukaemia still ranks in the top 10 forms of cancer and it remains one of the hardest to cure. It afflicts all age-groups and ethnic backgrounds and is the leading form of cancer in children while it is particularly difficult to treat in the elderly where aggressive chemotherapeutic approaches are not possible. The biggest obstacle to curing leukaemia has been preventing disease relapse. Initially, most patients diagnosed with leukaemia actually respond very well to conventional chemotherapeutic approaches and 70-80% go into remission. However, the majority of leukaemic patients eventually relapse and succumb to the disease. So the challenge for clinicians and medical researchers worldwide has been to not only understand why leukaemic relapse occurs, but to also develop new therapeutics that will prevent relapse and provide a long-term durable cure.

A team of researchers led by Dr. Mark Guthridge at the Institute of Medical and Veterinary Science (IMVS) here in Adelaide have discovered one of the mechanisms by which leukaemic cells may be able to survive and eventually become resistant to drug therapies leading to relapse. Using

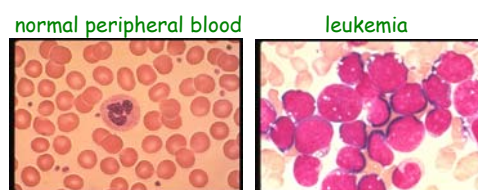
state-of-the-art techniques that allow them to look inside leukaemic cells, they have identified a “molecular switch” that controls cell survival and cell growth. This “molecular switch” in some respects resembles a light switch that in normal cells can be turned on and off to control cell survival, but in leukaemic cells is fundamentally faulty and remains permanently switched on. The consequence of this faulty switch is that leukaemic cells gain a survival advantage and have a prolonged life-span making them resistant to the cell-killing effects of chemotherapeutic drugs. It is this “molecular switch” that is thought to allow the long-term survival of leukaemic cells that leads to disease relapse.

The research team at the IMVS have begun to identify potential therapeutic targets of this deregulated “molecular switch” in leukaemia. In some cases, targeting this switch has resulted in killing leukaemic cells in the test-tube, however, the results are very preliminary and much more work will be required to validate possible targets and develop drugs for the clinic.

The work of Dr. Guthridge has been strongly supported by the Cancer Council of South Australia since he returned to Adelaide in 1998 from New York University Medical Center and he received a Peter Nelson Leukaemia Research Fellowship to continue his work in 2005. The findings of Dr. Guthridge together with Professor Angel Lopez, also at the IMVS, have been presented at major international scientific conferences and their significance for leukaemia research have been recognized with the award of a National Institutes of Health (NIH) grant from the United States Government.

One of the main reasons why the work of Dr. Guthridge and Prof. Lopez have managed to stay one step ahead of larger and better funded laboratories in the US and Europe is the unique combination of expertise, technology and pathology services available within the IMVS in Adelaide. For example, Professor Bik To and Professor Tim Hughes head the Therapeutic Product Facility (TPF) within the IMVS. Since the inception of the TPF in 1986, it has remained the largest bank of leukaemic samples in Australia. It is this bank of leukaemia samples that provides an invaluable resource that gives Dr. Guthridge the edge in his research.

As a recipient of a Peter Nelson Leukaemia Research Fellowship, Dr. Guthridge gives frequent public lectures on how leukaemia arises, the current treatments available and how medical research is finding potential new ways to treat this intractable disease. He also takes members of the public who have made financial donations aiding leukaemia research on tours of the laboratories showing how current research may impact on the way we treat cancer in the future.



OTHER RESEARCH PROGRAMS FOR 2006

TRAVEL GRANTS

\$41,547

Royal Adelaide Hospital

2,750

Joanne Bowen Department of Medical Oncology
18th Multinational Supportive Care in Cancer Symposium, Toronto Canada and visit Brigham and Women’s Hospital, Boston 20 June – 5 July 2006

Ann Yeoh Department of Medical Oncology
18th International Symposium, Toronto Canada 21 – 25 June 2006

1,100

Children, Youth and Women’s Health Service

A/Professor Gordon Howarth Gastroenterology Department
4th international Conference on Gastrointestinal Carcinogenesis held in Honolulu, Hawaii 18 – 28th May 2006

2,750

Dr Geoff Matthews
Centre for Paediatric and Adolescent Gastroenterology
4th international Conference on Gastrointestinal Carcinogenesis held in Honolulu, Hawaii 2006 \$2,750

2,750

“In August 2006 I was able to participate in the 4th international Conference on Gastrointestinal Carcinogenesis held in Honolulu, Hawaii. Together with my PhD student, Geoff Matthews, we presented our findings on the effects of short chain fatty acids (butyrate and propionate) on gastric and colonic cancer

cells. At the meeting Geoff and I discussed our results with international experts, including Professor Graeme Young from South Australia. The conference was very important as it gave us some important insights into the potential for probiotics expressing these fatty acids to modify the course of gastric and colonic cancer. This will be an important new initiative for the research of our group.”

The Queen Elizabeth Hospital

Dr Jennifer Hardingham Haematology-Oncology Department 867
11th proteomics Symposium, Lorne Victoria 2 – 5 Feb 2006

Flinders Medical Centre

Dr Antony M Hooker Department of Haematology and Genetic Pathology 2,750
Environmental Mutagen Society 37th Annual Meeting Vancouver 15 – 23 Sept 2006

Flinders University

Roger Yazbeck School of Biological Sciences 2,750
Digestive Diseases Week, Los Angeles 20 – 25 May 2006

University of Adelaide

Jane D Holland Special Molecular Signalling Conference 2,750
Dubrovnik, Croatia + visit institutions in Germany and Sweden 26 May – 1 June 2006

Hedyeh Hedayati Department of Medicine 2,750
Attending the 8th World Congress of Psycho-Oncology, Venice Italy 18 – 21 Oct 2006

Catherine Mackenzie 800
16th National Health Promotion Conference, Alice Springs Northern Territory 24 – 26 April 2006

Hanson Institute / IMVS

Rachel J Gibson Dame Roma Mitchell Cancer Research Laboratories 2,750
18th International Symposium of Supportive Care in Cancer, Toronto Canada 16 – 26 June 2006

This conference was extremely interesting and has given me new ideas to think about for my future research. There were dedicated sessions on mucositis, my main field of research, and these sessions highlighted new advances that were occurring both in the laboratory and in the clinical setting. Of particular relevance was a satellite symposium entitled Best practices for Oral Mucositis: Evaluating New Evidence and Exploring Management Strategies. This symposium was very important, and provided an update on the latest research in the field of mucositis, including epidemiology, research and practical strategies for reducing the burden of mucositis. Other important presentations that were of benefit to me were Mucosal Damage from Targeted Therapies, Clinical Relevance of EGFR1 Therapy Skin Toxicity and its Management and Current and Future Targets for Mucositis Therapy. Following these sessions I was able to meet with several leaders in the field of mucositis, and this gave me the opportunity to discuss my research with them. In addition, I was a co-author on seven abstracts, all of which received very positive feedback. Two of these abstracts were selected to receive Young Investigator awards, and one was selected as the Young Investigator of the Year. I feel that it was a very positive two weeks that will really benefit my future research.

Andrea Stringer Dame Roma Mitchell Cancer Research Laboratories 1,100
18th International Symposium of Supportive Care in Cancer, Toronto Canada 20 – 26 June 2006

Olga Sukocheva Signal Transduction Laboratory 2,750
American Association for Cancer Research 97th Annual Meeting, Washington DC April 2006

“The AACR Annual Meeting is the most important cancer research meeting in the world. ...and featured world leaders in the fields of cancer genetics, molecular diagnostics, chemistry, imaging, clinical research, and stem cell biology. (The grant provided the) opportunity to increase my knowledge, broaden perspective, and share insights with others with the same dedication to research on cancer. The important thing was that I had an opportunity to present work and attract attention of several International Cancer Research Funds.

Dr Mark Guthridge Division of Human Immunology 2,000

New Directions in Leukemia Research Conference, Sunshine Coast Queensland + visit Leukemia Foundation Laboratory at the Qld Institute of Medical Research, Brisbane 1 – 6 April 2006

Anna Tsykin Microarray Bioinformatics 2,750
 Multinational Association of Supportive Care in Cancer, Toronto Canada 22 – 24 June 2006 + XXIII International Biometric Conference, Montreal Canada 16 – 21 July 2006

Prem Dwivedi Endocrine Bone Laboratory 2,750
 13th Workshop on Vitamin D, Victoria Canada 6 – 14th April 2006

Clinpath Laboratories Adelaide

Marilyn Betchley (postponed from 2005) 2,680
 Training, support and organisation of Cytology knowledge in Thimpu, Bhutan July to October 2006

“I was able to fulfil a desire to volunteer in a developing country during July to October 2006. Both the personal and professional experience was rich in challenges and rewards. The Cervical Screening Programme in Bhutan is growing: In 1999, 2000 smears were screened. This year numbers will reach 12,000. My task was to help improve all aspects of it. After an initial period in the Cytology lab at Jigme Dorgi Wanchuck National Regional Hospital where I acquainted myself with procedures and screen some Pap smears, I devised a teaching programme to reflect the needs of the Pathologist and Cytotechs. I thoroughly enjoyed the challenge of assisting them to improve and everyone has sincerely adapted the changes which I suggested.”

Sheila Ward Travel Grant Recipient

Dr Cory Xian Department of Orthopaedic Surgery Children, Youth and Women’s Health Service 2,750
 33rd European Symposium on Calcified Tissue, Prague Czech Republic 10 – 14 May 2006

“Participating in the scientific programme has enhanced my knowledge of diseases of bone and basic bone biology, and its correlation to bone cancer, bone metastasis, and cancer treatment-induced bone defects. By actively participating in the scientific programme and interacting with international peers, this meeting has benefited my research career development. It has helped take research work to a new higher level and to the internationally competitive forefront, and it has generated helpful interaction and useful future collaboration.”

Distinguished Visitors \$6,000

Supporting Institution	Visitor
Professor Guy Maddern Department of Surgery University of Adelaide	Professor Murray Brennan Head of Memorial Sloan Kettering Cancer Centre New York, USA

Student Vacation Scholarships \$15,225

Note: Some institutions invoice in 2007, upon completion of student placement

Chair in Cancer Care – Professor Ian Olver \$25,000

Data Managers Program \$144,775

Microarray Bioinformatics \$37,559

Total of Other Research Programs	\$510,343
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TCCSA TOTAL RESEARCH FUNDED	\$2,466,735
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The Freemasons Cancer Research Scholarship *Administered by TCCSA	\$25,000
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