WAIKATO MEDICAL RESEARCH FOUNDATION

Discovery, Innovation, Progress.

2013 Annual Report

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Chairman's Report

Once again our Charitable Foundation has had a very busy year in terms of receiving and approving grants and in making substantial progress towards raising our fund to a capital base of $5m. This has been with the very able and professional help from Fundraising Solutions Ltd in the form of Russell Mayhew. Over the past months, Russell has put together our Corporate Fundraising Team. This team comprises a number of prominent Hamilton business professionals who are making remarkable progress towards our fundraising goal. I am most grateful to them for their selfless dedication to the fundraising appeal.

I would in particular like to thank Peter De Luca, Senior Partner of Tompkins Wake Hamilton for chairing the Corporate Fundraising Team.

I am also very gratified with the enthusiastic response of the senior medical staff, and am pleased to announce we have substantial support from our medical colleagues, in our aim of raising our funds so that we can better fund local research. I would like to thank particularly Dr Cam Buchanan, and Dr Linda Rademaker, for chairing the medical fraternity fundraising team meetings very ably.

I have been especially pleased with the willingness of trustees to spend a significant amount of time attending meetings for the purposes of fundraising. I am also especially grateful to Geoff McDonald our Deputy Chairman, who has most willingly and ably stepped in for me while I have been unwell.

Special thanks must also go to Dr Peter Rothwell, our Patron, who has given much time to our cause.

I would especially like to thank Sir William and Lady Judi Gallagher who have once again come out in support of our community and agreed to be patrons for our fundraising appeal.

I am also grateful to Jonathan MacKenzie, Editor of Waikato Times, for energetically supporting us via the Waikato Times Media suite.

We are delighted that PAN® Media & Advertising has very generously provided us with a major sponsorship which includes a new market brand and logo, a very professionally produced brochure and other documentation for the fundraising appeal and assistance with marketing the fundraising appeal. They have also kindly agreed to develop our website to make it more user friendly and interactive and have offered to help us maintain it as we go along. Many thanks also to Fusion Print for kindly sponsoring the printing of the entire suite of documents for the fundraising appeal.

Once again, we have received a significant grant from Trust Waikato for which we are most grateful. Trust Waikato has supported our Foundation for the past twenty years with very generous funding. We are also very thankful to the local branch of the Cancer Society, which has been very generous in assisting us to fund grants this year.

There are others I could thank for their huge assistance, but I thought that should wait for another time.

I would like to take this opportunity of congratulating Dr Marianne Elston for achieving funding from the Health Research Council of New Zealand to investigate ethnic differences in thyrotoxicosis relating to presentation and outcome. Marianne was an earlier recipient of our funds, and is an example of how we like to help people at the beginning of their research so they can get exposure to bigger funding bodies. Marianne is the sole local recipient of an HRC grant this year, which is particularly noteworthy because within the HRC assessing committee members, there is a sole representative from the Waikato who actually comes from Auckland – Dr Philip Wood: congratulations, Marianne.

Thank you everyone so much for your support this year in fundraising and matters relating to the Waikato Medical Research Foundation. I hope you enjoy reading the rest of the report.

Dr Noel Karalus
Chairman

Report of the Grants Committee

This year, the WMRF received 15 applications requesting a total of $341,000. The standard of applications was typically high, but a number of worthy applications were unable to be supported due to lack of funds. These applicants are encouraged to apply next year when, thanks to the productive efforts of the current fundraising initiative, there may be more money available. Nine applications were supported totalling nearly $162,000. Applicants are reminded that the committee decision is final and further discussion will not be entertained.

Applications supported proposed studies on: whether the ulcer drug cimetidine can block cancer metastasis; erosive vulvovaginal lichen planus; the outcome in heart failure patients with an intact left ventricular ejection fraction; identification the optimal selenium compound for use with cancer therapies; the prevention of hypoglycaemia in newborns with oral dextrose; the control of sexual dimorphism of skeletal muscle by growth hormone and the gonadal steroids; bioactive natural products from New Zealand marine and terrestrial sources and the transplantation of encapsulated choroid plexus cells to treat retinal degenerative disease. Several grants support postgraduate projects and are cross institutional.

I thank the grants committee (Maggie Fisher, Amanda Oakley, Roy Daniel, Michael Jameson and the WMRF chairman, Noel Karalus) for reviewing, scoring and ranking the applications, and Ian Jennings for providing input from a financial viewpoint. A special thank you goes to the WMRF administrator Robyn Fenneman for gathering, organising and meticulously presenting the applications to the committee and responding to applicants.

Adrian Molenaar
Chair, Grants Committee

WMRF Funded Projects Grant Round 2013 – Total: $161,900 **Financial Assistance via Sponsorship Grants from Cancer Society / Trust Waikato / Grassroots Foundation**	
• Expression of sialyl Lewis antigens in cutaneous and choroidal melanoma primary, nodal and metastatic lesions : a predictive marker with therapeutic opportunities - can the ulcer drug cimetidine block metastasis Dr Duncan Bayne (Waikato Hospital)	$14,000
• hPOD - hypoglycaemia Prevention in Newborns with Oral Dextrose Jo Hegarty (University of Auckland / Liggins Institute)	$21,000
• Phase 1b pharmacokinetic (PK) and pharmacodynamic (PD) trial to identify the optimal selenium (Se) compound for use with cancer therapies Stephen Evans (Pharmacy Department, Waikato Hospital)	$26,000
• Review of erosive vulvovaginal lichen planus : clinical presentation and impact on quality of life Dr Harriet Cheng (Department of Dermatology, Waikato Hospital)	$5,900
• Transplantation of encapsulated choroid plexus cells to treat retinal degenerative disease Dr David Worsley (Hamilton Eye Clinic, Hamilton)	$20,000
• The control of sexual dimorphism of skeletal muscle by growth hormone and the gonadal steroids Dr Ryan Paul (Endocrine Research Fellow, Waikato Hospital)	$13,500
• Prospective Evaluation of Outcome in Patients with Heart Failure with a Preserved Left Ventricular Ejection Fraction : The PEOPLE Study Dr Gerry Devlin (Cardiology Department, Waikato Hospital)	$25,000
• Bioactive Natural Products from New Zealand Marine and Terrestrial Sources : Discovery and Modes of Action Dr Michele Prinsep (Department of Chemistry, University of Waikato)	$22,500
• Tramadol V Morphine for refractory postoperative pain in the recovery room Dr Kelly Byrne (Waikato Hospital)	$14,000

Summary of Funded Projects from Grant Recipients 2013

Grant #213 // Dr Duncan Bayne, Plastic Surgical Department, Waikato Hospital

Expression of sialyl Lewis antigens in cutaneous and choroidal melanoma primary, nodal and metastatic lesions: a predictive marker with therapeutic opportunities

Malignant melanoma is an aggressive form of cancer that most commonly arises in the skin but is also sometimes found in the eye. It can spread to other parts of the body and is seldom cured at that stage. One of the mechanisms by which cancer cells can spread is through copying the way white blood cells leave the bloodstream and move into body tissues. This is through the cells having certain molecules on their surface that stick onto specific "adhesion molecules" on the lining of the blood vessels.

In this project we will look at the melanomas in 80 patients whose melanomas have spread to the local glands or elsewhere in the body (called metastatic disease) and look at whether the melanoma cells that have spread elsewhere are using the same surface molecules that white blood cells have. If this is the case then we can potentially use a safe ulcer-healing drug called cimetidine to block the spread of melanoma cells like these, because it takes away the "landing sites" on the lining of the blood vessels. There is already evidence that cimetidine can do this in people with bowel cancer and if this research suggests that melanoma cells spread the same way then we would plan clinical trials in patients with high-risk melanoma to try to prevent recurrence and reduce the death rate.

Grant #214 // Dr Kelly Byrne, Consultant Anaesthetist, Waikato Hospital

Tramadol vs Morphine for refractory postoperative pain in the recovery room

A few people have a lot of pain as they wake up after surgery. This still happens even though they had strong pain relief medicines while they were in theatre and more of these medicines in the recovery room. What we are aiming to find out is whether patients with a lot of pain post-operatively, despite having already had some Morphine, respond better to either more Morphine, or to another drug called Tramadol. Anecdotally in our institution there are reports of patients in the recovery room receiving large doses of morphing post-operatively without adequate relief of their pain. When these patients then receive a dose of Tramadol, their pain resolves rapidly. Is this an effect of Tramadol, or is the administration of Tramadol merely coinciding with the peak effect of the Morphine that they have already received?

The evidence from the literature is scarce and conflicting. One previous study of the use of Morphine and Tramadol has shown them to have an infra-additive effect when used in combination. One would then expect the exact opposite of the anecdotal reports. Other studies have shown a Morphine sparing effect of Tramadol, but the 2 drugs were used together rather than compared to each other.

The proposed study is to recruit patients from the recovery room who have ongoing pain despite receiving post-operative Morphine. They would then be randomised to receive either Tramadol or further Morphine for their ongoing pain. The primary outcome measure would be time from first administration of study drug to readiness for discharge from the recovery room. Other outcome measures that will be recorded will be pain scores and how rapidly these change and the presence of side effects of the administered medications.

Grant #219 // Dr Gerry Devlin, Cardiology Department, Waikato Hospital

Prospective Evaluation of Outcome in Patients with Heart Failure with a Preserved Left Ventricular Ejection Fraction: The PEOPLE Study

Heart failure is a common condition with high rates of hospitalisation and death. Most clinical trials involving patients with HF have focused on patients with poor heart pump function (low LV ejection fraction). However, the heart pump function may be normal among a significant subset of patients with heart failure. Currently, there is uncertainty regarding which patients with heart failure with preserved pump function will be at risk of dying or being readmitted to hospital.

The objectives of this study are to determine which of these patients will be at risk of these events. This large-scale, multicentre international study is aiming to recruit 2500 patients with HF in New Zealand and Singapore. The patients will then be followed for 2 years. Maori have high rates of hospital admissions for heart failure and are often under-represented in clinical studies. The study results will impact on the clinical management of patients with HF in New Zealand and Singapore and will lead to the development of clinical trials to test newer treatments for patients with heart failure.

Grant #220 // Dr Stephen Evans, Pharmacy Department, Waikato Hospital

Phase 1b pharmacokinetic (PK) and pharmacodynamic (PD) trial to identify the optimal selenium (Se) compound for use with cancer therapist

Chemotherapy and radiotherapy are dreaded by many patients due to their toxicities. Laboratory and early clinical research demonstrates that selenium (Se), a trace mineral in which NZ soils are deficient, can significantly reduce the toxicity of these treatments without compromising their efficacy. This would be a clinically important exciting advance in cancer treatment that would be widely acceptable to patients. However the optimal type and dose of Se has not been determined, with doses used in clinical trials varying from 100-60,000 mcg/day. Sodium selenite (SS) is commonly used but causes significant DNA damage at higher doses that could, when given in combination with radiotherapy or certain chemotherapy drugs, increase the risk of secondary malignancies; this has not been evaluated clinically. The organic Se compounds, Se-methylselenocysteine (MSC) and L-selonomethionine (SLM) appear to be both more effective and safer than SS, with MSC being optimal in laboratory studies, though it has not been evaluated yet in combination with cancer treatments in human trials. Nor has the relationship been established between Se dose and its effects on the relevant mechanisms that help Se compounds to protect normal tissues while enhancing death of cancer cells.

Preliminary work by this research group has established laboratory tests to measure these mechanisms in blood. This project is now being extended into clinical testing as a phase 1 trial, giving SS, SLM and MSC orally at 400, 1600 and 6400 mcg/day for 8 weeks to groups of patients with prostate cancer and chronic lymphocytic leukaemia. Full evaluation of safety, tolerability and laboratory tests will be made, as well as any response of the cancer or leukaemia to treatment.

The overall goal of this research is to determine which Se compound, and at what dose, can be most safely and effectively used in future clinical trials in cancer patients, evaluating its ability to optimise the efficacy and toxicity of anticancer therapies. This will help researchers to avoid less effective forms and doses of Se that could also cause serious acute and late toxicities.

Grant #225 // Dr Michele Prinsep, Department of Chemistry, University of Waikato

Bioactive Natural Products from New Zealand Marine and Terrestrial Sources : Discovery and Modes of Action

At the University of Waikato, Drs Michèle Prinsep and Ryan Martinus and their students propose to undertake a project to identify and then carry out further studies on bioactive and/or novel metabolites from natural sources. This work primarily focuses on two different types of organisms, marine bryozoans (moss animals) and cyanobacteria (blue-green algae), both of which have proven to be excellent sources of novel, bioactive compounds. Our main emphasis is on bryozoans but cyanobacteria occur in a wide variety of habitats, both aquatic and terrestrial.

For the project, bryozoans will be collected from New Zealand and Antarctic waters, while cyanobacteria will be collected from so-called "algal blooms" or as required, cultured at the Cawthron Institute, Nelson, and the University of Waikato. All samples will be extracted in a suitable solvent and the crude extracts then tested for biological activity against two tumour cell lines (HeLA cells, a human cervical cancer derived cell line and P388, a murine leukaemia cell line) and a panel of microorganisms. Each extract will also be analysed using liquid chromatography-mass spectrometry (LC-MS) under standard conditions. Once a promising extract has been identified by these means, it will be investigated further to isolate the compound/s responsible for the observed biological activity through large-scale extraction, then separation and isolation of the active component/s by various types of chromatography. The biological activity must be monitored at every step of the isolation process by whichever assay is appropriate to each case, to ensure that the activity is being concentrated and that no loss of activity occurs.

Once a pure biologically active compound is isolated, its structure will be determined using a variety of spectroscopic techniques, especially high field nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. If possible, analogues of the isolated compound will be prepared to see if the activity can be improved. Compounds that are active against one or both tumour cell lines will be examined further using a secondary screening process to fully explore their mode of action and their potential biomedical applications. This secondary screening process will examine if the compounds cause apoptotic or necrotic cell death and hence identify those most likely to be of therapeutic potential.

Grant #227 // Dr David Worsley, Hamilton Eye Clinic

Transplantation of encapsulated choroid plexus cells to treat retinal degenerative disease

In the eye there are support cells that release proteins called neurotrophic factors that control the function of the retina. Neurotrophic factors control retinal development, maintenance and defend against disease and degeneration. As the eye ages the retinal support cells decline and neurotrophic factor release ebbs. Consequently there is in declining neurotrophic factor support for the retina. Ageing with decline of neurotrophic factor support is linked to the emergence of the neurodegenerative retinal diseases. These are the commonest causes of vision loss in adults; dry macular degeneration, glaucoma, diabetic retinopathy and inherited retinal neurodegenerative diseases. There are currently no treatments for dry macular degeneration which is responsible for 50% of blindness.

In recent years, a large volume of experimental work has explored how delivering single neurotrophic factors might delay, diminish or even repair these neurodegenerative retinal diseases.

We have a novel concept of how multiple neurotrophic factors can be delivered to the retina in a long-term delivery system and are conducting a proof of principle study in a rabbit model of retinal neurodegeneration.

The retina is part of the brain, derived from, and functioning very like brain neural tissue. Brain support cells are the choroid plexus epithelium (CPE). Unlike retinal support cells, there is a large body of research on CPE. The CPE is a neuro-endocrine gland releasing a cocktail of neurotrophic factors. As in the eye, CPE function declines with age. And degenerative brain diseases and CPE senescence are linked. Transplantation of healthy CPE into the brain is effective in treating animal models of brain neurodegenerative diseases including Parkinson's disease, Huntington's chorea and stroke. CPE transplantation for Parkinson's disease commences phase 1 clinical trials in Auckland in April 2013.

The evidence that neurotrophic factors by CPE transplantation treats brain neurodegenerative diseases raises the question:

Will choroid plexus transplantation into the eye treat retinal neurodegenerative disease?

Sourcing human CPE is impractical. We use pig CPE from a unique pig breed approved for human transplantation. To prevent immune rejection and obviate the need for immunosuppression drugs, CPE are immune-isolated by being placed in a ½mm diameter capsule. The immune system cannot 'discover' the cells. The capsule is porous so neurotrophic factors can be released and oxygen and nutrients can enter. Thereby the encapsulated CP cells, called NTCELL, remain healthy and can function as a neuro-endocrine gland.

Figure 1. Cell encapsulation

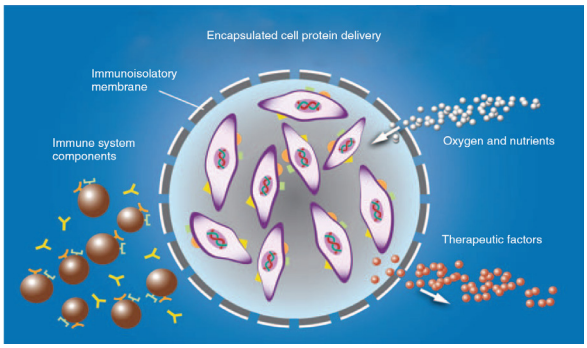
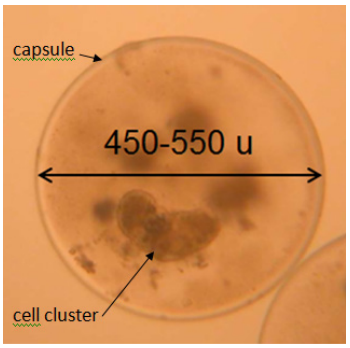


Figure 2. Encapsulated CP epithelium (NTCELL)



This proof-of-principle' study will assess the effect of implanting NTCELL into the eye of a rabbit model of retinal neurodegenerative disease. We have developed a technique for implanting NTCELL into rabbit eyes with a 20 gauge injector. Our preliminary work shows that NTCELL are well tolerated and the CPE remain healthy. We have done some work already, first with this model in rats which proved too small and recently with the rabbit; to validate the rabbit model in our laboratory and refine implantation.

If this study is successful, will then require study with another animal model (probably a dog inherited retinal neurodegeneration) before human study can be considered.

Past Grant Recipients
Final Reports and Findings 2011 – 2012

Grant #209 // Dr Nicola Starkey, School of Psychology, University of Waikato

The effects of traumatic brain injury on developmental outcomes in young children

Background

Traumatic Brain Injury (TBI) is one of the most common causes of death and long-term disability during childhood. Paediatric TBI may have wide-ranging effects across cognitive, behavioural, emotional and social domains, affecting normal developmental processes. However, the long-term effects of TBI in children, particularly mild TBI, are not well characterised and outcomes may be worse than those of similar injuries sustained by adults. Evidence suggests that the younger the age at injury, the worse the outcomes from TBI. Damage to developing brain tissue can disrupt both current skills and subsequent development; inhibiting the learning of new skills, and preventing the attainment of normal developmental milestones.

Aim

The overall aim of this project was to examine the effect of mild TBI on young children's (< 2 years at time of injury) cognitive, social, behavioural and emotional functioning two years after injury. The preliminary findings are presented here.

Results

The parental reports of their child's behaviour and the children's performance on the cognitive assessments are presented in Figure 1. The parent reports indicated that the TBI group showed slightly lower levels of internalising problems (such as anxiety and depression), poorer adaptive (social / communication) skills, and less well developed executive abilities compared to the control group (left panel Figure 1), however these differences were not statistically significant. In addition, there were no significant differences between the TBI and control groups in relation to parental mental health (specifically anxiety or depression).

Discussion

Together these preliminary findings suggest that a mild TBI during infancy has few negative effects on children's emotional, cognitive and behavioural development 2 years later. These findings support other recently published work with a similar follow up period (Crowe et al., 2012). While study findings suggest appropriate development across emotional, behavioural and cognitive domains at 2 years post-TBI, additional follow up of this cohort is recommended to fully ascertain the longer-term effects of TBI. As children mature they have to deal with increasing more complex situations (such as starting school) and it is possible that developmental difficulties may only become apparent in the face of increasing demands on the child. Such demands may exacerbate the current (small) differences observed between the TBI and control groups.

The current study is not without its limitations; the sample size is small and even though the age range is just 2 years, there are large developmental differences between the children who have just turned 2 and those who are almost 4 years old. In relation to this, there has been significant debate about the sensitivity of the questionnaires and measures that are typically used to assess children in this age group (2-4 years) and it has been suggested that most current measures are better suited for use in older children (Chapman et al., 2010). Recruitment of additional similar aged children with TBI and a longer term follow up with form the focus of future studies.

Grant #211 // Drs Tom Wheeler, Paul Harris, Adrian Molenaar, AgResearch

Histatherin, A Potential New Antimicrobial Protein From Milk

The number of effective antibiotics available to the medical profession is declining. As a result drug resistant bacteria are becoming increasingly problematic. The emergence of these resistant microorganisms requires the development of new and novel antibiotics. Screening of a bovine expressed sequence tag database for homologs of known antimicrobial proteins identified a small 6 kDa and 39 amino acid peptide. The peptide was named Histatherin and was patented by AgResearch in 2006. Since its discovery, research conducted into the detection and isolation of this peptide has proven challenging.

Over the summer period, attempts have been made to improve the recognition of Histatherin and to isolate a pure fraction from bovine milk. Investigation into glycosylation, a potential masking factor, produced conflicting results. While, for a number of reasons discussed in the main report, the glycosylation state of the protein could not be determined conclusively, the western blot of deglycosylated fractions containing Histatherin did not improve its detection. In fact is appears to have hindered it.

Experiments were also conducted to precipitate Histatherin from milk fractions, in particular lactoferrin. This method had been previously determined to be effective for this fraction as it isolates Histatherin from lactoferrin thereby generating a fraction with increased purity. However, this method may prove limiting if native Histatherin occurs in multiple forms as it may selectively capture only one variety.

Native gels were conducted in an attempt to produce a purified band of Histatherin for antibody generation. A new antibody to the native form may improve the western blotting method. However, the technique of native gels requires refining before this can be achieved.

Finally, liquid chromatography was employed on skim and trim milk to determine where Histatherin may be isolated from and to develop a suitable method for its purification. Hydroxyappatite columns have been previously employed due to calcium sites on the column. However, neither pH nor NaCl elution have generated the desired result to date. An alternative approach is now being taken; whole milk is being treated to obtain a fraction between 1 and 30 kDa. This should contain only a few proteins and be highly concentrated in Histatherin.

This work is now a starting point for continued research at an honours level occurring later this year. While many of the techniques have been recently learnt they have been employed correctly. Even so, this peptide seems determined to appear elusive at this stage.

Grant #194 // Professor Vickery Arcus, Biological Sciences, University of Waikato

Breaking the cycle of transmission of tuberculosis in New Zealand

Summary

This project aimed to develop a fast diagnostic test to confirm the presence of the 'Rangipo' strain of tuberculosis (TB) which is found throughout the Waikato/Bay of Plenty region. This strain of TB is readily transmitted between people and as such, requires fast identification in order to stop the spread of this disease.

Background to study

Tuberculosis (TB) is the second greatest killer worldwide due to a single infectious agent after HIV/AIDS. The incidence of TB disease in New Zealand was seven per 100 000 in 2010 which is higher than the rates for Australia, the United States, and Canada. Infection is usually curable with antimicrobial drugs, but relies upon strict adherence to the antibiotic regime. The Rangipo strain of Mycobacterium tuberculosis is responsible for the largest cluster of tuberculosis infections within New Zealand. This cluster has been active for over twenty years and its control has proved difficult owing to its high transmissibility and lack of a rapid test to identify the strain. Molecular typing is used to find and link source cases to contacts and to type strains allowing prompt identification and tracking of specific strains spreading through the population. We have developed a rapid PCR based diagnostic test to quickly identify this strain, based on Rangipo specific single nucleotide polymorphisms (SNPs) identified through whole genome sequencing, to aid in the diagnosis and treatment of this disease and limit its spread.

Rationale for study

There is a significant association between housing density, income levels, and TB, so although the wider community benefits from the intended diagnostic, there is a particular advantage for those community groups where large social gatherings and crowded living or working conditions are the norm.

Currently, there appears to be a large timeframe between patients presenting to doctors with suspected TB, and TB typing results from LabPLUS (the TB typing centre for New Zealand) identifying the infecting strain. By developing a simple and speedy PCR based diagnostic test, the Rangipo strain of TB could be quickly identified at the in-house hospital laboratory (initially Waikato Hospital) weeks prior to the full typing results coming back. This timesaving would be a significant factor in reducing possible transmissions between the infected patient and other contacts (especially given the usually crowded living conditions), thereby limiting the spread of this highly virulent strain of TB and improving the health and well-being of our local community.

Project design

Whole genome sequencing of multiple Rangipo TB isolates has been performed by our collaborators at the New Jersey School of Medicine and Dentistry. We have analysed the single nucleotide polymorphisms (SNPs) to identify candidates believed to be Rangipo specific, by comparing to sequences from various TB strains published on TB databases. SNP candidates were ranked according to the sequence homology between TB strains in the gene surrounding the SNP, and the function of the surrounding gene (eg, SNPs within genes that are highly homologous or known to be involved in virulence, pathogenicity etc were selected).

Regions surrounding the top ranked SNPs from both Rangipo and non-Rangipo TB DNA were amplified by polymersase chain reaction (PCR) and SNP sites analysed for unique restriction enzyme digest recognition sequences. Restriction enzyme digests of initially single SNPs and then multiple SNPs were performed under various conditions to identify the best candidate SNP/restriction enzyme combinations which clearly and robustly distinguish between Rangipo and non-Rangipo strains of TB.

The diagnostic was tested on a range of both known and blind control samples to ensure sensitivity of the test before its implementation in the hospital laboratory.

Results

Seven Rangipo isolates from the Waikato hospital strain collection covering cases from 1991-2010 were fully sequenced with whole genome sequencing by our collaborators at the New Jersey School of Medicine and Dentistry.

We analysed the single nucleotide polymorphisms (SNPs) to identify candidates believed to be Rangipo specific, by comparing to sequences from various TB strains published and reported in international TB databases. 159 Rangipo specific SNPs were identified (these were SNPs which were found in all seven Rangipo isolates but not any other TB strains in the databases). Candidate SNPs were ranked according to the sequence homology between TB strains in the gene surrounding the SNP, and the function of the surrounding gene (eg, SNPs within genes that are homologous or known to be involved in virulence, pathogenicity etc were selected).

To develop the Rangipo specific diagnostic, we designed PCR/Restriction Enzyme digests to distinguish between the 2 SNP variants (Rangipo v non-Rangipo). Using genomic DNA from Rangipo and Non-Rangipo strains, we amplify a region of the genome we know to contain a SNP. This amplification product when run on an agarose gel is represented by a single band of DNA for both of the SNP variants. To distinguish between the two SNP variants, we exploit the nucleotide difference at the SNP position. We identified restriction enzymes which specifically recognise the DNA sequence of only one of the SNP variants.

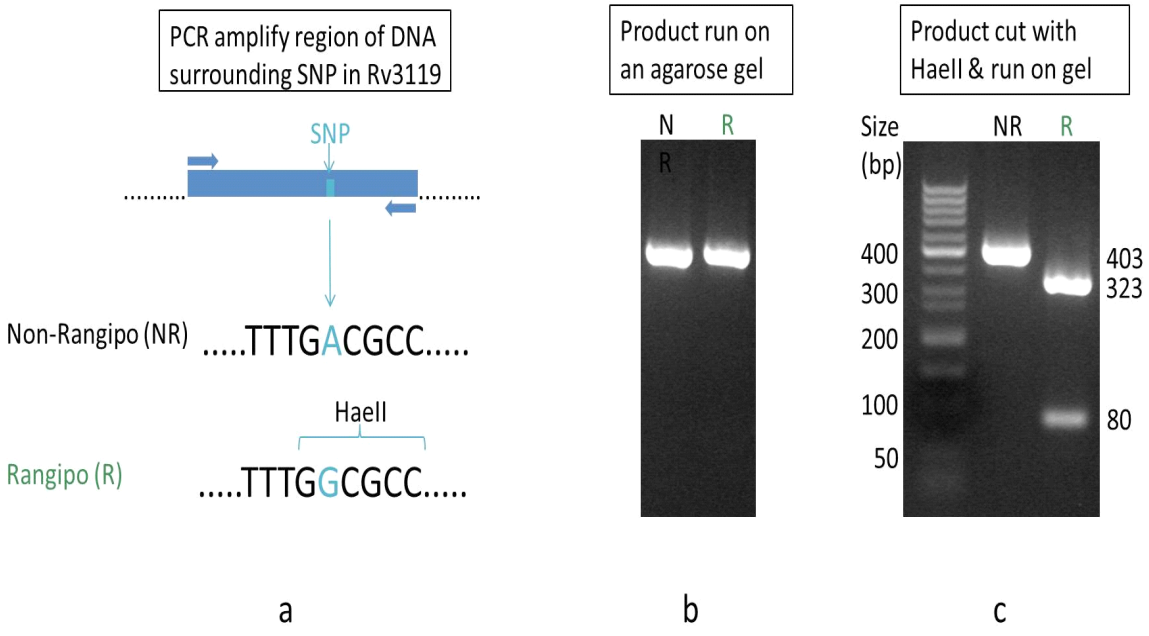


Figure 1. Development of a PCR/RE digest for Rv3119.
a) The DNA sequence surrounding a SNP in the gene Rv3119 for both a Rangipo (R) and Non-Rangipo (NR) strain. The different nucleotide at the SNP position is shown in blue (A in NR, G in R), the Restriction Enzyme HaeII recognises the sequence in the R strain (GGCGCC) but not in NR (GACGCC). b) When the PCR product is run on an agarose gel, both R and NR strains show the same 403bp band. c) When the PCR product is digested, if the strain is NR there is no HaeII recognition sequence at this SNP so the 403bp product is still intact, but if it is an R strain, HaeII recognises the sequence and cuts so 2 bands of 80 and 323bp are produced.

Restriction enzyme digests of initially single SNPs were performed, and then multiple SNPs were performed to strengthen the diagnostic. These were performed under various conditions to identify the best candidate SNP/Restriction Enzyme combinations and digests which didn't clearly discriminate between Rangipo and Non-Rangipo, required digestion at different temperatures, or produced too many fragments when combined in multiplex were ruled out.

The triplex assay contains three regions of DNA which have been PCR amplified in a single reaction, resulting in three products covering three different SNPs. Each product is the same size in both Rangipo and Non-Rangipo strains, but once digested a different banding pattern for Rangipo and Non-Rangipo can be seen (as with the single Rv3119 assay). The final

triplex assay used in the Rangipo diagnostic uses SNPs in Rv3119, Rv2504c and Rv1821. A Restriction Enzyme combination was chosen which cuts Rangipo for Rv3119 (HaeII), and Non-Rangipo for Rv2504c (DraIII) and Rv1821 (Sau3AI).

DNA from the seven fully sequenced Rangipo isolates were subjected to the diagnostic along with H37Rv (the tuberculosis lab strain) as a negative control. All Rangipo strains produced the same expected banding pattern after digestion and this pattern differed substantially to that of the Non-Rangipo control. Results for isolate 'R' were faint so the diagnostic was repeated with more input DNA later to confirm.

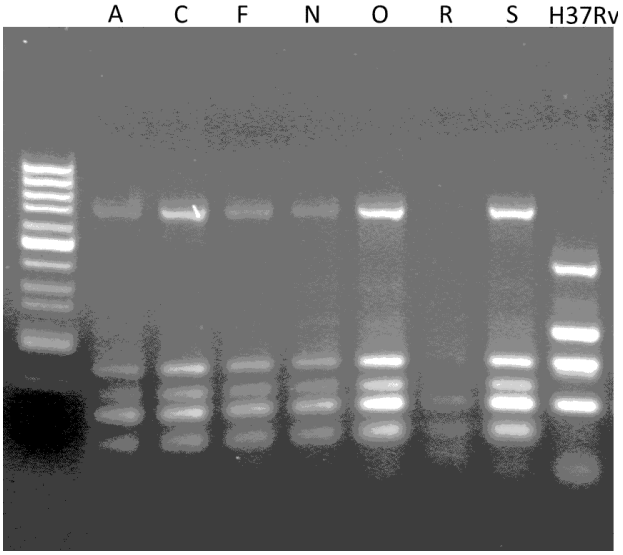


Figure 2. Aplication of the Rangipo diagnostic on the seven fully sequenced Rangipo isolates. Samples A, C, F, N, O, R and S have all been fully sequenced by whole genome sequencing, and are confirmed as Rangipo by the diagnostic. H37Rv is used as the Non-Rangipo control.

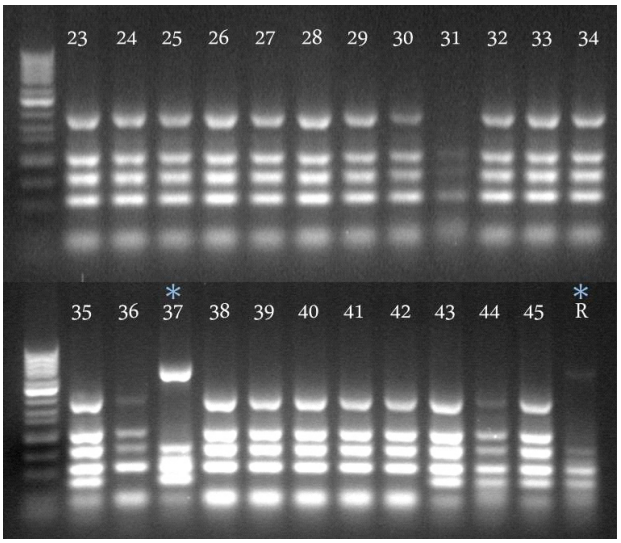


Figure 3. Application of the Rangipo diagnostic on 23 blind control samples. Samples 23 – 45 were 'blindly' tested and 'R' was our Rangipo control. The blue star above sample 37 indicates the only Rangipo isolate that was included in blind samples.

The diagnostic was initially tested with 21 'known' isolates from LabPlus, all from within the same MTB lineage as the Rangipo strain (Euro-American), so they were closely related isolates. All five of the Rangipo samples were correctly identified. Next, 23 'blind' isolates from LabPLUS covering four MTB lineages, East Asian, Indian Oceanic, East African Indian and Euro-American were tested. Here, the single Rangipo sample was correctly identified

A further 21 Euro-American isolates from LabPLUS were screened blind using the Rangipo diagnostic, the three Rangipo isolates were correctly identified. In total, 44 isolates were screened blind, and all four of the Rangipo isolates were correctly identified.

In the future, we hope to implement this diagnostic in the hospital laboratory which will reduce the current diagnostic time (from the DNA extraction stage) from up to eight weeks, to four and a half hours and at little cost. By Identifying Rangipo isolates faster, we can potentially limit patient contacts earlier, thereby reducing the spread of this virulent strain of TB.

A second goal of future research is to analyse SNPs that change the amino acid sequence of proteins thought to be related to the pathogenicity of the bacterium. In particular, several SNPs have been identified in the ESX genes which encode known tuberculosis antigens. This may point towards particular characteristics of the Rangipo strain such as increased transmissibility and virulence.

This work was presented at The Biannual Waikato Medical Research Meeting held at Waikato Hospital on the 14th March 2013. We sincerely thank the Waikato Medical Research Foundation for their support of this research.

Grant #188 // Dr Marianne S Elston, Endocrinologist, Waikato Hospital

Somatostatin receptor subtypes in *SDHB* germline mutation positive and sporadic phaeochromocytomas and paragangliomas

Introduction

Many neuroendocrine tumours, including phaeochromocytomas and paragangliomas (PGLs), express one or more somatostatin receptors (SSTR) 1-5. A number of studies have reported SSTR expression in phaeochromocytomas and PGLs. However, receptor expression patterns have been conflicting and until recently specific monoclonal antibodies were not available against SSTR1-5. The aim of this study was to compare SSTR1-5 expression in *SDHB* germline mutation-associated phaeochromocytomas and PGLs with sporadic tumours.

Methods

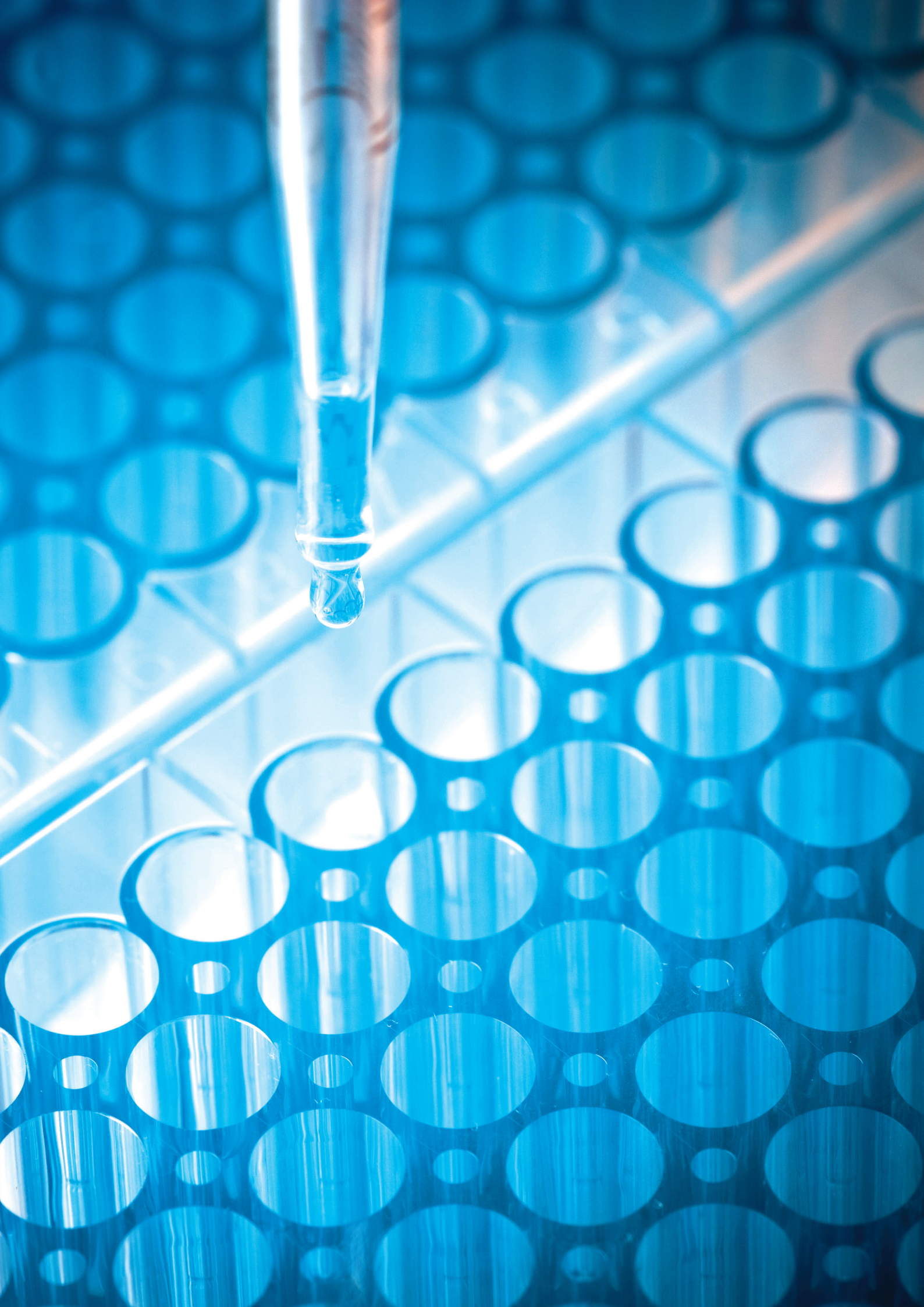
Written informed consent was obtained from all patients. Immunohistochemistry for SSTRs 1-5 and SDHA and SDHB was performed using specific monoclonal antibodies on archived formalin-fixed paraffin-embedded tissue.

Results

Twenty-one tumours were included (13 phaeochromocytomas, 8 PGLs); 6 tumours were from patients known to have an underlying *SDHB* germline mutation (3 with malignant disease) and 15 were sporadic tumours. All *SDHB*-associated tumours had absent staining of SDHB on immunohistochemistry with preserved SDHA staining, consistent with the known *SDHB* germline mutation. All sporadic tumours demonstrated positive staining for both SDHA and SDHB consistent with the absence of an underlying *SDHA* or *SDHB* mutation. All *SDHB*-associated tumours demonstrated strong staining for SSTR2A. In contrast 9/16 sporadic tumour demonstrated weak or absent staining for SSTR2A. With the exception of one sporadic tumour, which demonstrated weak SSTR5 staining, all tumours had absent SSTR5 staining.

Conclusion

This study confirms the utility of using SDHA and SDHB immunohistochemistry to screen for an underlying *SDHx* germline mutation in patients with phaeochromocytoma/PGL. SSTR2A appears differentially expressed in *SDHB*-associated tumours as compared with sporadic tumours. If these findings are confirmed in a larger group of *SDHB*-associated tumours it would suggest that octreotide therapy (unlabeled or radiolabeled) might have a therapeutic role in these patients.



Statement of Financial Position as at 31 May 2013

	2013	2012
Accumulated Funds	**$1,846,941**	$1,322,504
Represented by:		
Current Assets		
Westpac Cheque Account	**86,064**	26,171
Westpac Appeal and Bonus Saver Accounts	**594,033**	-
ASB	**121,240**	312,390
GST Refund due	**6,750**	-
	808,087	338,561
Investments		
Cash and Equivalents	**577,545**	307,108
NZ Fixed Interest	**363,422**	590,456
NZ Listed Property	**104,611**	90,158
Australian Investments	**145,921**	133,712
American Investments	**8,678**	6,725
British Investments	**994**	644
	1,201,171	1,128,803
Total Assets	**2,009,258**	1,467,364
Current Liabilities		
Accounts Payable	**159,567**	141,755
Related Party Payable	**2,750**	3,105
	162,317	144,860
Net assets	**$1,846,941**	$1,322,504

Statement of Financial Performance for the year ended 31 May 2013

Income	**2013**	2012
Dividends	**11,648**	9,732
Donations – Appeal	**574,000**	-
Donations – General	**26,260**	40,145
Donations – Trust Waikato	**65,000**	65,000
Foreign exchange gain	**-**	167
Grants refunded	**1,700**	-
Interest – Appeal Funds	**20,024**	72,399
Interest – Investment Funds	**55,669**	-
Unrealised gain on investments	**26,866**	-
	781,167	187,443
Expenditure		
Administration expenses including website	**11,596**	13,864
Advertising and promotion expenses	**2,954**	3,010
Audit fee	**2,779**	3,651
Fees paid to auditor for other services	**5,789**	5,106
Foreign exchange loss	**1,925**	-
Fundraising expenses	**95,670**	11,593
Grants	**123,190**	136,963
GST adjustments	**518**	-
Loss on realisation of investments	**4,936**	5,524
Portfolio management fees	**5,212**	5,681
Printing, stationery and postage	**2,161**	-
Unrealised loss on investments	**-**	8,979
	256,730	194,371
Net surplus/(deficit)	**$524,437**	$(6,928)

Waikato Medical Research Foundation History

In 1986 the Waikato Medical Research Foundation (Inc) was established and incorporated to promote, encourage and sustain medical research in the Waikato Region. At the time, Professor Michael Selby explains:
The aim was to undertake research that would be of benefit to the Waikato. Obviously we were hoping that the research would have wider applications than the Waikato. Inevitably, if you make any advances, the very nature of scientific work is that it gets published, and therefore you hope that the benefit will be widespread and therefore the people of the Waikato would benefit along with everybody else – that was the aim. So, we did put emphasis on publication, and therefore, of course quality – so that was part of the initial requirement.

The Waikato Medical Research Foundation has been established to enable ethical medical research to take place within the region. Medical Research will benefit everybody, and it warrants the support of all citizens.

In forming the Foundation, and going to the general public in the early years of fundraising, it stressed the importance that this is a local body. When initially formed, The foundation stressed to members of the general community in Hamilton and outlying areas that there were medical or health problems specific to the Waikato area, and that it was important to have a locally administered fund – and now 25 years on, the purposes of this Foundation are still as it was when initially formed.

The Trust was founded in 1986 with a capital pool of $1m.

"The legacy of the past is the seed that brings forth the prosperity of the future"

Board of Trustees (1986)
Standing (left to right) Denis Jury, Andrea Donnison, Don Llewellyn, Ross McRobie
Sitting (left to right) Ken Mackay, John Gillies, Michael Selby, Brian Smith, James Grace

WAIKATO MEDICAL RESEARCH FOUNDATION
Discovery, Innovation, Progress.

Those who signed the Trust Deed in 1986 were:

Charles Beresford, Physician, Waikato Hospital,

John Gillies, Paediatrician, Waikato Hospital,

Jack Havill, Anaesthetist, Waikato Hospital,

Jim Grace, Solicitor,

Dryden Spring, a company director,

Michael Selby, Professor at the University of Waikato,

Jack Wilson, Head of TECH,

David Braithwaite, a company director,

Brian Smith, a chartered accountant and

Valerie O'Sullivan of Matamata.

To our Donors – Thank you

The Trustees of the Foundation wish to thank all who have generously donated since our inception in 1986. From 1986 to 2013, the Foundation has supported researchers in the following institutions:

Waikato District Health Board	$957,000
University of Waikato Faculty of Medical Health Sciences,	$442,000
University of Auckland – Waikato Clinical School	$349,000
AgResearch	$589,000
Polytech	$41,000
Private researchers	$151,000

Without your generous donations, the Foundation would not have been able to support 25+ years of research in the Waikato.

Donation Form

This form can be downloaded from our website: www.wmrf.org.nz

I wish to make a donation to the Waikato Medical Research Foundation

Please tick one :

O $50 O $100 O $200

O $500 O $1000 O $2000 Other (Amount $_____)

O I enclose a cheque made out to: Waikato Medical Research Foundation

O I have made a direct payment to WMRF Bank Account: Westpac 030 306 0208170 01
(Please include your name as reference for the payment)

O Please send me a receipt

As we are registered with the Charities Commission (Charities Commission No: CC20443), all donations to Waikato Medical Research Foundation over $5.00 are tax rebatable.

Please complete your details and post / fax for a receipt.

Name of donor: _____

Address: _____

Daytime telephone: _____

For future contact, we would like to e-mail interested parties, and if you wish to receive information from us, please complete below:

Email address: _____

WAIKATO MEDICAL RESEARCH FOUNDATION
Discovery, Innovation, Progress.

Post to: Private Bag 3200, Waikato Mail Centre Hamilton 3240
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Web: www.wmrf.org.nz

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