Scientia

INNOVATIONS IN HEALTHCARE

HIGHLIGHTS:

- NIGRAAN: Helping children reach their fifth birthday in Sindh, Pakistan
- Stem cell therapy for the treatment of sepsis and organ injury
- Engineering to improve healthcare: Quenchbody for rapid diagnosis
- The power of sleep: management of obstructive sleep apnea hypopnea syndrome

October 2015

WELCOME...

Our healthcare system depends and thrives on innovations, both old and new. Indeed, medical equipment that one can find in any doctor's office, a stethoscope, was once a revolutionary innovation that we now routinely use to do simple, yet critical tasks such as measuring blood pressure and listening to normal/abnormal noises of the heart and lungs. One may notice state-of-the-art equipment in the operating room as well, such as the complex system of monitoring machines that is used to measure various vital signs during surgery. Scientific innovations that help increase our understanding of molecular processes underlying diseases can also help us identify targets that can be used for drug development or disease monitoring. For example, the development of insulin, which is the primary treatment for individuals with diabetes, stemmed from Dr. Frederick Banting's finding that molecules released from the pancreas are critical for the regulation of carbohydrates in the body and development of diabetes.

As it is clear from these examples, innovations in healthcare have had a direct impact on our quality of life. Recent innovations are no different, as scientists and physicians are tirelessly discovering new ways to improve our lives, whether such findings impact specific populations, such as those with rare cancers, or the general population, such as women and families. In light of these new discoveries, it is our responsibility to maintain our knowledge of upcoming innovations that have the possibility of significantly changing our lives. Therefore, this issue focuses on innovations in healthcare that aim to change diagnosis, treatment and management of various disorders and conditions, ranging from sepsis to skin damage from UV rays.

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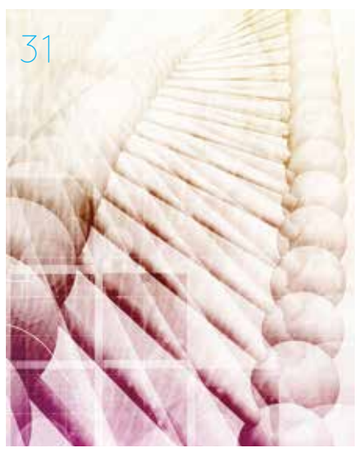

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FIGHTING SEPSIS

Sepsis is a term broadly used to describe a generalized severe infection of the organism in response to a pathogenic agent. The infection can begin in the lung, urinary tract, and the brain, and secondarily affect the blood, which can lead to organ failure and death. Up to one third of patients affected by severe sepsis will die, making sepsis responsible for the death of over 20000 people per year in the United States. Furthermore, sepsis increases the length of stay in Intensive Care Units (ICUs), causing economic and social damage.

Despite efforts in studying this well-known disease, the mortality rate due to sepsis remains very high because its progression occurs very rapidly for doctors to diagnose and treat adequately. For this reason, researchers and doctors have been searching for better therapeutic interventions for this condition. This section will discuss novel treatment alternatives for sepsis, their power in improving healthcare in ICUs and ultimately reducing mortality. Interestingly, two completely different approaches have been studied by Dr. Claudia dos Santos and Dr. Damon Eisen. While the first focused on highly advanced techniques, such as stem cell therapy for tissue regeneration and repair for these patients, Dr. Damon Eisen and his group studied the role of a well-known and widely prescribed medication in sepsis: acetylsalicylic acid. Dr. Eisen further added that that in addition to the use of aspirin being cost-effective, it could also be integrated in the health care system as a preventative measure.

Stem Cell Therapy for Critical Illness involving Sepsis and Organ Injury

Professor Claudia dos Santos is interested in studying the disease processes and the therapeutic targets related to critical illnesses involving sepsis and organ injury. Here we discuss the research background of professor dos Santos and her work on stem cell therapy in animal models of sepsis and lung injury.

Your research is focused on studying the critical illness caused by sepsis and organ injury. So to start, can you describe to the readers the nature of these conditions and their potential impacts on health and survival?

Sepsis is a life-threatening condition that occurs when the body has an overwhelming immune response to infection. Severe sepsis patients are often admitted to the intensive care unit, where they receive life supportive care. Most people who die following sepsis or a sepsis-like disorder call systemic inflammatory response syndrome (SIRS) die from Multiple Organ Dysfunction Syndrome (MODS), which accounts for up to 80% of all deaths in modern ICUs. Any organ can be a target of MODS including the lungs, the kidneys, the brain, the liver, the gut or the blood coagulation system. Although advances in care, diagnosis and management of infection have reduced early mortality, no specific treatments have been identified to treat MODS. Moreover, survivors experience significant disability after discharge from ICU, and mortality risk are increased for survivors long term.

How did your interest in studying critical illnesses begin and what has been the motivation behind it?

During my medical school training, I noticed that I gravitated towards the sickest patients. I was particularly drawn to the challenge of diagnosing and managing life-threatening conditions requiring sophisticated organ support and invasive monitoring. Many critically ill patients are in extremis, and their biology is complex and requires extensive knowledge of medicine to understand how multiple crucial variables such as past medical history, medication, psychosocial and economic factors can contribute to the clinical outcome and the success of the treatment. I was especially drawn to being on the cutting edge of research and technology, and I found that I enjoyed the technical aspect of a procedure-intensive subspecialty. I also liked working with critical care nurses, doctors and other adjunct medical professionals in intensive care units. These are a wonderful group of dedicated and hard working professionals who shared with me a strong sense of purpose.

In the course of your research, you have studied the application of stem cells in the treatment of sepsis and associated tissue injury. Can you explain what stem cells are and why are they of therapeutic value in these cases?

Stem cells are undifferentiated biological cells that retain the potential to differentiate into specialized organ cells (e.g. muscle, liver or lung cells). This particular feature has raised the interest in stem cells as a therapeutic tool for tissue regeneration and repair. Stem cells also possess other biological characteristics such as the ability to release molecules with the potential to regulate the function of the immune system. The research done at my laboratory has shown that bone marrow-derived mesenchymal stem cells ameliorated the inflammatory and injurious immune responses in animal models of sepsis and acute lung injury. These therapeutic effects are believed to be mediated by the release of anti-inflammatory molecules rather than by the tissue regeneration properties of stem cells. In addition, recent findings from my group suggest the ability of stem cells to reprogram the immune cells to produce less inflammatory mediators, and to enhance the clearance of the bacteria causing the inflammation.

Are you planning to extend your research on stem cell therapy further? What might be the scope of the next step?

Yes. The major goal of my research is to translate our current knowledge of stem cell biology to the development of novel therapeutic strategies for the treatment of sepsis in critically ill patients. To this end, we have established collaborations with the Canadian Critical Care Trials and Translational Biology Groups. Both groups are involved in the first human trial of stem cells for the treatment of severe sepsis. Our role will be to profile genetic material from septic patients who received stem cells and compare it to patients who received placebo to determine how patients respond to treatment and determine the markers that can predict response to therapy as well as other important clinical outcomes such as surival. We are also working on improving stem cell technologies by optimizing and enhancing the anti-inflammatory and anti-bacterial potential of cells. In the future, we hope cell-free approaches may also become available for the treatment of sepsis, acute lung injury and multi-organ failure.

Promise and Challenges of Stem Cell Therapy in Sepsis and Organ Injury

Professor dos Santos and her group have recently validated the therapeutic potential of stem cells in controlling the complex inflammatory and immune responses associated with sepsis and sepsis-induced organ injury in clinically relevant animal models.

A STORY OF CRITICAL ILLNESS AND ORGAN DYSFUNCTION

Severe sepsis is a leading cause of morbidity and mortality worldwide. It is the most common reason for admission to the intensive care unit (ICU) accounting for 20% of such admissions. Although early identification, aggressive resuscitation, administration of antibiotics and supportive care has reduced mortality, this remains unacceptably high. From a health care perspective the aggregate healthcare cost reaches $20.3 billion per year, sepsis represented 5.2% of the national costs for all hospitalizations in 2011 in the US. Moreover, post-acute care of survivors is estimated to be as high as $3.5 million per individual at 1 year post-ICU discharge.

The vast majority of ICU deaths occurring among critically ill septic patients are caused by Multiple Organ Dysfunction Syndrome (MODS). The latter is a complex condition in which the normal physiological function of the affected organ cannot be maintained without intervention. It usually involves two or more organs, including vital organs such as the lungs (acute respiratory distress syndrome), the kidneys (acute kidney injury), the brain (septic encephalopathy), the liver (acute liver failure), the gut or the blood coagulation system. The degree and severity of organ failure is profoundly dependent upon the patients' age and pre-morbid condition, with older and frailer patient doing worst despite the use of proper medication and life support.

Sepsis and organ dysfunction involve a series of abnormal reactions of the cellular and soluble components of the innate immune system, the biological system responsible for coordinating our response to injury and infection. In progressive cases, and for yet unclear reasons, over- or under-activation of the innate immune system combined with microcirculatory failure results in MODS. This intricate pathogenesis represents a challenge for identifying effective treatments against sepsis and MODS. Targeting a single pathway is unlikely to be effective in modulating the complex inflammatory and immune responses. Indeed, numerous specific pharmacological agents have failed to produce significant benefits in clinical trials of severe sepsis.

STEM CELLS: ORIGIN AND BIOLOGICAL CHARACTERISTICS

Stem cells are undifferentiated cells that retain the potential to differentiate into a variety of cell types with different functions (a feature referred to as pluripotency). They are found in multicellular organisms, but are most commonly isolated from mammals. There are two broad types of mammalian stem cells: embryonic stem cells, which are isolated from embryos at early developmental stages, and adult stem cells, which are found in various adult tissues. In terms of clinical application, stem cell may be derived for either allogeneic use (from a donor to a patient) or for autologous use (from the patient's own body). Autologous cells, however, require significant preparation time (weeks) and they would not be adequate for use in critically ill patients who will likely need the cell therapy immediately after admission to the ICU. 'Therefore a bank of stem cells collected from healthy donors will have to be made available for clinical use' says Professor dos Santos.

Mesenchymal stem cell therapy reduced the complex inflammatory reactions underlying sepsis in a clinically relevant mouse model, and reconstituted the components of the immune system to enhance the clearance of the causative microbes

STEM CELL THERAPY FOR SEPSIS AND ORGAN INJURY

Among the various subtypes of adult stem cells, bone marrow-derived stem cells, which are commonly known as Mesenchymal Stem/Stromal Cells (MSC), have gained increasing interest for the treatment of diseases involving inflammation and organ injury. The research done by Professor dos Santos and her group has shown the ability of MSC to ameliorate the inflammatory responses associated with acute lung injury and sepsis in mouse models, indicating their therapeutic potential. Importantly, these therapeutic effects were achieved after the onset of experimental sepsis/organ injury, mimicking the natural disease and

a clinically relevant therapeutic approach.

Regarding the mechanisms underlying the therapeutic effects of MSC in models of sepsis-induced MODS, it is uncertain whether the pluripotent nature of these cells plays the most significant role. 'Frankly, pluripotent mechanisms involving stem cell differentiation are not thought to underlie the beneficial effect conferred by MSC in sepsis', says Professor dos Santos. In contrast, other characteristics of stem cells, particularly their ability to release anti-inflammatory mediator molecules, have been proposed as effectors of the MSC-conferred protection from injury. Moreover, recent findings by Professor dos Santos and her team have postulated a role of MSC in 'reprogramming' the inflammatory response in sepsis, indicating that the beneficial effects of MSC extend beyond the suppression of inflammation. These combined immunoregulatory and immunomodulatory effects suggest MSC therapy has the pivotal advantage of addressing the complexity of immune abnormalities observed in sepsis and MODS, and may represent a promising novel treatment strategy affecting the inflammatory response at multiple levels.

In addition, the work of Professor dos Santos has also shown that MSC could reconstitute the physiologic status of the immune system, allowing the immune cells to fight and clear the bacteria causing the sepsis. This effect adds an extra level to the therapeutic potentials of MSC in sepsis, addressing the removal of the definitive cause of the disease.

CHALLENGES FOR THE MSC-BASED THERAPY

Despite the promising therapeutic effects of MSC in treatment of sepsis and organ injury, various important issues remain to be resolved before the full benefits of the technology are realised. 'The need to address these barriers to clinical translation is underlined by the limited clinical experience with MSC in critically ill patients to date', says Professor dos Santos.

The optimal route of administration of MSC is not known, with evidence supporting the intravenous, intratracheal and intraperitoneal administration routes. The optimal dosage regimen for MSC, including the lower effective doses, is also not clearly determined. Preclinical studies to date have used relatively poorly defined, heterogeneous MSC. Recently, more specific MSC subpopulations were identified, however, their capacity in attenuating organ injury remains to be determined. A standardized protocol for isolation and characterization of MSC versus other stem cell subpopulations is currently lacking, a consensus on minimal criteria for product development needs to be standardized for its use in sepsis patients. Furthermore, there is no validated method of measuring MSC bioactivity in the host after administration. Despite advances in MSC research, our understanding of the mechanisms of action remain incomplete. Of great concern is the tolerance of the immune system of the recipient to autologous MSC administration. MSC have long been believed to be hypoimmunogenic or 'immune privileged'—meaning that they do not provoke the innate immune system to elicit defence responses against them. This property will make it possible for stem-cells grown in culture, if proven safe, to be transfused similarly to how we transfuse other blood products today. It is not known whether rejection responses might influence the efficacy of allogeneic MSC therapies, and no definitive clinical advantage of autologous MSC over allogeneic counterparts has been demonstrated. In fact, MSC may exert therapeutic functions through a brief 'hit and run' mechanism. Notwithstanding, because this treatment will require cell delivery the remote risk of tumorgenicity exists, although no studies have shown any risk of malignancy.

CURRENT STATUS AND FUTURE PERSPECTIVES OF MSC THERAPY

Despite our incomplete understanding of MSC biology after delivery, their promising therapeutic effects in animal models of sepsis and organ injury have encouraged Professor dos Santos and other scientists to conduct initial clinical trials on human patients. The laboratory of Professor dos Santos is currently collaborating in an undergoing clinical trial with Dr. Lauralyn McIntyre and Dr. Duncan Stewart at Ottawa Hospital Research Institute in Canada to evaluate the safety and efficacy of MSC therapy in septic shock patients. The role of Professor dos Santos and her group is to profile genetic material from septic patients to identify biological markers of patient classification, disease prognosis and therapeutic effects. This work can potentially contribute to filling a part of the gap in the current knowledge of the functional and the therapeutic biology of MCS.

Researcher Profile

Professor Claudia dos Santos M.D. M.Sc. F.R.C.P.C.
Associate Professor of Medicine, Interdepartmental Division of Critical Care, Scientist, Keenan Research Centre for Biomedical Science of the Li Ka Shing Knowledge Institute of St. Michael's Hospital Scientist, Institute of Medical Sciences, Collaborative Program in Genome Biology and Bioinformatics and Laboratory Medicine and Pathobiology, School of Graduate Studies, University of Toronto

Professor dos Santos is an associate professor of Medicine at the University of Toronto and a scientist at the Keenan Research Centre for Biomedical Science and the Li Ka Shing Knowledge Institute of St. Michael's Hospital. Her major interest is in the pathogenesis of sepsis, acute lung injury and multiorgan dysfunction syndrome. Her laboratory employs integrated systems biology and functional genomics approaches to: (a) understand host-dependent molecular mechanisms of acute organ failure, and (b) develop an "informed" approach to the discovery of novel molecular targets for therapy of sepsis and acute lung injury. Professor dos Santos has developed various model systems from basic epithelial cell stretch models to animal models of critical illness. Her group exploits whole genome approaches, such as microarray technology in vitro and in vivo, to identify novel molecular targets for therapy.

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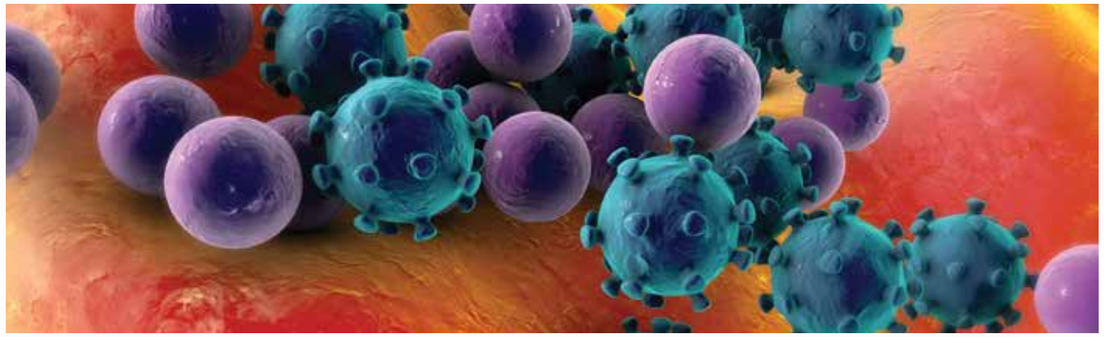
Using aspirin will show us that prevention is better than cure, even for sepsis

Dr. Damon Eisen has had a long career studying infectious diseases and their subsequent treatment. Here he discusses the use of aspirin as a preventative measure against the life-threatening condition known as sepsis.



To start with, could you explain how your research background led to your current interest in sepsis?

As an Infectious Diseases clinician researcher one learns to question ways in which common conditions are treated. This in turn prompts one to think if serious infections could be managed better. This certainly applies to sepsis.

Early recognition and treatment improves the outcome in sepsis. Antibiotics, intravenous fluids and other supportive therapies have greatly improved the survival in severe sepsis, but a stubborn 20-30 per cent of cases don't respond. It is these patients who need more treatments, ones that target the body's response to severe infection, to survive this incredibly common problem.

Where does your research fit in the larger context of current biomedical research?

The so-called 'adjuvant' treatments for sepsis trialled to date have all been used when the reaction to severe infection has commenced. An alternative approach is to prevent the consequences of severe infection.

Low-dose aspirin is an important candidate treatment, working not to prevent infection but by reducing the scale and intensity of the immune response. Aspirin can inhibit well-characterised biochemical pathways that mediate sepsis. Doses as low as 81 mg a day can prevent further development of sepsis-related inflammation, as well as ameliorating established sepsis. These factors may be the key to effective use of aspirin to prevent sepsis. It can be taken daily, safely and cheaply, and it may dampen the life threatening immune response sepsis.

What have you discovered so far from the aspirin/sepsis trials?

Our main results will derive from the ANTISEPSIS study, a ground-breaking, world-first trial using a population of patients in a randomised controlled trial, where we will be able to analyse the association between aspirin usage and mortality from sepsis in participants aged over seventy.

We have completed a large observational cohort study of patients with systemic inflammatory response (SIRS), many of whom had proven sepsis. A total of 7945 ICU admissions to St Vincent's Hospital, Melbourne, between April 2000 and November 2009 were examined. The probability of in-hospital death for individuals who were identified as having SIRS or sepsis was analysed according to whether they were on aspirin prior to their admission. Among the 5523 elderly patients with a first episode of SIRS, 2082 were administered aspirin in a 24-hour period around the time of SIRS recognition. Propensity analysis showed reduced numbers of deaths with 10.9 per cent mortality for aspirin users and 17.2 per cent mortality in non-users. There were potential side effects though as aspirin administration was associated with increased risk of renal injury (6.2 per cent versus 2.9 per cent). In the 970 patients with proven sepsis, aspirin administration was associated with a lower mortality (27.4 per cent vs. 42.2 per cent). This observational study provides vital preliminary data to make us examine this important issue prospectively.

How do you envision aspirin-based treatments will be implemented?

If low dose aspirin is shown to be effective and safe in preventing death due to sepsis it would be quickly adopted for that indication and it would be incorporated into the preventive medicine strategy for adults at risk of severe infection.

What long-term consequences might this study have on future work and potentially on healthcare, society and policy?

Results from our large observational study of critically ill patients indicated the number needed to treat with low dose aspirin to prevent death due to SIRS or sepsis was between seven and 16. If these data are indicative of the benefit found in the ANTISEPSIS study, then the use of low dose aspirin would be enormously cost-effective. Even if lower reductions in mortality are shown then this along with evidence that low-dose aspirin affects sepsis-related outcomes in the elderly in terms of hospitalisation, ICU admission, and death would be a new and significant scientific advance.

Poisoned blood and willow trees

James Cook University is the second-oldest in Queensland, Australia. They are well known for their pioneering work on both tropical infectious diseases and marine biology.

We sometimes forget in this age of advanced medicine, of transplanted organs and genetic therapies, that many truly classic medicines still hold their own. One of these, steeped in history, is aspirin, a compound found almost ubiquitously in medicine cabinets around the world. Its origins lie in treatments made from willow-tree bark, known since ancient Greek times to be effective against pain and fever, and stretch to the discovery in the 1800s of acetylsalicylic acid. Acetylsalicylic acid, better known by the brand name Aspirin, is converted within the body to the active ingredient in willow bark, itself known as salicylic acid.

The long history of aspirin has led to a number of studies into the impressively wide range of diseases it can treat. This naturally includes pain, fever, and inflammation, but also the prevention of heart attacks, strokes and blood clots – indeed aspirin is often given after heart attacks to reduce the likelihood of another attack. Further support for aspirin's broad efficacy comes from clinical researchers such as Dr. Damon Eisen, who has spent several years examining its effect on one of medicines long-known foes: sepsis.

The Egyptians wrote about Sepsis, it was commented on by Hippocrates yet it's still a scourge of modern medicine. But what is it? It begins with an infection, in the lungs, the urinary tract, the brain, etc. This sets off an inflammatory response in the body, essentially a far larger version of the swollen redness seen around infected wounds. Internally, inflammatory signalling proteins are circulating throughout the body, inducing this response wherever they go. Within the bloodstream, platelets begin to activate, thickening the blood and leading to hypertension and clotting. Lymphocytes and neutrophils, two types of white blood cell, begin to commit cellular suicide, harming both the protective immune response and extending the inflammatory process. The combination of these effects can lead to organ failure and death. Despite numerous studies focusing on this age-old disease, it remains deadly: approximately one third of patients with severe sepsis will die, killing over 200,000 each in year in the USA alone.

Why is sepsis so deadly? In part this is due to the speed at which it progresses, often too fast for doctors to adequately react. As such the ideal treatment would be a preventative one, helping to support the body prior to disease onset. As such, much attention has been focused on the expected results of a trial known as ASPREE. ASPREE, short for Aspirin to Prevent Events in the Elderly, is a preventative trial, essentially asking whether daily low doses of aspirin can reduce the risk of elderly patients developing heart problems or dementia, amongst others. This trial is running in both Australia and the US, and will follow 19,000 patients over the course of 5 years.

Aspirin is able to treat a wide range of diseases, should sepsis be added to the list?

A subset of this trial is known as ANTISEPSIS, an acronym derived with some flexibility from AspiriN To Inhibit SEPSIS. This sub-trial will use patient questionnaires alongside hospital records to determine whether this low-dose aspirin treatment acts to prevent sepsis. Dr. Damon Eisen, head of this sub-study, is confident that it will, as he has previously conducted smaller observational studies in the field. One of these studies, examining almost 8000 hospital admissions, indicated that patients who had been given aspirin in the 24 hours surrounding the diagnosis of sepsis had a significantly lower chance of dying – 10.9% vs 17.2%. Given the number who die of sepsis each year, reduction could lead to thousands of lives saved.

How is aspirin able to achieve this? Aspirin is a member of the non-steroidal anti-inflammatory drugs, (NSAIDs for short), other members of which include ibuprofen and naproxen. It acts by targeting and modifying enzymes known as cyclooxygenases, which produce a number of signalling molecules and hormones to control various aspects of physiology. Once aspirin has altered cyclooxygenase-2 the enzyme increases production of the molecule known, simply enough, as 15R-hydroxyeicosatetraenoic acid. This then passes through a complex chain of enzymes, cell types, and signalling processes, with the end effect of reducing inflammatory signals and encouraging the clean-up of dead cells. In doing this it reduces the length and severity of sepsis attacks, allowing doctors time to support the patient and bring them through with better chances.

If the ANTISEPSIS trial shows the expected benefits from aspirin treatment, it will have a major effect on current sepsis treatment plans. Aspirin is both cheap (as Dr. Eisen comments, "use of low dose aspirin would be enormously cost-effective") and has a long history of safe use, as such it would be readily integrated into preventative regimens. Time will tell if Aspirin will have yet another chapter added to its long and storied history.

Researcher Profile

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After completing his Bachelor of Medicine/ Surgery, Dr. Eisen focused his talents on the blood-borne parasite Plasmodium falciparum, research that led to a Doctorate of Medicine from the world-class University of Melbourne. This focus on infectious disease led to studies on the effect of aspirin on S. aureus infections, and in turn to work on the ASPREE study. Dr Eisen has had a fruitful career, having his name on 80 publications, including three in the Lancet and one in Nature Medicine, and undoubtedly many more to come.

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Victorian Infectious Diseases Service and the Intensive Care Unit, Royal Melbourne Hospital
Townsville Hospital and Health Service

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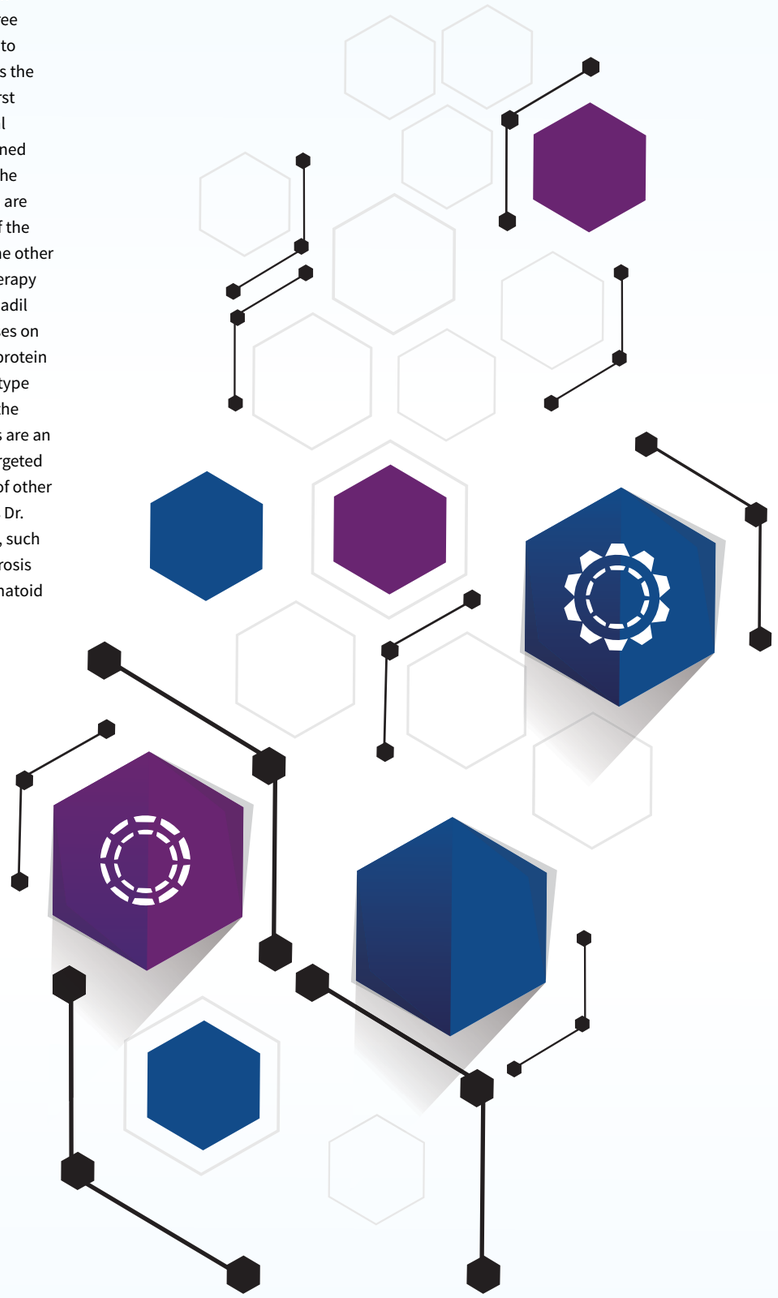
TARGETED THERAPIES

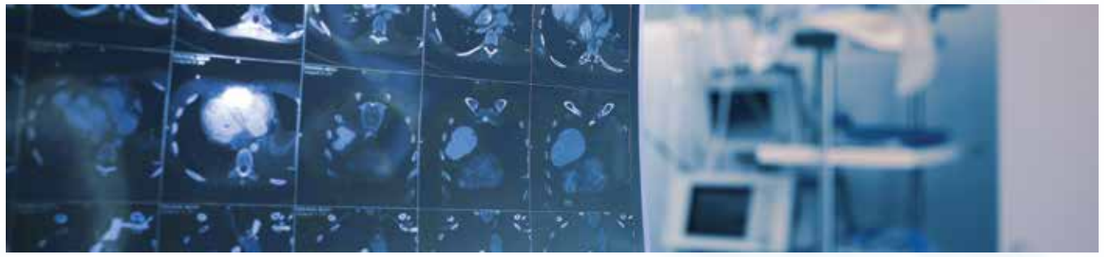
Today, cancer is one of the leading causes of death in developed countries. Importantly, mortality rates from cancer have been increasing primarily due to the aging population and lifestyle changes. The last world report from 2014 showed 14.1 million new cases and 8.2 million deaths globally. There are numerous risk factors already recognized for cancer, the most significant factor being old age. Factors that could explain the association between aging and cancer are attributed to immunosenecense - errors accumulated in DNA over a lifetime and age-related changes in the endocrine system. Even with age being the most common risk factor, younger individuals are also diagnosed with cancer. Diverse environmental factors have been proposed to contribute, such as tobacco, diet and obesity, infections, radiation, stress, lack of physical activity and environmental pollutants. This makes cancer a very complex multifactorial disease where it is very difficult to prove the cause of the disease in a certain individual. Because of its multifactorial nature, the treatment of cancer is often a combination of different therapies, such as radiation, chemotherapy, surgery, as well as targeted therapies. Targeted therapy is also known as molecularly targeted therapy, as it is based on the use of molecular medicine focusing on blocking >

the growth of cancer cells by interfering with specific molecules needed for carcinogenesis and tumour growth. Although the combination of traditional chemotherapy and targeted therapies have been widely used, targeted therapies alone are expected to be more effective because they are less harmful to normal cells. It is different from traditional chemotherapy that simply interferes with all rapidly diving cells. This section comprises of three articles that will discuss targeted therapies applied to specific types of cancers, and one article that details the use of targeted therapy in another disease. In the first article, Dr. Kazuhito Suzuki comments on his clinical trial results with thymidine kinase and other combined targeted agents (bortezomib and lenalidomide) in the treatment of multiple myeloma. Targeted therapies are also examples of immunotherapy, which consists of the use of immunomodulators for therapeutic goals. The other three articles of this section will discuss immunotherapy for the treatment of different types of cancers. Dr. Nadil Ahmed and Dr. Stephen Gottschalk's research focuses on unique immunological manipulations of the HER2 protein in osteosarcoma. Dr. Nakamoto examined another type of targeted therapy, which evolves around making the immune system believe that hepatocarcinoma cells are an infection, thus acting against the tumour. Lastly, targeted therapies have also been applied to the treatment of other diseases. The last article of this section will address Dr. Shunsuke Mori's work with immune system players, such as cytokines, particularly IL-6, IL-1, and tumour necrosis factor (TNF) in the treatment of patients with rheumatoid arthritis.



A Winning Combo

With a long term goal of abolishing multiple myeloma, Dr. Kazuhito Suzuki of the Jikei University School of Medicine, Japan, spends his time trying to identify the best treatments. Here, we ask him about the challenges in his work.



Could you discuss your background? What brought you into the field of oncology, and multiple myeloma research in particular?

I was greatly interested in multiple myeloma because I was entering the field just as we had significant progress in treatments for multiple myeloma. Bortezomib became available to patients with relapsed and refractory multiple myeloma in Japan just as I became a clinical resident, which really changed the treatment strategies available.

What was your most significant research finding so far?

My research findings from this trial indicate that the CLD regimen was very effective and tolerable in relapsed multiple myeloma patients. Moreover, if biochemical relapse occurred after entering the lenalidomide maintenance therapy, the CLD regimen became active again.

My findings from previous research demonstrated that thymidine kinase activity was related to overall survival in patients with newly diagnosed diffuse large B cell lymphoma or mature T cell lymphomas. I would like to extend this work by analysing the correlation between thymidine kinase activity and survival in patients with newly diagnosed multiple myeloma.

Have you experienced any obstacles doing your research and how did you overcome them?

Recruiting patients into this study was difficult because the inclusion criteria were very strict. Recently, we extended our candidate pool by decreasing the necessary haemoglobin concentration from 8.0 g/dL to 7.0 g/dL in our inclusion criteria. In general, I consider inclusion criteria to be one of the most important issues surrounding success in a clinical trial.

Your work focuses on therapy combinations, and their synergistic effects. Why did you choose to examine CLD + lenalidomide maintenance therapy? Do you intend to examine other therapeutic combinations in future?

In my opinion, bortezomib and lenalidomide are standard antimyeloma agents and thus should be optimally administered just before myeloma cells can develop resistance to anti-cancer compounds. Why did we focus on the combinations that we studied? Firstly, a goal of first-line and second-line chemotherapy is, naturally, to achieve as optimal a response as possible. A lot of studies had demonstrated that better response was achieved with this combination, including longer progression-free-survival and overall survival rates. Secondly, triplet chemotherapy regimens tend to show a superior response in patients when compared with doublet regimens.

Bortezomib had only been approved as an initial, first-line, chemotherapy in Japan. So, we selected bortezomib-containing regimens as our initial chemotherapy, and lenalidomide-containing regimens as the second line of treatment. Several studies had previously demonstrated that combinations of cyclophosphamide and lenalidomide were active and well tolerated.

One combination, comprising the Bortezomib, lenalidomide and dexamethasone (BLD) regimen, is considered a good option for salvage chemotherapy. However, for several reasons, I chose not to select the BLD regimen for salvage chemotherapy. First, a Phase 1/2 trial of BLD demonstrated that the maximum tolerated dose for lenalidomide was 15mg, whereas I consider that optimal salvage therapy dose of lenalidomide is 25mg daily for 21 days, as seen in the MM009/ 010 trial. Second, the BLD regimen is extremely expensive. Third, long-term lenalidomide maintenance therapy is controversial and in need of further research. Fourth, we omit dexamethasone wherever we can because long-term administration of dexamethasone can lead to increased infection rates. We wanted to demonstrate that dexamethasone is not necessary for maintenance in patients showing a good response to the CLD regimen. Because of this, I selected CLD regimens from the wide range of lenalidomide-containing options to identify whether it provided better results for patients.

How would you like to extend your research once this clinical trial is finished?

If I had a lot of research funding, I would like to perform a clinical trial in which anti-myeloma agents are selected in accordance with clonal evolution by next generation sequencing methods – personalised medicine for myeloma. For example, proteasome inhibitors are selected for XBP-1 over-expressing myeloma cells, or IMiDs is selected to treat cereblon-overexpressing myeloma cells. However, for the present, we would like to collaborate with other specialist teams in order to relieve patients' symptoms. Our next project involves performing plasma exchange combined with bortezomib containing chemotherapy to treat cast nephropathy in myeloma patients.

What would be your 'dream' research project?

My dream is to cure multiple myeloma. However, current treatments utilising proteasome inhibitors and IMiDs alone are not enough to cure all patients. Immunological approaches, such as CART therapy, are necessary stages even after patients demonstrate a good response to chemotherapy such as proteasome inhibitor and IMiDs.

Multiple Medicines For Multiple Myeloma

One of the major private medical schools in Japan, the Jikei University School of Medicine is involved in a number of facets of medical research. Work in the Clinical Oncology group has led to significant improvements in cancer treatment, such as that of multiple myeloma.

Multiple myeloma is the second-most common blood-derived cancer in the USA, caused by uncontrolled growth of the antibody-producing white blood cells (known as plasma cells) within the bone marrow. This results in a variety of symptoms: normal blood cell production is affected, leading to anaemia; persistent bone pain occurs as they are broken down by activated cells; while the large numbers of antibodies secreted by the overgrown plasma cells accumulate in the kidneys, damaging them and eventually leading to kidney failure. The disease remains incurable to this day, over half the patients who are diagnosed with multiple myeloma will be dead within five years.

Treatment of multiple myeloma involves therapies which can reduce the number of growing plasma cells, thus slowing the progression of symptoms. High-dose chemotherapy using compounds such as lenalidomide and bortezomib is used to prevent cell replication, sometimes assisted by targeted radiation therapy to wipe out the most persistent infestations. Patients can also be injected with stem cells (their own, or those from a healthy donor) which will then recolonise the now-empty bone marrow – going on to produce healthy cells.

However, while this process can lead to symptom relief and better quality of life, it is in many ways just a mirage, a temporary stay of execution. Plasma cell growth can be halted for a time, via drugs, radiation, or stem cell therapy. However, much like the monster from a bad horror film, the cancer will almost always come back. Even worse, the selective pressure exerted by the anti-cancer drugs will ensure that, much as bacteria develop antibiotic resistance, the surviving tumour cells will be resistant to the drugs which could previously kill it.

SILVER BULLETS

One approach to minimise this zombie-like resurrection of the cancer is known as maintenance therapy. First, the initial chemotherapy treatment knocks the number of plasma cells down to a reasonable level, some patients then have transplants of healthy stem cells to help them recover. Next, the patients begin to take a continuous, but lower drug dosage – the aim of this being to hold back any attempted revival by the remaining tumour cells. On the whole this approach works well, significantly increasing the time patients have in remission.

Unfortunately, there are some major downsides, particularly in that anti-cancer therapies are, by their very nature, toxic. As such patients on maintenance therapy often have numerous side effects which lower patient quality-of-life and can sometimes require cessation of the therapy. The question then becomes: how can we develop maintenance therapies such that they are effective, but with minimal long-term side effects?

Multiple myeloma remains incurable to this day, but research into optimal treatments is helping to extend patient survival time.

HUNTING MONSTERS

To answer this question we need to turn to clinical trials, in which researchers and physicians work together to identify the best therapies for patients in need of help. Two such trials are currently being supervised by Dr. Kazuhito Suzuki of the Jikei University School of Medicine, Japan. In particular, his group is studying the knockout effect of a triple-treatment known as CLD, followed by the lighter maintainance treatment of lenalidomide alone in relapsed or refractory myeloma patients

What have they discovered from this work? First, the CLD triple-treatment is very effective at knocking down the number of plasma cells, even in cases where patients have relapsed after prior treatment. Second, the lenalidomide maintenance treatment appears to be both effective (with a significant increase in patient survival after 4 years) and very well tolerated. As would be expected, some patients needed slight reductions in their long-term dosage, but on the whole the lenalidomide therapy caused minimal side-effects.

This is valuable information for scientists and physicians, as they need to balance both efficacy, side-effects, and cost. A common alternative to CLD, known as bortezomib, was at one stage rejected by the British National Health Service for its excessive cost (indeed, Dr. Suzuki chose to test CLD in part due to the expense of bortezomib). Having shown that the triple-treatment and maintenance program was both effective and well tolerated, this study allows doctors to confidently choose the less expensive treatment option, free of worries that it is somehow inferior.

Dr. Suzuki's dream is to find a cure for multiple myeloma. While this moment lies far in the future, getting to that point requires us to constantly improve our knowledge of treating the disease. Studies such as this are thus invaluable steps in our inexorable journey towards an ultimate remedy.

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Dr. Suzuki graduated from the Jikei University School of Medicine in 2006, after which he began his successful research career within the Clinical Oncology & Haematology Department. For 2 years he studied treatment strategies and research mind in the Cancer Institute Hospital of the Japanese Foundation of Cancer Research. His research focuses on drugs targeting multiple myeloma, in particular the ways in which these can synergise for greater efficacy.

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Taking Immunotherapy To The Next Level

Cancer researchers Dr. Nabil Ahmed and Dr. Stephen Gottschalk are tweaking immunologic technology to attack osteosarcoma, a cancer that can hide from the usual immune cell strategies.

A NASTY CANCER

Osteosarcoma, although not a very common malignancy, is the most common human primary bone cancer, accounting for over 900 new cases in the U.S. and about 200 cases in the U.K. each year. Osteosarcoma characteristically attacks the long bones, like the legs and arms. More importantly and more distressing, osteosarcoma is most prevalent in children and younger adults less than 25 years of age. Current therapeutic strategies usually consist of radical surgery, often an amputation, and systemic chemotherapy. However, despite improvements in outcome for patients with local disease, say, isolated in the leg bone, the survival rates for patients with metastatic or recurrent disease remain very poor. Given these facts, scientists like Nabil Ahmed and Stephen Gottschalk have been researching novel biologically-based and targeted therapies using clever immunologic manipulations aimed at a protein called HER2.

Some cancer types, most famously breast cancer, can express HER2, or human epidermal growth factor receptor 2. HER2 is so named because it has a similar structure to normal protein human epidermal growth factor receptor—HER1. HER2 production, like the production of HER1 and other forms of epidermal growth factor receptor, can promote cell growth and division when it is functioning normally. Indeed, these proteins are crucial for the in utero development of the heart and major vessels. On the other hand, when HER2 is overexpressed after birth, cell growth accelerates beyond its normal limits and in some types of cancer it can promote rapid cell growth and proliferation—hence tumour formation. It is well known that HER2-positive breast cancer tumours are more invasive and aggressive than HER2-negative tumours. HER2-positive tumours are associated with increased disease recurrence and poor prognosis. Over-expression of HER2 is also associated with more aggressive types of ovarian, stomach, and uterine cancers. Thus, HER2 has been targeted in recent years with a variety of immunological cancer drugs.

For example, trastuzumab (sold under the brand names Herclon and Herceptin) is a monoclonal antibody that binds to and interferes with the HER2 receptor. This antibody, combined with other chemotherapeutic drugs, has been show to improve overall survival and disease-free survival relative to treatment regimens involving treatment with placebo or chemotherapy alone. But to work, trastuzumab must bind to the receptor—HER2—on the outside of the tumour cell, preventing it from being activated and "doing its thing." HER2-negative tumours and tumours with low expression of HER2 are not vulnerable to trastuzumab and similar monoclonal antibody drugs. This is precisely the problem with osteosarcoma. Although HER2 is expressed in over 60% of primary osteosarcoma tumours and correlates with a poor outcome, its expression on osteosarcoma cells is low, rendering HER2-specific antibodies like trastuzumab less effective. This low profile condition in osteosarcoma is what Ahmed, Gottschalk and their colleagues are attempting to circumvent.

CHIMERISM: RESURRECTING A MYTHOLOGICAL HYBRID

In order to kill a cancer cell, the tumour cell must exhibit certain antigens on its surface that can be processed to activate cytotoxic T cells, those T lymphocytes that attack cancer cells and cells that are infected by viruses. However, cancers like osteosarcoma do not attract and allow activation of cytotoxic T cells, thus evading the usual anti-tumour immune process. This is where Ahmed and Gottschalk plan to use a hybrid—a chimera—to immunologically attack osteosarcoma cells. The hybrid in question is a chimeric antigen receptor, or CAR.

The chimera, a creature from Greek mythology, is an animal with a body made up of parts of more than one animal. Homer's Iliad describes "a thing of immortal make, not human, lion-fronted and snake behind, a goat in the middle, and snorting out the breath of the terrible flame of bright fire." Ahmed and Gottschalk's chimera, on the other hand, is not so fearful, but it is just as fantastic. The CAR that Ahmed and Gottschalk wish to use to treat osteosarcoma is a molecule that seems to be made of different parts—one side consisting of the extracellular receptor domain of a monoclonal antibody and the other a so-called transmembrane and cytoplasmic signalling domain derived from molecules that are known to activate T cells so they become cytotoxic. In other words, one side of the CAR is an antibody to bind to HER2 receptors in the tumour—the head of a snake, able to bite and hold on to the tumour—and the other side is a T cell activating molecule—the head of a lion, able to roar out an invitation

calling T cells to join the party. Even more, T cells isolated from the patient's own blood cells can be grafted with this hybrid molecule, making it that much more of a hybrid, but insuring the T cell is close by when the antibody portion attaches to the tumour cell. This is the HER2-CAR T cell, designed to attach to the HER2 receptor and kill cancer cells. This amazing immunologic chimera, then, is not something for Bellerophon to kill—it is the basis for Ahmed and Gottschalk's strategy to defeat osteosarcoma.

STARTING SMALL: HOW ABOUT MICE?

Talking about CAR T cell therapy—unleashing the chimera on cancers—is fine and good, but what about data? Ahmed and Gottschalk are not simply putting down ideas on paper; they have experience—first in mice. Ahmed, Gottschalk and their colleagues developed a lung metastatic model of osteosarcoma in mice, with very promising results. They demonstrated in mice that various subtypes of osteosarcoma express HER2 at levels that, in contrast to HER2 antibodies like trastuzumab, can be efficiently targeted using CAR T cells decorated with the HER2 targeting CAR. Indeed, the investigators found that there is a higher level of HER2 expression on osteosarcoma stem cells makes them particularly susceptible to HER2-CAR T cells despite being generally resistant to chemotherapy typically used in osteosarcoma regimens. In the mouse model, HER2-CAR T cells exhibited a potent anti-tumour effect even against bulky, established lung and other vascularized osteosarcoma metastases. They published their data in the journal Molecular Therapy in 2009, and numerous researchers since then have referred to it. Ahmed and Gottschalk used this data as the justification for launching a first-in-man trial using autologous HER2-CAR T cells for advanced osteosarcoma.

THE CHIMERA IS SAFE AND EFFECTIVE

With the efficacy of HER2-CAR T cells in mice established, Ahmed and Gottschalk turned to human disease. They and their colleagues took patients with recurrent or refractory HER2-positive sarcoma received escalating doses of HER2-CAR T cells to assess effectiveness and safety of the approach. After all, the chimera may attack the cancer—which is a good thing—but it may also attack the patient's own cells. In a study named ***HEROS,*** that is HER2 for OSteosarcoma, that was later published in the May 2015 issues of the Journal of Clinical Oncology, they took 19 patients with sarcomas—16 of them with osteosarcoma—and treated them with HER2-CAR T cells. These HER2-CAR T cells were derived from T cells removed from the patients' own blood. These hybridized cells were tested from each patient and all were found to be toxic for HER2-positive target cells in vitro. After treatment, they detected HER2-CAR T cells for at least 6 weeks in the majority of patients, especially those who received higher doses of HER2-CAR T cells.

The development of effective targeted therapies for osteosarcoma is hampered by the limited number or low expression of known tumour antigens, such as HER2. HER2, one of few antigens expressed by osteosarcoma, is quite attractive for immunotherapy since it is expressed in > 60% of primary osteosarcoma and correlates with a poor outcome. But although HER2 is a validated target for breast cancer immunotherapy, its expression on osteosarcoma cells is low, rendering HER2-antibody therapy less effective. That's where the HER2-CAR T cell comes in.

The results of the study were exciting. Two of the patients actually had their tumours biopsied six and 12 weeks after the infusion and HER2-CAR T cells were detected at tumour sites. So the chimeric cells actually made it to the target. Of 17 evaluable patients in the study, four had stable disease for 12 weeks to 14 months after the treatment. Three of these patients had their tumours removed. In one, the tumour had actually undergone necrosis by almost 90%. So the chimeric cells actually did the work they were designed to do.

The median overall survival of all 19 infused patients was 10.3 months, with a range of 5.1 to 29.1 months, which was good considering these were patients who had recurrent or refractory disease and most had bulky disease at the time of HER2 CAR T cell administration. With this study, Ahmed, Gottschalk and their associates had the first evaluation of the safety and efficacy of HER2-CAR T cells in patients with sarcomas. They showed that the cells can be given systemically without evident toxicity to the patient, setting the stage for studies that combine HER2-CAR T cells with other immunomodulatory approaches to enhance their expansion and persistence and combat difficult cases of cancer, including osteosarcoma.

WHERE DO THEY GO FROM HERE?

Based on their encouraging work with HER2-CAR T cells and sarcoma, both in mice and in humans, Ahmed, Gottschalk and their associates now want to expand their scope to develop an effective immunotherapeutic approach to progressive osteosarcoma. In a study design that has already been approved by the National Institutes of Health's Recombinant DNA Advisory Committee (RAC), the Institutional Biosafety Committee of Baylor College of Medicine and by Baylor's Institutional Review Board, the researchers have initiated a study that builds on their exciting findings, entitled Administration of HER2 CAR Expressing T cells to Subjects with Advanced Osteosarcoma: NCT00902044. This study, named ***HEROS 2.0,*** Ahmed and Gottschalk will give autologous HER2 CAR T cells after creating a "lymphocyte space" that will be permissible of expansion the administered T cells and their persistence past the 6 week period seen with The HEROS Study.

With this study, Ahmed, Gottschalk and their colleagues plan to enrol patients with progressive HER2-positive osteosarcoma who have failed standard-of-care therapy. These patients will be treated with HER2-CAR T cell infusion, provided that: their T cell lines can be properly modified to work; they have a life expectancy of more than six weeks; they an appropriate physical activity scale; and acceptable organ function for the therapy. In the first human study, Ahmed and Gottschalk assessed about eight to twelve patients per year for enrolment, with approximately 80% of them being found to have HER2-positive tumours. With the same attrition factor as the first, smaller study—which was about 25%—recruitment and infusion of 12 to 15 patients would be feasible over a projected two-year study period.

One important question the researchers need to answer in The HEROS 2.0 Study is the question of lymphodepletion. When T cell therapies are used in patients—such as here, with HER2-CAR T cells being administered to fight the cancer—the native T cells in the patient's body tend to inhibit the proliferation of the administered therapeutic T cells via a normal T cell regulatory mechanism mediated by regulatory T cells, or Treg cells. Obviously, a person's T cells cannot replicate out of control—that would be tantamount to a case of leukaemia. The body has mechanisms in place to prevent such proliferation. However, if you plan to inject someone with T cells designed to kill the cancer cells, you would like them to multiply and spread throughout the body, essentially on a search-and-destroy mission. The more soldiers in your army, the better you are at searching out the enemy. One strategy to achieve this, is to treat patients before HER2-CAR T cell infusion with the chemotherapy medications cyclophosphamide (CY) and fludarabine (FLU), drugs that kill of many of the native T cells in the body. That way, the HER2-CAR T cells are free to multiply unregulated—at least until the normal T cells can regenerate themselves--and search out cancer cells to attack. The researchers will use different regimens of CY, FLU, or both to see if lymphodepletion is truly necessary during HER2-CAR T cell therapy, and if so, how much depletion is necessary.

WHAT DO THEY HOPE TO FIND?

In the end, though, Doctors Ahmed, Gottschalk and their co-workers plan The HEROS 2.0 Study to answer three questions:

First, is it safe to use autologous HER2-CAR T cells in patients with advanced HER2-positive osteosarcoma after lymphodepleting chemotherapy? In their original study, HEROS, the researchers found no ill effects. Here, however, when you deplete the patient's own T cells so you can infuse them with HER2-CAR T cells, will that cause any problems?

Second, is lymphodepletion necessary—and how much of it—in using HER2-CAR T cell therapy against osteosarcoma? In their smaller study, they did not use lymphodepletion, but some authorities feel that it gives a better result, even though it might expose the patient to obviously toxic chemotherapy. Using several levels of CY/FLU lymphodepletion therapy should help answer that question.

Finally, how long will the HER2-CAR T cells persist in the patients' bodies, will they multiply and expand their numbers and will they attack the tumour cells successfully? In HEROS, things looked promising, but they only have data on a small number of patients. Here is the major aim of the study. After all, advanced osteosarcoma is the target and HER2-CAR T cells are the bullets. Ahmed and Gottschalk need this study to show how close they can hit the bull's eye.

Researcher Profile

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Dr. Nabil Ahmed received his MD and an MPH in Paediatrics from Cairo University Kasr El Aini School of Medicine, Cairo, Egypt He did residency training in Paediatrics at the Lincoln Hospital, Weill-Cornell Medical College, in New York City and Children's Hospital of New Jersey, Mount Sinai School of Medicine, in Newark, New Jersey. He also received extensive fellowship training in Paediatric Haematology and Oncology from the National Cancer Institute at Cairo University, as well as the Texas Children's Cancer Centre at Texas Children's Hospital, Baylor College of Medicine in Houston, Texas. Dr. Nabil Ahmed's research interests include novel immunologic and gene therapies for the treatment of solid malignancies. His preclinical efforts focus on the development of novel cell based therapeutics for these malignancies, with an emphasis on targeting the tumour profile, in a manner that is inclusive of the heterogenous tumor antigenic landscape as well as elements of the tumour microenvironment.

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Dr. Stephen Gottschalk received his MD from the Georg August University in Göttingen, Germany. He did residency training in Paediatrics and Fellowship training in Paediatric Haematology and Oncology at Baylor College of Medicine in Houston, Texas. He is currently the Director of the Translational and Basic Research Division of Texas Children's Cancer Centre in Houston, Texas. Dr. Gottschalk's research interests include T-cell derived therapies for treatment of a variety of cancers. He is actively conducting Phase I clinical studies with antigen-specific T cells for patients with cancer. In the laboratory, he is overseeing a group of MD and PhD researchers working on several peer-reviewed preclinical research projects focused on overcoming current limitations of T-cell therapies using genetic approaches.

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Fighting a cold, fighting a tumour

The Faculty of Medical Sciences at University of Fukui has been providing medical training and research for many years, using their close links to the most advanced treatment hospitals in Fukui Prefecture. Amongst the life-saving treatments being developed are those for hepatocellular carcinoma.

Hepatocellular carcinoma is one of the most common forms of liver cancer, and usually occurs as a secondary disease following a viral infection (by Hepatitis B or Hepatitis C) or in patients with cirrhosis, the liver failure often caused by alcohol abuse. As with many tissue tumours, hepatocellular carcinoma (HCC for short) tends to grow in discrete lumps, known as nodules.

The survival rate for HCC is low, with death usually following 3-6 years after diagnosis. Treatment involves the typical approaches: cutting the tumour out (only an option in 15% of patients), destroying it with locoregional therapies with modalities such as radiofrequency ablation and transarterial chemoembolization (effective, but can only target the known tumours) and liver transplants, (which is very dependent on how far the cancer has spread). Unfortunately, the majority of treatments do not prevent recurrence, in which the cancer revives itself from a few stray cells which were not destroyed and goes on to spread once more throughout the body.

What is needed then is a way to fight cancer at the cellular level, rather than with the broad cut of a surgeon's scalpel. Conveniently, the body has a number of mechanisms designed to fight and kill cell-sized objects, mostly as a way to defend itself from bacterial or viral invasion. Is it possible to recruit our own immune system in the fight against cancer? Going from recent clinical trial results, the answer is yes.

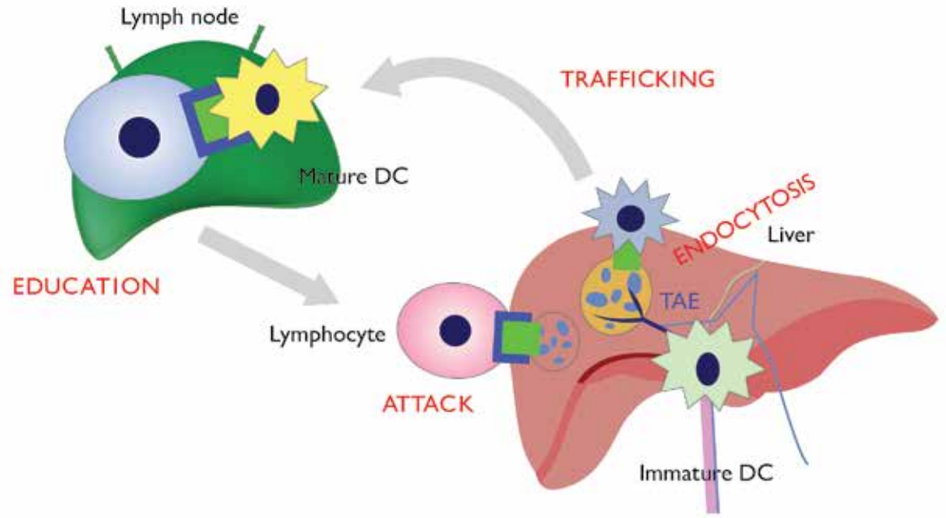
IMMUNOLOGICAL TEENAGERS

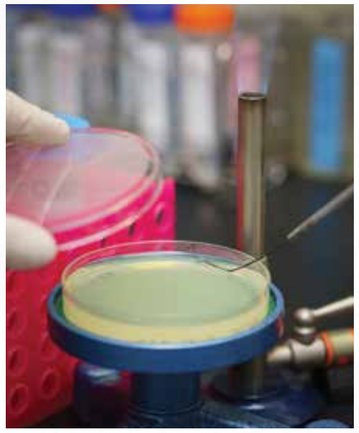
Understanding how this works requires us to delve slightly into the complex world of immunology, a world made up of a number of cell types, all communicating and working together to fight infections. It begins with immature dendritic cells (DCs for short), which spend their youth essentially as most teenagers do, sitting around and eating. Located in areas at risk of infection, such as the skin, lungs, and intestines, they constantly sample the environment by taking up and digesting proteins and other particles from their immediate surrounds. When the immature DC detects evidence of an infection in the vicinity, as determined by the presence of several pathogen-specific chemicals, it begins the process of maturation.

Again like teenagers, maturation involves moving out of home, although in this case the DC merely moves down the hall to the lymph nodes. Here they halt, displaying on the cell surface a snapshot of all the proteins which were in the vicinity when they first began to activate. In effect, this acts as a signature of the infection, a signature which is then displayed to the other immune system cells which reside within the lymph nodes. Through a series of complex interactions, the mature DCs are thus able to activate T-cells, the workhorses of the immune system, inducing them to reproduce and hunt down the invading pathogen. Importantly, because the DC has 'warned' them of the identity of the invader, the targeting of the immune response is extremely specific.

Can our immune system fight cancer? According to recent studies, yes it can.

So how does this process apply to cancer? Through some clever scientific methods it is possible to trick the immune system into believing that the cancer cells are an infection, thus mobilising it into action against the tumour itself. The first stage of this process involves taking a sample of the patient's own blood and purifying a specific type of cell known as a Peripheral Blood Mononuclear Cell (a PBMC for short). It is important that the cells be purified from the patient's own blood, cells from other people would be recognised as 'foreign' by the immune system, and would be rapidly targeted for destruction. PBMCs are able to differentiate, or mature, into a variety of different cell types following chemical treatment, and so these cells are converted in the lab to immature dendritic cells.





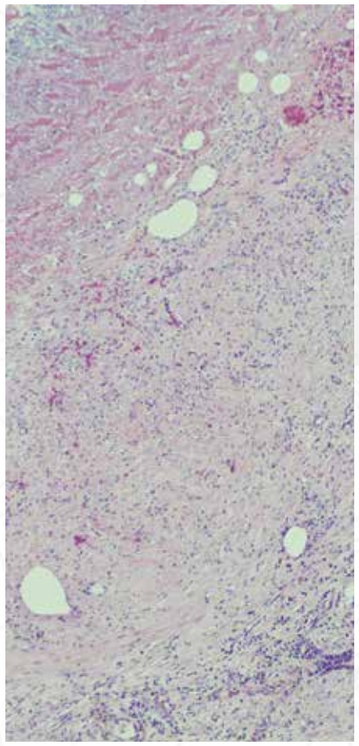
One of the patient's HCC tumour nodules is then injected with a chemical which begins to induce apoptosis, the programmed cell death which leads to the cells essentially committing suicide. This process leaves large amounts of tumour-cell proteins in and amongst the dying cells. At this point the immature dendritic cells are injected into the HCC tumour nodule, along with a chemical which encourages them to begin the process of maturation. The effect of this is that the immature DCs save a 'snapshot' of the tumour environment, including a number of proteins or molecules which are highly indicative of tumour cells. As mentioned earlier, these DCs will then migrate to the lymph nodes and begin to prime the immune system to target and eliminate any cell which expresses these tumour antigens, effectively clearing out the cancer as though it were a normal infection.

EFFECTIVE ADOLESCENTS

This approach is undoubtedly clever, but how effective is it? Work by researchers such as Professor Yasunari Nakamoto at the University of Fukui, Japan, is beginning to show that it leads to significant improvements in patient survival time. Professor Nakamoto graduated from Kanazawa University in 1989, just as Hepatitis C was being discovered. He spent several years as a postdoc at the prestigious The Scripps Research Institute (TSRI) in California, studying both Hepatitis C and its link to hepatocellular carcinoma. Upon returning to Japan, he commented that "since there were so many patients suffering from HCC in Japan, I decided to use my immunology experience for the treatment of liver cancers. Dendritic cells (DC) were an interesting therapeutic tool for many malignancies, potentially including HCC."

From this initial decision Professor Nakamoto made strong progress, in 2007 he reported on an early stage clinical trial treating 10 patients with hepatocellular carcinoma. PMBCs were taken from each patient, and the process of maturation into DCs took approximately 7 days. On the seventh day the patients were treated by trans-catheter hepatic arterial embolization (TAE), a method for inducing apoptosis in the tumour nodes. This was followed by injection of the freshly prepared, patient-derived DCs into the tumour. Professor Nakamoto's group was able to show that once injected, these cells were able to remain within the region for up to 17 days, collecting a molecular signature of the tumour itself. They then induced the other parts of the patients' immune system to target the tumour cells, developing an immune response against the typical 'tumour antigens' such as Her-2/neu (the target of the ground-breaking anti-cancer drug sold as Herceptin). Most importantly, there were no side effects associated with the treatment, a very important factor when treating already-sick patients.

The group followed up on this success with a new clinical trial, treating thirteen patients



with hepatocellular carcinoma. This trial used a slightly varied method, the cells were extracted and converted into DCs, but this time were treated with a chemical known as OK432 during the conversion process. OK432 is a dried powder made from killed bacteria, Streptococcus pyrogenes to be precise. Because this powder is recognisably 'bacterial', and thus dangerous, it essentially encourages the dendritic cells to 'wake up' and begin the process of maturation, along with several other helpful downstream effects. This new therapeutic method proved to be very successful, while the earlier trial had shown some promise, the use of OK432 led to a significant increase in patient survival. Over half of the patients given standard treatment saw the cancer return within a year (recurrence being a significant problem for hepatocellular carcinoma), however eleven of the 13 DC-treated patients remained cancer-free after one year. The role of the dendritic cells was supported by the observation that the DC-treated patients had higher levels of both immune system signalling proteins and tumour-killing activity. Most importantly, the treatment seemed to be well tolerated by all of the patients. Taken together, this suggests that the DC therapeutic approach is able to significantly extend the life of patients with hepatocellular carcinoma.

ADULTHOOD

These results are very promising, and Professor Nakamoto is confident about the future of these treatments. As he comments, "DC is a very safe and widely applicable therapeutic tool for malignancies. I believe it will become a standard treatment option in combination with current therapies such as trans-catheter hepatic arterial embolization (TAE), in particular for advanced stage cancers. I think the DC therapies can be combined with various treatments, including immune checkpoint inhibitors, for advanced malignancies." Time will tell, naturally, but these initial clinical trials have shown sufficient success that further studies are almost certain to be approved.

Where would he like to take his research after this work is complete? He would like to examine the variability of cancer, as he commented: "During our research into the development of cancer immunotherapies, I noticed that the therapeutic effects were largely different from patient to patient, they were not predictable prior to the treatments." His hope is to extend his work into the field of personalised medicine, essentially using our knowledge of the body at a patient-by-patient level in order to provide the most effective treatment possible.

This process is already quite 'personal', as DC-treatment involves the use of our own cells to fight cancer, but could be improved even further as we determine which tumour cell factors are most important in affecting the chance of the treatment working. Work to this effect is already being conducted, as the Nakamoto group and collaborators attempt to identify a number of chemicals which can substitute for OK432. Although OK432 remains the most effective DC-activator, it is possible that others may be more useful in certain subsets of hepatocellular carcinoma tumours. This type of research is, unfortunately, significantly more complicated to perform, and indeed Professor Nakamoto comments that "there are many questions to be answered in order to solve these problems". Answering these questions and solving these problems, however, remains a goal for many dedicated scientists, with the challenges of cancer heterogeneity and personalised medicine being very hot fields of research indeed. As Professor Nakamoto brings the expertise of his team into play, this field is bound to become hotter still.

Researcher Profile

Professor Yasunari Nakamoto, M.D., Ph.D.
Head of Gastroenterology and Neurology
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After completing his MD at Kanazawa University of Japan, Professor Nakamoto began his long career in research by completing his PhD in Medical Science. After a stint at the prestigious The Scripps Research Institute (TSRI) in California, USA, he returned to Japan and continued climbing the scientific ladder. He is currently the Head of the Second Department of Internal Medicine at the University of Fukui, Japan, with a wide range of research interests.

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UNIVERSITY OF FUKUI

A novel treatment for rheumatoid arthritis with renal insufficiency

Dr. Shunsuke Mori has been involved in developing novel treatments for rheumatoid arthritis. In this interview Dr. Mori elaborates on the current state-of-the-art in the field and recent contributions from the Mori laboratory.

To begin with, please explain how your current interest in rheumatoid arthritis was built upon your research background.

In the 1980s, a number of cytokines were identified as key mediators between the innate immune system and the adaptive immunity. My first achievement as a researcher was molecular cloning of a cDNA encoding rabbit interleukin-1ß (IL-1ß) and its receptor antagonist, a natural inhibitor of IL-1. I played a part in the IL-1 project during the early period of cytokine research in terms of the pathogenesis of inflammatory arthritis, which, in turn, aroused my interest in rheumatoid arthritis (RA) pathogenesis and treatment.

Where does your research fit in the larger context of current biomedical research?

A number of novel biological antirheumatic drugs have been approved for use in RA treatment. These drugs are targeted at specific molecules and pathways in the immune system, and their emergence has changed the course of RA and improved patient and social outcomes. My studies were carried out with the intention of providing information to rheumatologists who make therapeutic decisions.

Have you encountered any challenges in your research? How did you overcome them?

To draw definite conclusions, I planned multicentre studies with several cooperating institutions. A great deal of time and manpower were required to collect appropriate data regarding clinical variables and outcomes in target patient populations. I formulated research questions and conducted pilot studies, and then I coordinated the active participation and collaboration of candidate institutions.

Has your work led to any major discoveries to date, and if so, what could that mean for the patients and doctors alike?

My aim is to provide a safe, effective treatment option for individual RA patients who are suffering from complications and comorbidities. There are currently no reliable, evidence-based guidelines for deciding whether biological antirheumatic drugs can be safely introduced

and continued in RA patients who have RA-associated pulmonary diseases, chronic infectious diseases, renal insufficiency, etc. and for whom biological drugs are the first treatment option.

Tocilizumab (TCZ), a humanized monoclonal antibody against the IL-6 receptor, is effective and generally well-tolerated when used for RA patients. Although there was extra concern that TCZ may have an influence on protective antibody response to vaccinations, we showed that RA patients receiving TCZ can benefit from influenza and pneumococcal polysaccharide vaccinations. In fact, our findings are being used in the most recent European League Against Rheumatism (EULAR) recommendations for the management of RA.

Renal insufficiency is not uncommon in RA patients. It is recommended that most biological agents be used in combination with methotrexate (MTX), but MTX is contraindicated in patients with severe renal insufficiency. We showed that TCZ therapy has good efficacy parameters regardless of whether or not MTX is also used. In addition, TCZ effectively improved erythropoietin-resistant anaemia.

Do the pathways and proteins you are studying play any other roles in the organism?

Cytokines play a key role in communicating with the immune system and host tissue cells, possess pleiotropic functions across a broad range of tissues, and mediate a variety of normal physiologic and pathophysiological processes. They are also involved in growth, differentiation, drug resistance, and death of tumour cells. Inhibition of IL-1, IL-6, and tumor necrosis factor (TNF) through the use of biological drugs is effective in the treatment of autoimmune, inflammatory diseases.

Do you envision any type of TCZ-based treatments and how they might be implemented?

While monotherapy with anti-TNFα inhibitors has considerable efficacy in the treatment of RA, a combined use with MTX is highly recommended for additional benefits and better outcomes. When that is not possible, TCZ can be a promising antirheumatic drug. I am now also planning a clinical study addressing the risks and benefits of TCZ therapy for polymyalgia rheumatica.

What long-term consequences might your studies have on future work and potentially on healthcare, society and policy?

I believe that our findings, namely the utility of TCZ therapy in RA patients with renal insufficiency and the availability of vaccinations in RA patients receiving TCZ therapy will provide important information for rheumatologists and may contribute to the development of guidelines and recommendations for biological antirheumatic therapies and vaccination policies.

Are you planning to expand your research interests? Where might you focus your attention and why?

I am planning to identify markers for predicting therapeutic responses to different types of biological antirheumatic drugs, which will be useful when selecting the most appropriate biological drugs for individual patients.

Towards developing new guidelines for treatment of rheumatoid arthritis

Rheumatoid arthritis, a commonly encountered autoimmune, inflammatory disease, has become prevalent amongst the elderly. Current research in the field, such as Dr. Shunsuke Mori's work focuses on treating rheumatoid arthritis when accompanied by complications such as pneumonia and renal insufficiency.

RHEUMATOID ARTHRITIS

The immune system comprises two major arms, namely innate and adaptive immunity. Inflammation forms a part of the innate immune system, namely a first-line defence of a living tissue to disease or infectious agents. Acute inflammatory responses are essential for the removal of external insults from the body and the repair of tissue. However, genetic defects in the regulatory mechanism of the immune system and a repeated or persistent environmental stress may produce chronic inflammation and autoimmunity as a result of defective self-tolerance, which can cause severe tissue damage and dysfunction.

I believe that our findings will provide important information for rheumatologists and may contribute to the development of guidelines and recommendations for biological antirheumatic therapies and vaccination policies.

Rheumatoid arthritis (RA) is one of the most common autoimmune, inflammatory diseases, in which abnormal systemic immune responses cause chronically inflammatory and persistently destructive processes in the synovium and other organs. It typically results in warm, swollen, and painful joints, which may result in low red blood cells, inflammation around the lungs, and inflammation around the heart. RA affects between 0.5 and 1 per cent of adults in the developed world with 5 to 50 per 100,000 people newly developing the condition each year. There is currently no cure for RA, but treatments can improve symptoms and slow the progress of the disease.

TREATMENT AND COMPLICATIONS

While the cause of RA is not clear, it is believed to involve an approximately equal contribution of genetic and environmental factors. The underlying mechanism involves the body's immune system attacking the joints. The goals of treatment are to minimize symptoms such as pain and swelling, to prevent bone deformity, and to maintain day-to-day functioning. This can often be achieved using two main classes of medications: analgesics and disease-modifying antirheumatic drugs (DMARDs).

Antirheumatic drugs act by altering the underlying process that causes RA, and using these drugs has changed how RA is managed, while improving patient outcomes. There are two main classes of antirheumatic drugs. Biological antirheumatic drugs were designed to target specific molecules and pathways in the immune system. Synthetic antirheumatic drugs include methotrexate (MTX), which is the most important and useful DMARD. MTX is used as the first-line antirheumatic drug, but in most cases, combinations of synthetic antirheumatic drugs with biological drugs are required to achieve low disease activity or remission.

Dr. Shunsuke Mori's research efforts attempt to bridge a gap in the knowledge regarding the best use of currently available antirheumatic drugs for optimal care. RA patients often have complications or comorbidities. While guidelines and recommendations regarding biological therapy for RA patients have been developed worldwide by the scientific societies and healthcare authorities, doctors worldwide are not provided with reliable guidelines for deciding whether biological antirheumatic drugs can be used as treatment for RA patients who have RA-associated pulmonary diseases, chronic infectious diseases, renal insufficiency, etc. and for whom biological is the first treatment option. As an example, renal insufficiency is not uncommon in RA patients. It is recommended that most biological agents be used in combination with MTX, which is, however, contraindicated in patients with severe renal insufficiency.

TOCILIZUMAB: A STEP FORWARD

Vaccinations for influenza and pneumococcal pneumonia are strongly recommended for RA patients, because these patients are at an increased risk of contracting infectious diseases. Tocilizumab (TCZ), a humanized monoclonal antibody against the interleukin-6 (IL-6) receptor, is effective and well tolerated by RA patients. IL-6 was considered an essential factor for antibody production, and therefore there was special concern that TCZ may have an influence on the protective antibody response to vaccinations. Dr. Mori's work showed that TCZ did not impair immunogenicity of either of these vaccines in RA patients and that no severe adverse effects were observed. Thus RA patients receiving TCZ can benefit from influenza and pneumococcal polysaccharide vaccinations. Dr. Mori's findings are currently being used in the most recent European League Against Rheumatism (EULAR) recommendations for management of RA.

Cytokines, a class of small proteins involved in cell signalling, are known to mediate communicating between the innate immune system and the adaptive immunity. Cytokines, particularly IL-6, IL-1, and tumour necrosis factor (TNF), are essential in driving healthy inflammatory processes. Dr. Mori's team performed a multicentre study which indicated that TCZ therapy is efficient in RA patients with renal insufficiency whether or not MTX is also used. In addition, TCZ effectively improved erythropoietin-resistant anaemia in this patient population. Anaemia associated with chronic inflammatory disease is common and typically mild in patients with normal functioning kidneys. In patients with renal insufficiency, however, there is primary deficiency in erythropoietin production, which leads to more severe anaemia. Dr. Mori's findings showed that blocking of the IL-6 activity by TCZ is beneficial to RA patients with renal insufficiency and anaemia, and TCZ may be a first-choice biological agent for such patients.

While monotherapy with anti-TNFα inhibitors has considerable efficacy in the treatment of RA, a combined use with MTX is highly recommended for improved outcomes. In the cases where MTX is contraindicated or not tolerated, such as in patients with renal insufficiency or in those with hypersensitivity to MTX, anti-TNFα inhibitors have to be used without MTX, often with no disease remission. Dr. Mori found that in contrast, TCZ, even in a single use, can be a promising antirheumatic drug for these patient groups. Therefore, Dr. Mori is currently evaluating the long-term safety and efficacy of TCZ monotherapy for RA patients with severe and end-stage renal insufficiency.

HEALTHCARE APPLICATIONS

The drugs used to treat RA have individual advantages and disadvantages. Some patients fail antirheumatic treatment with a particular biological drug (primary lack of efficacy), but may show a satisfactory response to another drug. In some cases, patients respond well to a biological drug at its first use, but its efficacy can disappear during the treatment (secondary loss of efficacy). Nevertheless, no guidelines have been established that outline how to use these drugs for optimal patient care. Although it is currently difficult to maintain clinical remission without continued biological antirheumatic therapy, a window of opportunity may exist in some RA patients for inducing long-term remission. Dr. Mori's future research aims are to identify clinical and genetic markers for predicting therapeutic responses to different types of biological drugs, which will prove useful when considering tailor-made therapies suitable for each individual patient.

The utility of TCZ therapy in RA patients with renal insufficiency and the availability of vaccinations in those receiving TCZ, will, in Dr. Mori's opinion, provide a treatment option for rheumatologists in real-life medical practice and will also contribute to the development of vaccination policies. Since 2014, Dr. Mori has participated in a project aimed at producing a set of guidelines for management of polymyalgia rheumatica (PMR), a different type of inflammatory rheumatic disease that affects elderly individuals, and the results of this project have been published, with the title "2015 recommendations for management of PMR: a EULAR and American College of Rheumatology collaborative initiative." Dr. Mori is additionally planning a clinical study addressing the risks and benefits of TCZ therapy for PMR.

Researcher Profile

Shunsuke Mori
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Dr. Shunsuke Mori is the Director of Clinical Research at the Department of Rheumatology, Clinical Research Center for Rheumatic Diseases, NHO Kumamoto Saishunsou National Hospital. Currently Dr. Mori is interested in studying rheumatoid arthritis and has made significant advances in the treatment of rheumatoid arthritis with renal insufficiency.

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TECHNOLOGICAL INNOVATIONS FOR HEALTHCARE

Since World War II, we have experienced tremendous technological advances worldwide. Technological advance is characterized by a combination of different processes, such as research, development, demonstration, and finally, deployment. Production of innovation requires the involvement of laboratories, firms, as well as financing organizations to get to the final product. Even government initiatives and policies can shape the innovation processes.

Products of innovation can be applied to widespread use. We can think of numerous products of innovation that were able to promote huge changes in life style, such as the internet, smartphones, digital cameras, GPS, and many others. In healthcare, technological innovations can be applied to disease prevention, diagnosis, treatment, and rehabilitation. In this section, we will see four studies that are using advanced technologies with the aim of improving healthcare. These studies each describe one of the four steps (research, development, demonstration, and deployment) of the innovation process. Dr. Hiroshi Ueda's work aims to improve diagnosis, with his study focusing on the development of Quenchbody, a fluorescent dye-labeled antibody that can detect antigen for fast diagnosis of illnesses. Dr. Scott Tenenbaum focuses on the use of structural RNA technology for the development of novel therapeutics by aiming to destroy virus-infected and cancer cells using RNA technology. The use of these highly advanced technologies are extremely important for innovation and improvement of healthcare. However, low- and middle-income countries have limited access to novel technological innovations, as they cannot afford certain health technologies. Dr. Ed Schmidt has been working on a cost-effective tool called "The Embryo Cradle", which will advance genetic and epigenetic modifications in embryonic stem cells, as well as manipulation of embryos. It is evaluated that this tool can be 90% cheaper than the previous commercially available tool in the market. Lastly, this section will show examples of technological innovations applied to the treatment of chronic diseases that are more advanced along the innovation process. Novaremed, a pharmaceutical company, will discuss novel drugs that have been successfully tested for the treatment of neuropathic pain.

PROCESS OF TECHNOLOGICAL INNOVATION

DEVELOPMENT




Involvement of:

- laboratories
- firms
- financing organizations
- government initiatives and policies

DEMONSTRATION



Applications in healthcare:

- prevention
- diagnosis
- treatment
- rehabilitation

Improving Medical Care with Engineering

Professor Hiroshi Ueda is a researcher at the Ueda Laboratory of the Tokyo Institute of Technology. Here he describes his work developing Quenchbody, a fluorescent dye-labeled antibody that can detect antigen for fast diagnosis of illnesses.

Ueda Laboratory members

To start, when did you develop an interest in biochemistry?

Since my father was a medical doctor, I have been interested in the working mechanisms of the human body since my high school days. However, I chose my major in chemical engineering, since I was also interested in engineering. I also happened to meet a very interesting professor, who had been a radical anti-pollution activist but had just started a career teaching biotechnology. Since then, I focused my studies on the biochemistry and biotechnology of antibodies. This interest was a gift from the professor, and is my life's work. Fortunately, antibodies continue to be one of most useful proteins in this area, in view of diagnostics and therapeutics.

Can you tell me more about the Ueda Laboratory? How and why was it established?

I was given my "Protein Engineering" lab as a PI about 12 years ago in the School of Engineering, University of Tokyo. There I worked for 10 years, and moved to my current affiliation (Chemical Resources Laboratory, Tokyo Tech) 2 years ago. One of my key interests has been the effect of antigens on the stability of antibody fragments. In many antibodies, when the antigen binds to it, the antibody structure is stabilized through the bridging effect of its two (heavy and light) chains. Based on this phenomenon, I found that antigen concentration can be measured by measuring the interaction strength of the two chains. This principle also constitutes the background of our new biosensor "Quenchbody (Q-body)".

Do you or your research team have any plans to help disseminate Quenchbody into hospitals or other medical facilities?

Not yet at this moment. However, since we are developing Q-bodies to several diagnostic markers in collaboration with a diagnostic company, it is probable that our team will go for this direction in near future.

Who funded the Quenchbody research? Did you have any difficulties gaining funding?

The Q-body was first discovered during the academia-industrial collaborative "Metropolis Area" project funded by the Japanese government about 6 years ago. At that time, we were collaborating with a small venture company "Proteinexpress" that has a specialty in site-specific chemical labelling technology using a cell-free protein synthesis system. However, the problem was that the company was not making enough profit due to small sales of reagents. After the end of the project, a most difficult time followed. What happened was that the key collaborator (Dr. Ryoji Abe) in the company could not get a salary for months, and a researcher in my lab (Dr. Hiroyuki Ohashi) had to quit the lab due to the same reason. Afterwards, the company found an angel who decided to buy the Q-body division of the company, and the R&D of Q-body has been funded and continued by the angel (Ushio Inc.) to date.

Do you have any key collaborators? What has been their involvement?

As already written, Ryoji and Hiroyuki are now working with Ushio Inc., and we are collaborating mutually in order to develop practical Q-body assays and associated technologies for the sake of our bright future. I think the keys to success exist both in science (biochemistry and chemical biology) and in the market. It is quite an exciting challenge. Hopefully, we can find more collaborators for the latter aspect.

Do you have plans for future projects? If so, can you say what they are?

The future of Q-body is a secret for now :-). However, I am also interested in bioluminescent protein-based sensors. The concept is to divide the enzyme (luciferase) reaction into two by introducing mutations, not by the dissection of polypeptide, to make two mutants. Based on this novel concept, I already published two papers for protein-protein interaction detection in *Analytical Chemistry.* I hope this concept is utilized to regulate the activity of many other enzymes.

Is there anything else you would like to add?

About 16 years ago, I was a visiting researcher in Cambridge, MRC-LMB, UK, for two years under the guidance of Dr Sir Gregory Winter (One of the founders of human antibody technology and also Master of Trinity College now). Although my work at that time was not very successful, to be honest, I really enjoyed the academic and also innovative atmosphere of the lab and the Cambridge area. This attitude constitutes the backbone of my research going forward.

Quenching Patient Doubts

The Ueda Laboratory at the Tokyo Institute of Technology, with funding from Ushio Inc., recently developed a new, award-winning biosensor that uses fluorescence quenching to quickly detect antigens. Its speed has the potential to greatly reduce stress for patients who may need a diagnosis.

WHAT IS FLUORESCENCE QUENCHING?

Scientists have discovered certain dyes that fluoresce, or glow, when they undergo certain chemical reactions or while traveling through the human body. In medical science, they are often attached to specific molecules so that the molecules' progress through the body can be tracked. When the molecules get physically close to a certain substance, or substances, the fluorescence is "quenched", or weakened, and does not glow as brightly as it previously did. With the knowledge of where and when quenching occurred, scientists and doctors can determine what is happening inside a patient's body. Fluorescence quenching happens one of two ways; it is caused either by energy transfer or electron transfer. In the case of energy transfer, two dyes of different colours are tracked as they travel through the body. Where the two dyes interact or get very close to each other, the fluorescence reduces and it is possible to directly see their interaction or measure the distance between the dyes all the way down to the nanometre level. Electron transfer, on the other hand, is only possible with molecules that give electrons to other molecules or receive electrons from other molecules through chemical reactions. Electron transfer is measured at the angstrom level. Angstroms are typically used to express the size of single atoms or molecules, making these measurements very small and precise.

Professor Hiroshi Ueda's lab at the Tokyo Institute of Technology recently used this concept to develop an extremely useful new biosensor device, named Quenchbody, which is a fluorescent dye-labeled antibody, detecting antigen. Medical tests that look for the presence of certain molecules are called "immunoassays", and they are often used to detect antigens. Antibody-based diagnoses are advantageous to medical professionals because they can be used to prepare personalized medicines that target specific, disease-related molecules. Antibodies interact strongly with other molecules, and the reactions are very specific, making antibodies good for the disease they are attempting to fight. Currently, however, the immunoassay process takes hours to complete and receive results. Not only are there multiple steps to complete the tests, but they also require a long incubation period to wait for certain chemical reactions to complete. Waiting for a diagnosis can be an incredibly stressful life event, so a quick test result goes a long way to improving the health care experience for patients. It also frees doctors from the laborious, time-consuming testing process, allowing them to spend more time with patients or on more difficult projects.

In many antibodies, when the antigen binds to it, the antibody structure is stabilized through the bridging effect of its two (heavy and light) chains. Based on this phenomenon, I found that antigen concentration can be measured by measuring the interaction strength of the two chains. This principle also constitutes the background of our new biosensor "Quenchbody".

QUENCHBODY

Quenchbody operates using electron transfer fluorescence quenching. It allows doctors or researchers to detect the binding of an antigens to an antibody fragment. Antigens are substances that cause the body to produce antibodies. In this case, tracking which antibody the antigen becomes attached to indicates a certain disease. Quenchbody can detect both small and large molecules like proteins, so the biosensor will be able to diagnose a very wide range of illnesses. In theory, it can detect most of the commonly diagnosed diseases today, so its potential application and impact on the medical system is tremendous. What used to take hours now only takes seconds or minutes, so diagnoses can be made desk-side or bedside, while the patient is with their doctor.

One or more dyes are chemically attached very near to the antigen binding site of an antibody. The dyes are placed on specific spots depending on what antigens need to be tracked. If no antigen attaches to the site, the dye stays where it was chemically placed, and will be quenched by tryptophan residues in the antibody. If an antigen binds, the dye gets "kicked off" the antibody molecule, because the antigen stabilizes the antibody and displaces the dye molecules. When the dye is expelled, it emits fluorescence. Tracking the activity of the dye allows medical professionals to quickly determine what antigens are attaching to which antibodies. Different colours can be used for different antibodies to distinguish between them, for example, or a single Quenchbody test can determine the presence or absence of a single antigen.

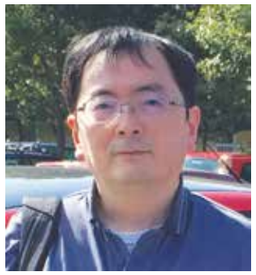
In addition to diagnosing disease, collaborator and project funder Ushio Inc. has used Quenchbody to detect and identify many drugs including narcotics and toxins. The potential applications of Quenchbody are enormous, and may improve the day-to-day operations of many different medical industries.

AWARD-WINNING TECHNOLOGY

Quenchbody has already won the Student Award at the annual meeting held by the Society of Chemical Engineers. The prize was awarded to Takuya Kawamura, a student of the Ueda Laboratory. The project specifically focused on the detection of a cancer-related membrane protein, called claudin, by Quenchbody. The project was originally developed by Professor Masuo Kondoh, a collaborator of Professor Ueda's who specializes in claudin research. The two met at the Antibody Engineering meeting held in San Francisco three years ago, while Professor Ueda's student was working unsuccessfully on a very similar project. He subsequently decided to change his research plan to focus on claudin. With help from Professor Kondoh's group, who provided antibody clones and generously shared previous work with Kawamura. Now, doctors helping patients who may have cancer can simply stain the claudin antigen on the cells just be applying Quenchbody to the supernatant (the supernatant is the liquid that cells are suspended in during culture and growth). Quenchbody claudin tracking does not require any washing steps, a process that significantly slowed previous immunoassay diagnosis tests. In addition, Ushio Inc. and Ueda Lab members recently received an Innovative Technology Award from a Japanese press Fuji Sankei Business I (July, 2015). In the ceremony they received the prize from Hisako, a Japanese Imperial family member who recently addressed the IOC, delivering Tokyo's opening speech of the presentation.

It is not surprising that Quenchbody has already begun receiving awards. Its ability to relieve patient stress is significant. Even in the event of a positive diagnosis, patients can begin treatment as opposed to waiting in suspense. More significant, however, may be its impact on medical professionals. Expertise is a resource that should not go to waste, and time-consuming testing procedures take up valuable time, especially when there is a better option available. In addition, doctors and patients can be together for the few seconds or minutes it takes for the results to appear, allowing for more communication between the two and relieving patient stress even further. The Ueda Laboratory and their collaborators quickly and competently reacted to the needs of the medical community, and their continuing contributions will play an important part in shaping the future of medicine.

Researcher Profile



Professor Hiroshi Ueda
Chemical Resources Laboratory
Tokyo Institute of Technology

Professor Hiroshi Ueda studied at The University of Tokyo, where he earned his PhD in chemical engineering. He has received both the Research Award and Promotion Award from the Society of Chemical Engineers in Japan, the Hot Article Awards from the Japan Society for Analytical Chemistry, and the Nagase Science and Technology Promotion Award. He currently teaches at the Tokyo Institute of Technology where he specializes in protein engineering of antibody and other molecules with the aim of biosensing and diagnostics. Professor Ueda is also an expert in immunoassay, bioluminescence, and combinatorial technology.

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Structurally Interacting RNA: a Novel Therapeutic and Diagnostic Tool

Dr. Scott A. Tenenbaum is a molecular biologist who is interested in studying RNA biology, particularly RNA-protein interactions inside the cell. His work has led to the development of the structurally interacting RNA technology, which has important implication for the development of novel therapeutics.

To begin, what is your research background, and how did it lead you to your current position?

My PhD was in Microbiology and Immunology from Tulane University Medical Center, under the guidance of Dr. Robert Garry. In the process, we developed several new diagnostics and identified important interactions between RNA and proteins, which are major biological molecules of cells. We particularly identified some members of what we call 'RNA-binding proteins' (RBPs), which play an important role in regulation of gene activity in the cell. To further understand the function of these proteins, I joined the lab of Dr. Jack Keene at Duke Medical Center as a Post-doctoral fellow. Dr. Keene is one the leading RBPs researchers in the world, and has made great discoveries in this area. Together we developed and adopted new genomic-based techniques to identify and understand networks of RNA-protein interaction. This led to a whole new understanding of gene regulation and the birth of a new area of research.

I joined the University at Albany-SUNY as a faculty member in their new Genomic Cancer Center in 2003 and then moved to the College of Nanoscale Science and Engineering at SUNY-Polytechnic Institute in 2009 as an the Acting Vice President for Research and the Associate Director of Nanobioscience Constellation. My lab has continued to study RBP-RNA interactions, which have led us to the discovery of the structurally interacting RNA or what we have termed 'sxRNA'.

Whatis RNA and which biological functions is it responsible for?

RNA is one of the central building blocks of life. It plays a major role in protein synthesis going from DNA to RNA to Protein, the process known as the 'Central Dogma' of biology and genetics. Messenger RNA has traditionally been viewed as a molecule that simply transfers the coding information from the DNA to the final protein product. However, during the past several decades research has revealed many other cellular functions of RNA, including enhancement of enzymatic reactions, providing a structural scaffold for protein, RNA and DNA interactions, and regulation of protein synthesis from DNA (gene expression). Most importantly for our research, mRNA appears to not only contain the code for the 'what' but also has the regulatory code for the 'when, where and how much' a protein should be made.

As described in your publications, the sxRNA technology allows the control of expression of an ectopically delivered, RNA-based gene within a targeted cell type or tissue. What are the scientific principles underlying the sxRNA technology and how was it developed?

We first developed informatic and molecular tools that allowed us to identify naturally occurring sxRNA interactions, which appear to have the potential to interact and modify mRNA and are involved the regulation of generating the protein products produced from these mRNAs. This led us to commandeer the concept and try to design costum sxRNA molecules that can target chosen gene systems in order to switch their activity ON or OFF. There is a tremendous amount of informatic design that goes into the process followed by empirical testing of the switches for activity.

Are you planning to extend your research on sxRNA further? What might be the scope of the next step?

At the moment we are focused on three areas, anti-virals, biomanufacturing and advances in stem cell derived differentiated tissue. In the future we plan on developing sxRNAs for other disease targets and for gene therapy, as well as continuing to study the biology behind the natural occurrence of interacting RNA in the cell, and how they may be playing a role in regulation of gene expression.

Throughout your research on sxRNA, have you collaborated with other research groups or institutes? If so, what has been the role of your collaborators?

We have major collaborations with multiple groups including SUNY-Stony Brook and SUNY-Upstate Medical for development of anti-viral drugs, and The Neural Stem Cell Institute to develop sxRNA based stem cell therapeutics and The Austrian Centre of Industrial Biotechnology to improve biomanufacturing methods. Our approach has been to introduce the basic sxRNA technology and then provide the informatics and technical support to develop customized sxRNAs for their specific needs and applications.

Principles and Applications of Structurally Interacting RNA

Structurally interacting RNA (sxRNA) is a novel technology developed by Dr. Tenenbaum to control the expression of specific genes in the cell. Here we discuss the scientific principles of the technology and its potential applications for the development of therapeutics and biological research tools.

THE STORY OF RNA AND GENE EXPRESSION

RNA is a biological molecule that plays a central role in the generation of protein from DNA in the cell, a process that is essential for maintaining all forms of life. The genetic information required for protein synthesis is stored within the DNA in units known as the genes. During a process known as 'transcription', a particular gene is replicated into a homologous copy of RNA, called the messenger RNA (mRNA). Specific cellular machines called ribosomes then use the mRNA code to build the corresponding protein in a process known as 'translation.' The overall process of generating the protein product of a certain gene is referred to as 'gene expression'.

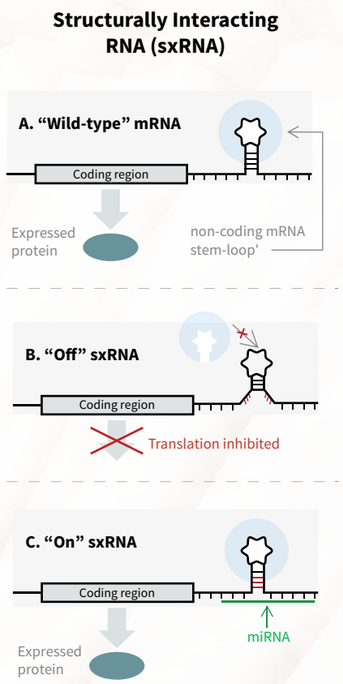
Eukaryotic cells carry a wide array of genes that encode a multitude of proteins of various structural and dynamic functions. However, throughout the developmental stages of the cell, and depending on the changes in the ambient physiological conditions, the expression of these genes might be either abundantly needed or undesired. Thus, the cell adopts a variety of regulatory mechanisms to control gene expression.

Besides being a core element in protein synthesis, RNA also plays a role in the regulation of gene expression. This regulatory role is mainly implemented by a group of RNAs collectively known as non-coding RNA, which modulate gene expression by controlling the process of mRNA translation. Examples of non-coding RNA include specific regions of the transcribed mRNA itself that do not undergo translation or what is referred to as the untranslated region (UTR), as well as other independently expressed small RNA molecules known as microRNA (miRNA). Much of the non-coding UTRs in mRNA is typically folded in numerous distinct conformational structures, that frequently include 'stem-loops', which can form in a manner that controls the availability of the adjacent coding-mRNA for translation into protein. This process is controlled through the effect of a large family of regulatory proteins known as RNA binding proteins (RBPs), which upon binding to the various stem-loop structures can trigger the induction or repression of translation as well as regulate the stability of the mRNA. Recently, it was discovered that the regulatory capacity of the stem-loop mRNA on gene expression can be further complemented by the interactions of miRNA, a second class of regulator non-coding RNA. miRNA molecules can bind to mRNA at specific regions, concealing or revealing binding sites for RBPs that can then modulate the translation process.

The structurally interacting RNA technology provides a basis for a novel class of therapeutics able to destroy virus-infected cells and cancer cells.

STRUCTURALLY INTERACTING RNA AS A MODULATOR OF GENE EXPRESSION

Using modern molecular technologies, Dr. Tenenbaum and his group have identified naturally occurring miRNA-mRNA interactions, which in contrast to the conventional linear binding of miRNA with mRNA, resulted in structural modification of stem-loops in the UTRs of mRNA targets. Inspired by the phenomenon, they have designed artificial RNA molecules that mimic miRNA in their capacity to modify mRNA structures, with the aim of developing a tool to control gene expression. These RNA molecules, which were designated "structurally interacting RNA (sxRNA), are designed so an embedded regulatory stem-loop is modified so its structural configuration can be regulated so that the binding of cellular RBPs that control mRNA translation is enabled or prevented. This mechanism provides a discrete 'ON-OFF' switch for the expression of the sxRNA target gene. Furthermore, the sxRNA technology can be engineered to take advantage of the cell's naturally occurring miRNA, which can be exploited to restore the optimal stem-loop structure and RBP binding, making an interchange of the ON-OFF gene expression statuses possible. This feature can be realized by designing sxRNA molecules that are engineered to structurally target a miRNA of interest. In such cases, after the integration of sxRNA with the regulatable stem-loop, a subsequent miRNA-sxRNA interaction will restore the optimum stem-loop structure, permitting the RBPs binding. Unlike the existing technologies that turn off gene expression, the switchable gene expression control system offered by the sxRNA technology provides a means to turn ON gene expression, providing a solution for the transient control of the ectopic production of any protein product with a variety of amplitudes.



Panel A. An RNA binding protein (RBP) binds to its naturally occurring "wild-type" stem-loop target sequence, which results in increased translation of an upstream gene by as much as an order of magnitude.
Panel B. We rationally design an sxRNA "switch" with a mutated stem-loop that prevents RBP binding and inhibits translation of the gene.
Panel C. In the presence of a targeted miRNA "trigger", a trans-acting, 3-way structure forms that stabilizes the stem-loop target structure. The RBP binds to the new sxRNA stem-loop target sequence resulting in increased translation of the upstream gene.

By coupling the translational regulation of gene expression with the unique microRNA signature patterns in the various cell types, the sxRNA technology can enable cell-specific expression of a desired protein or reporter gene for a diseased cells (e.g. virus-infected and cancerous cells) or even a specific developmental cell stage. The ability to target and control the expression of a desired gene at the level of mRNA rather than DNA opens up many new possibilities for vaccine, molecular and gene therapeutic tools. 'We have received very encouraging market feedback for this platform technology, which we believe has immediate commercial opportunities in important diagnostic and therapeutic areas', says Tenenbaum.

POTENTIAL APPLICATIONS OF SXRNA IN BIOMEDICINE

sxRNA technology represents a innovative way to control gene expression, with promising therapeutic applications such as the development of anti-viral therapies. 'Our sxRNA technology has advanced to the point that we are optimistic about targeting a number of significant and unique RNA molecules in infected cells in the hope of producing a new class of anti-viral therapeutics', says Tenenbaum. The technology has been recently proposed as a novel sxRNA-based antiviral drug against the Epstein-Barr virus. This virus is known to cause infectious mononucleosis (the kissing disease), and is commonly associated with the occurrence of various types of cancers such as lymphoma, nasopharyngeal and gastric carcinoma, and breast cancer. Currently, there are no specific antiviral agents to treat latent EBV infection, despite decades of research. However, Dr. Tenenbaum and his fellows have proposed promising sxRNA-based approaches to develop anti-EBV drugs. One of these approaches is based on the fact that some viruses such as EBV make their own miRNA in the cells. Here, these viral miRNA are exploited to interact with the sxRNA component of ectopically delivered custom-designed mRNA triggering its translation. In this case, the sxRNA-mRNA is designed to encode for a lethal protein that kills the cell when generated. Since the production of the lethal protein is limited to the presence of specific viral miRNA, the approach selectively kills infected cells, while sparing the healthy ones. A second approach to develop sxRNA-based anti-EBV drugs involves the 'anti-microRNA' technology, in which sxRNA is used to modify a stem-loop structure with which a specific cell-derived miRNA interacts to trigger the generation of an endogenous lethal protein. EBV typically use this miRNA to prevent the death of the host cells, which can be reversed by the anti-miRNA sxRNA, leading to death of the infected cells.

sxRNA is also of specific interest in stem cell research in which the desired homogeneity of cell populations is technically hindered by lengthy culturing periods and methods for selection of individual clones of interest. Dr. Tenenbaum and his team propose sxRNA as a tool to express antibiotic resistance or reporter genes, which serve as selection markers for the target clones during stem cell culture and differentiation. For such purpose, a mRNA-sxRNA complex encoding the reporter gene will be activated in response to interaction with complementary miRNA which is present only in the target cell population.

The Present and Future of the sxRNA Technology

As discussed, sxRNA is a well-developed technology that is close to commercial introduction, especially in areas of stem cell research and anti-viral therapy. However, according to Dr. Tenenbaum, some aspects of the technology are still being optimized. 'We are still adjusting the switching activity of our sxRNA constructs. We are also trying many new genes, including cell death genes and antibiotic resistance genes, so we can select for, or against, survival'', said Dr. Tenenbaum. The biggest current challenge for the therapeutic application of sxRNA is the issue of the delivery. Fortunately, several miRNA and mRNA therapeutic companies are currently putting efforts forth to develop strategies for robust delivery of RNA molecules.

The current focus of Dr. Tenenbaum and his group is mainly on anti-viral therapies and solutions for biomanufacturing and stem-cell production and homogeneity. In the future they will explore other therapeutic areas including gene therapy and will continue to study the natural occurrence of these sxRNA and how they might be playing a role in translational control of gene expression.

Researcher Profile

Dr. Scott A. Tenenbaum
Associate Professor
SUNY- Polytechnic Institute, College of Nanoscale Science & Engineering, Albany, NY

Dr. Tenenbaum's research focuses on understanding some of the basic aspects of how the human genome works with an emphasis on post-transcriptional gene regulation. Along with his research team, Dr. Tenenbaum has helped to advance cutting-edge technology and computer-based informatic approaches to studying RNA biology, specifically RNA-binding proteins and how these molecules regulate information contained in RNA. Dr. Tenenbaum's research methods also include working on nano-based technology to make the research more robust and high-throughput. Dr. Tenenbaum is involved with HocusLocus Inc., a start-up biotechnology company that was spun-out of SUNY-Polytechnic Institute in Albany, NY. Hocus Locus is working to commercialize SUNY owned sxRNA intellectual property.

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SUNY POLYTECHNIC INSTITUTE

Modification of Existing Technology to Aid Research

Dr. Ed Schmidt is a scientific researcher interested in understanding intricate gene regulatory mechanisms of complex organisms. For the past 8 years he has worked with GeneSearch developing "The Dracula Pipette" recently renamed "The Embryo Cradle" to innovate and propel forward his line of work.

To aid our readers better understand your work, please tell us how your research background led to your interest in gene regulatory mechanisms?

Much of my career has focused on investigating mechanisms and consequences of gene regulation. My PhD work at Oregon State University investigated the gene regulatory mechanisms that shut-down a gene involved in producing the DNA precursors needed to replicate the genome as fetal cells destined to become muscle transitioned from a state of proliferative expansion to become mature contractile muscle. At the University of Geneva in Switzerland I investigated the gene regulatory programs underlying the unique stable functions of mature liver cells in rat and mouse livers. All of these studies, however, were based on describing the natural state of different cells under different conditions, and did not actually attempt to investigate the underlying mechanisms. I moved to the University of Utah to do a second postdoc to learn how to manipulate the mouse genome such that I might be better able to test the roles of specific genes in physiological processes. These technologies require very sophisticated embryo manipulations, and thus it was at this stage in my career that I first became actively involved in embryology technologies.

You've done a lot of research into redox biology as well as developing a mouse liver model. How has this previous work influenced your current R&D work for GeneSearch?

About 8 years ago my lab became interested in cellular antioxidant systems and how they regulate cellular physiological processes. For our studies, we need to occasionally develop novel mouse lines with specific mutations that are expected to help us understand these processes. This brings us back quite frequently to needing the sophisticated embryological manipulations that underlie development of novel mutant mouse lines. We generally out-source this work to committed experts who do nothing else and can therefore accomplish this much more quickly and efficiently than we could. However, it also made me wish that the technology could improve to the point that generating novel mouse lines could become more routine, and could be done in-house by less specialized labs. It was this latter desire that piqued my interest in the Dracula embryo manipulation tool.

You and GeneSearch have improved the original "Dracula Pipette" for embryo handling. Can you discuss the limitations of the original design?

In a word, "size"; however with this came very demanding technological hurdles. The original Dracula was designed for manipulating llama embryos. In scaling the Dracula tool down it was necessary to adapt it for use under a microscope, to drive the tool with advanced micromanipulators and hydraulic systems, and most importantly, to incorporate high performance materials that could provide the physical and optical properties needed for this tool on this small size-scale.

How does your new "Embryo Cradle" compare to the "Dracula Pipette?"

My understanding is that the name "Dracula" was chosen based on the utility of the tool for puncture-and-aspirate procedures. Now that our small-scale tools are becoming highly functional and showing great promise for applications in human clinical medicine, the name has become rather a handicap. In recognizing this, GeneSearch changed the name to the "Embryo Cradle" to emphasize the gentle way it holds the embryo as it preforms very delicate manipulations.

How will the new design benefit mouse and human embryo procedures?

For mouse, our goal is to make procedures easier, such that they can be performed easily and routinely by a broader cross-section of research scientists. For the human clinic, this tool holds great promise for developing improved technologies for no-harm pre-implantation genetic testing.

How do you expect your current research into embryo handling to affect your future work?

My goal is to make all of our mouse models in-house in the future, cutting time and costs needed to make novel mouse models, giving us more freedom to make and test some more cavalier allelic designs without worrying that investing in a non-useful design might bankrupt the lab.

Bringing Big Technology to Even the Smallest Of Laboratories

Advanced approaches for the genetic or epigenetic modifications of embryonic stem cells have revolutionised our ability to modify the cells from which novel mouse lines are produced. However, procedures for utilizing these cells in embryos have remained the same for decades. Dr. Ed Schmidt's work together with GeneSearch on "The Mouse Dracula Pipette" looks to amend this.

A MAJOR HURDLE IN A KEY PLACE

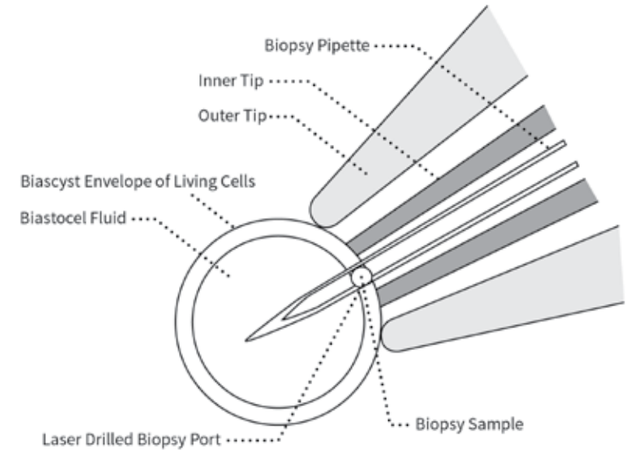
In the field of biomedical research mouse embryo cell based manipulation is a critical pillar for the foundation of any research. Such undertaking is very technologically and economically challenging. The cost and difficulties in generating chimeric mice is a major reason why many researchers avoid making novel mouse lines to test important hypothesis. Also, harm-free biopsy of embryonic stem cells remains difficult if not impossible using contemporary tools and technologies.

Mouse embryology goes far beyond just biomedical research. It also extends itself into human genetics as a "trial" or "prototype" for clinical research and for clinical procedures in both human genetics and reproduction clinics. Agriculture in the form of improved dairy animal management and conservation efforts for better reproductive management of endangered species are some other fields that can see themselves benefitted as well, however, these are fields with more cost prohibitive budgets and technical limitations that have up to now been left out of mouse embryology.

Its elegant coaxial design holds what I perceive to be great promise for putting highly sophisticated embryological procedures into the hands of a much broader cross-section of biomedical laboratories.

CURRENT MANIPULATION TOOLS

To this day the field of mouse embryo manipulation has been utilising pipettes for holding and pipettes for manipulation positioned by separate micromanipulators with 3 axes. In order to penetrate the embryo the injection pipettes and holding pipettes are presented in exactly opposite directions to create a conventional compression strategy. The problem with this is that the mouse oocyte and early embryo have a notoriously hard zona pellucida, often requiring the use of a piezo hammer and sharp injection pipettes for dependable penetration. As the embryo expands, the zona is stretched thinner, has less resistance to deformation, and this strategy becomes increasingly difficult.



As a way to help with the penetration, other instruments such as chilled stage microscopes or piezo hammer can be brought in to help facilitate the penetration of the embryo with conventional equipment. These extra instruments and steps required for conventional methods to work add layers of economic and technical strain to the research. Laser or piezo assistance in the penetration of the zona pellucid can cost laboratories between 10,000 and 50,000 U.S. dollars per unit. It is also worth mentioning that dependency on such technology for this fundamental part in mouse embryo manipulation opens up the research to be susceptible to work schedule problems and delays that can arise and are more prevalent the more factors are introduced.

The technical prowess needed to manage mouse embryo manipulation with conventional methods and technology is extreme. Most embryological procedures require skilled expertise to perform; some, like those involving hatched or manipulated blastocysts, are inaccessible to even expert embryologists.

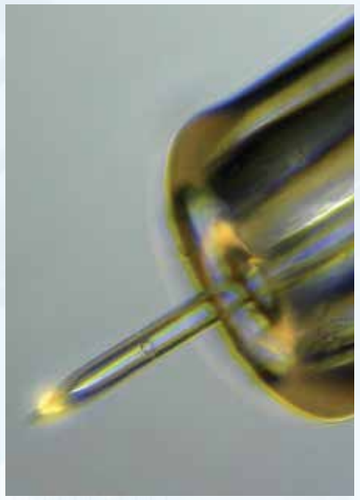
A DIFFERENT SPIN

The "Dracula Pipette" was originally conceived and developed as a tool for cryopreservation of llama embryos and it was used to produce the first live births from frozen/thawed lama embryos. Early version was hand operated. Human and mouse embryos are 1/1000th the volume of a llama embryo, to accommodate for this, diameters of all tips were scaled down to 1/10th of the original size.

The Mouse Dracula Pipette looks to be the solution to the problems that have hampered biomedical research for decades, bringing economic and manageable technology to the hands of all researchers.

THE INNOVATION THE MOUSE DRACULA OFFERS

The innovation of the Dracula system is the simple elegant coaxial design developed and continually refined by GeneSearch Inc. The Dracula has two concentric tips with separate



vacuum or pressure control for the space between the two tips, the space between the inner tip and the injection probe, and the space inside the injection probe, itself. Embryos are held firmly yet without damaging stresses by a ring of vacuum between the two tips. A brief pulse of vacuum inside the inner tip can aid in penetration as the injection probe is advanced. The Dracula system draws the injection surface taught, like the surface of a drum.

The Dracula Pipette has another unique ability, simultaneous injection and aspiration, or "flushing". Once the puncture has been made fluid contents of the embryo can be removed very quickly by applying a vacuum inside the inner tip. This causes fluid to flow out around the injection pipette, though the opening in the inner tip.

A biopsy port can be laser-drilled on the side of the probe. When the probe is in use it is pushed through the zona pellucida and trophoblast layers, the biopsy port is aligned with the trophectoderm layer and a vacuum is applied. This allows a few cells to be drawn into the probe and then sheared off of the trophectoderm layer as the probe is withdrawn.

Price looks to be another innovation for the Dracula system. The current cost production of a single Mouse Dracula RC-1, a versatile research tool with accessories including several sets of tips, biopsy probes and injection pipettes is ~$2,500 U.S. dollars. At present, GeneSearch is evaluating an all-plastic version of the Dracula device, which will cut this cost to ~$250 U.S. dollars. In the future, additional cost reductions may allow production of a single-use "Disposable Dracula" tool for human ART/genetic diagnosis clinics.

A FIRM PLAN LOOKING FORWARD

The Mouse Dracula Pipette for all it is now has a steady aim on where it wants to be. A two phase plan each with two aims has been set up to be realized during the next three years.

Phase one of the plan looks to establish the effectiveness in the manipulation of mouse embryos, specifically through the optimization for the insertion or extraction of a small number of stem cells to/from the trophectoderm and the inner cell mass to improve chimera production. To do this the following Specific Aims must be fulfilled:

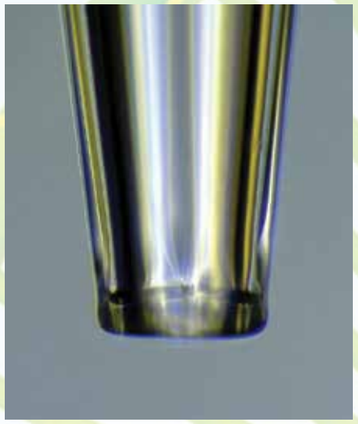
Phase One Aim One: optimize the Mouse Dracula for delivering embryonic cells into mouse embryos. The secure yet gentle handling by this tool allows even large probes to be easily inserted into the blastocoel of blastocysts.

Phase One Aim Two: optimize the performance of the Dracula system for no harm biopsy. The coaxial design of the Dracula allows delicate embryos to be handled.

In phase two of the plan the tool will be refined for more effective commercialization. This phase will initiate once aim one of phase one has been fulfilled. In phase two, the following will be fulfilled:

Phase Two Aim One: optimize ease of use and adaptability of the Mouse Dracula. This aim is to make the Mouse Dracula adapt to a large range of microscope and micromanipulator platforms.

Phase Two Aim Two: refine the Mouse Dracula for more effective commercialisation. Components are currently individually manufactured and hand assembled, this maximizes our ability to address our feedback and improve performance. Once we have met our desired requirements we look to adapt this process to higher volume production.



Researcher Profile

Dr. Ed Schmidt
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Dr. Ed Schmidt received his Ph. D. in Biochemistry & Biophysics from Oregon State University. He is currently a Professor for the Department of Microbiology & Immunology, Montana State University. His primary research interests are to understand the intricate gene regulatory mechanisms that function in development and maintenance of complex organisms. He has been the recipient of numerous national and international grants and awards. For the past 8 years he has collaborated with GeneSearch, Inc. in "The Dracula Project".

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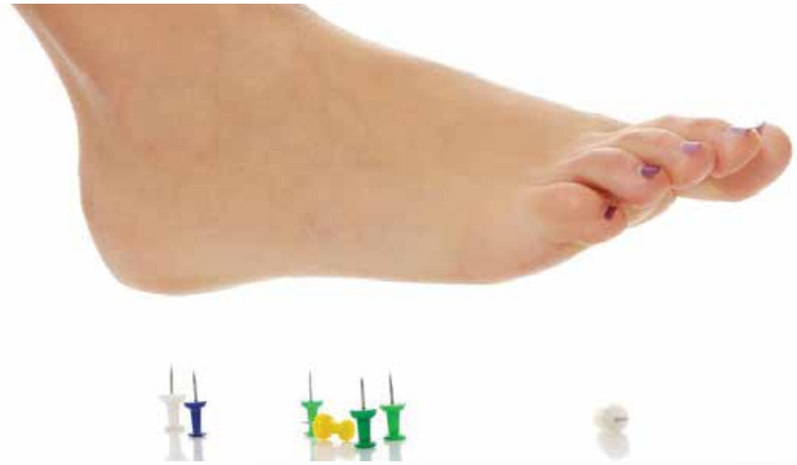
GENESEARCH, Inc.

No pain, certain gain

Novaremed is a pharmaceutical company developing a number of new drugs against chronic diseases. At the head of the pack is their proposed treatment for neuropathic pain, known as NRD135S.E1. Here we discuss their plans and recent successes.



There are very few things in life worse than constant, unforgiving pain. Whether it occurs as the hitch in the small of your back as you sit down to work again, the creak in your knuckles as you go outside on a cold day, or even the deep burning within your bones from the chemotherapy, chronic pain continuously makes itself known to the sufferer.

Chronic pain can be divided, based on the causative reason, into two types, neuropathic and nociceptive. Neuropathic pain is a continuous pain where the damage has been done to the nervous system, making the specific cause difficult to identify and treat: think of back pain, of peripheral neuropathy in diabetics, shingles. Neuropathic pain makes up around 25-40% of chronic pain cases, depending on country, age group, etc. Nociceptive pain, by contrast, comes from damage to the body tissues, to muscles, joints, bones. Think of the pain you feel after breaking a bone, in arthritic joints, after an operation. This pain, thankfully, usually goes away once the underlying injury has healed.

The current state of treatment for chronic pain is less than optimal. Only a few drugs have actually been approved for use in humans, the big 4 being Gabapentin (Neurontin), Lyrica, Cymbalta and Lidocaine, all of which were launched between 1999 and 2004. Unfortunately, besides their limited efficacy, all of these drugs have significant side effects – most commonly dizziness, drowsiness, ataxia (lack of muscle control), and digestive problems. These side effects can sometimes be so bad that patients would prefer to drop the medication and simply cope with the pain itself. Beyond this there are three major factors which need improvement: current pills need to be taken several times a day; they do not have sustained medicine release for long term-treatment; and they often show unwanted interactions between different drugs.

To add to these difficulties, chronic pain is itself a growing problem. In 2008 there were between 9 and 16 million sufferers in the US and Western Europe. In three years, in 2018, there is expected to be between 16-22 million, a significant increase. This is also, naturally, a growing market for therapeutics, with the chronic pain field expected to have sales between 7-10 billion US dollars in 2018. It is this growing field, and knowledge that the current medication is somewhat lacking, which has brought new entrants into the field.

ENTERING THE RING...

One of these entrants is Novaremed, an Israeli company dedicated to developing drug for chronic pain with sustained efficacy. The company began, back in the early 90's, with an observation by the founder and CEO, Dr Eli Kaplan. At the time he was observing the common use of Antiviran as a remedy for hepatitis and various gastrointestinal disorders. Antiviran, before you ask, is the name given to a crude powder extracted from cultures of non-pathogenic microorganism, which has been used as an herbal tea/folk remedy for quite a long time. He suspected, as is often the case, that this mixture acted as a crude form of numerous interesting medicine-like molecules, and thus set out to purify and identify them.

After almost six years of teasing out the contents of this mixture, splitting it into fractions, testing each part, sub-dividing further, Dr Kaplan finally found the first of the active substances. A simple molecule, relatively small, it was neither a peptide, nor a lipid, not a lipoprotein, but chemically appeared to act by simulating a small peptide. Once this original compound had been identified, it was possible to use targeted molecular design to create a leaner, more effective version which was only half the size of the original substance (a mere 357 Daltons).

Novaremed itself was founded in 2008 to follow up on these early discoveries, with the initial work being performed in the Ashkelon Technology Incubator. These start-up incubators provide a vital role in helping innovative companies begin, providing facilities and equipment for early development and research. They were also assisted in this process by the traditionally entrepreneur-friendly Israeli government, as Michal Silverberg, Clinical Manger, commented "the continuous financial support of the Chief Scientist enabled Novaremed, like many other young companies, to cross the barrier of early product development".

Their current lead compound is known as NRD135S.E1, and has already shown extremely promising results in a number of tests. Indeed one of the only down-sides is that the name

itself is quite a mouthful, (Liat Hochman, Director of Clinical Operations, comments “internally we call it E1”, which is definitely easier to say). E1 is what is known as a New Chemical Entity, a molecule that is unique and has not been previously seen in therapeutics. Alongside this E1 has a novel Mechanism of Action, (the manner in which it works), which relies on modulating the activity of a protein known as Lyn. Lyn is a tyrosine kinase, a type of signalling protein which acts as a mid-point between an outside stimulus (i.e. pain) and the cells own reaction to this occurrence. No other pain drug targets tyrosine kinases, nor does E1 bind to the common pain receptors – this means that it does not overlap with other treatments and thus could be extremely useful for patients who are not responsive to typical treatment.

“developing a first in class, efficacious and safe remedy, which has a unique mechanism of action, for this indication” (neuropathic pain)

ROUND 1

The process of developing a drug is long and often convoluted, but falls into three main stages. First come the pre-clinical experiments, making sure that your drug does what you want in cell lines, in biochemical assays, and in animal models. Next comes the First-in-Man studies, known as Phase I, which act as safety testing for the new drug: how well is it tolerated? How long does it stay in the body? How is it removed? Last are efficacy studies, Phase II and Phase III, which test how well the drug works at solving its target disease. As the potential medicine works through each stage more and more information is gathered, helping to inform the final use when it reaches the clinic.

Novaremed began with pre-clinical studies on E1, determining how it worked – not just that it affected Lyn, but how much, how quickly, and how long, all in excruciating detail. Once the biochemical characterisation was out of the way, they moved on to animal studies, examining various different models of chronic pain. Each of the studies showed not only that the drug was safe and well tolerated, but that it was often more effective at pain-reduction than a number of well-established medicines on the market.

ROUND 2

Following their excellent pre-clinical results, Novaremed moved on to the next stage, first-in-man trials. These trials, intended to determine safety and tolerability of a new drug, are usually entitled Phase I or, as in this case, Phase Ia. This was an ascending dose study, which means that the 32 healthy male volunteers (in 4 cohorts), were given gradually increasing doses of drug to see how it was tolerated. As would be expected, this is a slow and careful process, with a maximum level of care for the health of the volunteers. It was followed by a Phase Ib trial,

in which volunteers took a single dose for five days, to accurately model the typical chronic pain sufferer.

As expected, E1 was safe and well-tolerated, a few mild and transient side effects were seen in both the placebo and the drug group, indicating that they were most likely not caused by -E1- nerves. Alongside this, biochemical studies showed that E1 was rapidly absorbed into the bloodstream (within 1-3 hours) and then eliminated over the course of 24 hours. This is, naturally, exactly what the designers of a one-tablet-a-day medicine would like to see.

The next stage, Phase IIa, involves testing efficacy in actual patients. In this case the researchers are working with patients suffering from diabetes-associated neuropathic pain. This process has just begun, and results will not be known until the end of the process in March 2016, but everyone at Novaremed is confident that their initial observations will be borne out. As they comment, they are “developing a first in class, efficacious and safe remedy, which has a unique mechanism of action, for this indication”.

ROUND 3, TAG TEAM?

So where to from here, for this company? Naturally there is the long process required to get their new drug, NRD135S.E1, to market. This not only requires human clinical trials, but also that they show the ability to safely manufacture their chemicals in the quantities needed. According to Neta Pessah, Pre-Clinical Manager, this is no problem, they have already “scaled up with relatively high yields, high purity, and under GMP (good manufacturing procedures) as required by ICH guidelines”. Beyond this, from a purely prosaic view, the firm needs to defend its discoveries via patenting, and Noveremed has managed to secure strong patent protection for E1 until 2031.

Nor are the team at Novaremed happy to rest on their laurels after their success so far with E1. They are using it as a base to develop “other compounds in the pipeline that are chemically related to NRD135S.E1” comments Pessah, “which may be developed for indications other than neuropathic pain”. The compound pipeline, of course, is one of the most important assets that a pharmaceutical company has, and thus the existence of these novel molecules is a strong sign for the company’s future development.

Based on these strong fundamentals, and plans for the future, the founders of Novaremed are looking around for others with similar goals. As Liat Hochman, Clinical Operations, makes clear, “we are looking for collaboration with pharma companies interested in pain, and potentially other indications related to E1’s mechanism of action.” Their hope is that, by working with collaborators, they will be able to spread the benefits of their discoveries far further than otherwise possible. This is a company with lofty goals and a plan to launch themselves upwards. After all, once we can defeat pain, the sky is the limit.

Researcher Profile

Novaremed

Founded in 2008, the Israeli pharmaceutical company Novaremed is a rapidly impressing investors with their pipeline of compounds for treatment of chronic diseases. At the head of the list is their proposed treatment for neuropathic pain, known as NRD135S-E1. Headed by Dr. Eli Kaplan, and with members from well-known multinationals on their side, they are definitely a group to watch.

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OCS – Office of Chief Scientist and Angels

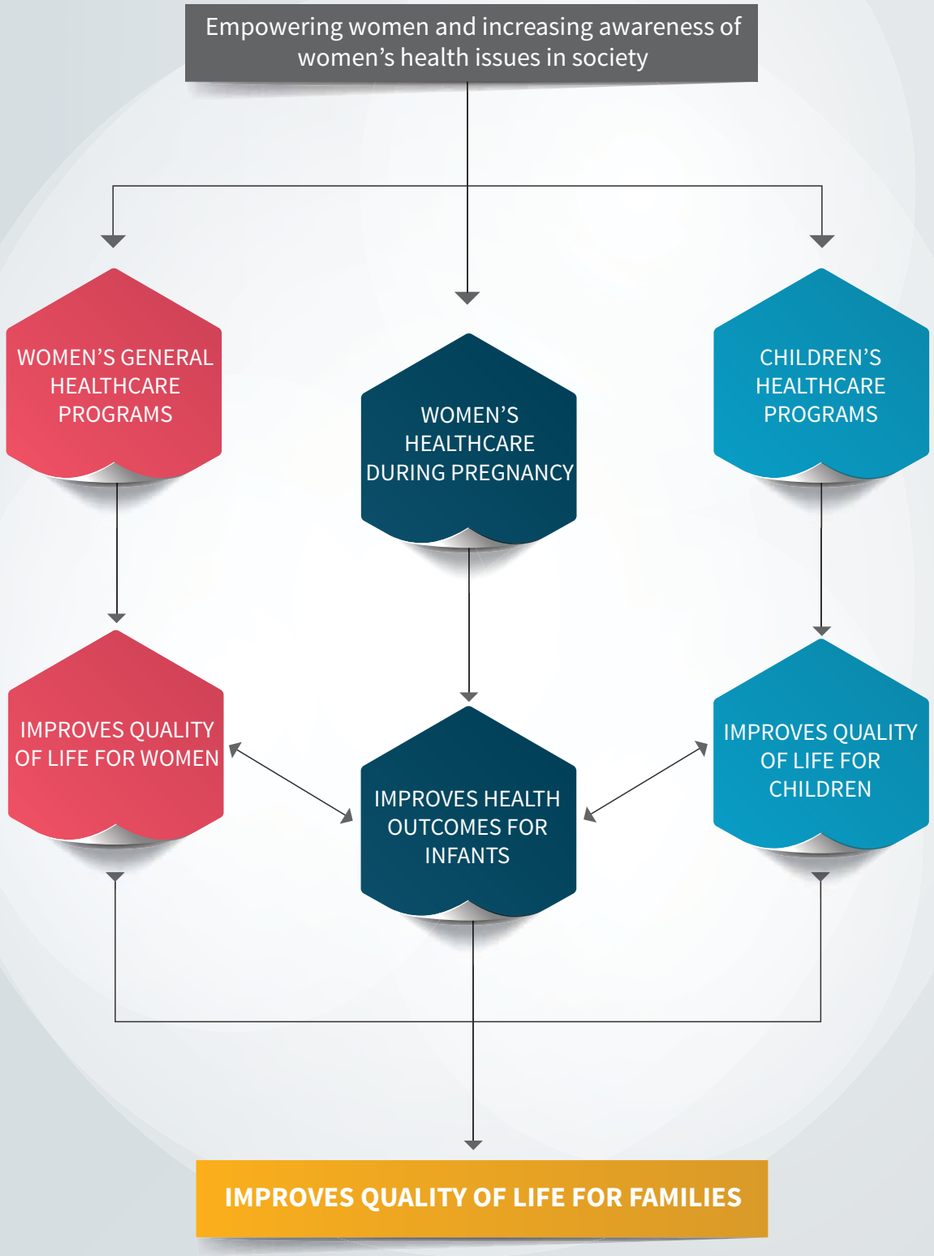
NOVAREMED

WOMEN'S HEALTH, PREGNANCY AND EARLY CHILDHOOD

For over 30 years we have seen much talk of narrowing the gender divide, mainly focused on economic measures such as the equality of pay. However the need to look specifically at women's health remains, not least because inextricably linked to improved health for women is the improved life chance of their offspring. Increasingly we see numerous social programs that focus on women's health and the resultant positive impact on the wellbeing of children and communities. Amongst these programs are those that focus on the health of women during pregnancy and their foetuses during gestation, those that focus on childhood health problems, and others that work on improving the interaction between parents and their children. Although great advances have been observed, economic and government initiatives for women and children are still scarce, and here we showcase research and health interventions that seek to address health issues that result in decreased infant mortality. >

> The first two articles in this section will discuss specific programs aiming to improve healthcare during pregnancy. Dr. Marita Lynagh's article examines incentives to lower smoking rates amongst pregnant women, and Dr. Jodie Benson comments on the importance of treating vitamin D deficiency during pregnancy to prevent deficiencies in newborns. In the third article, Dr. Hagit Friedman discusses the development of Infant Neuro Aquatics, which is a treatment for premature babies suffering from developmental disorders. This work is salient at a time that premature births have been increasing due to modern medical interventions such as In Vitro Fertilisation (IVF). Surprisingly, children are still dying from easily treatable conditions, such as diarrhea and pneumonia in rural, remote, and under-developed areas. Dr. Fauziah Rabbani's work provides greater insight in to a system wide health intervention called project NIGRAAN. This community based health programme was designed to improve skills and communication amongst healthcare workers in order to reduce childhood mortality. Lastly, Dr. Sophie Havighurst and Ms Ann Harley present a parenting program that focuses on the establishment of better relationships between parents and children.

WOMEN, CHILDREN AND FAMILY HEALTHCARE INTERACTIONS



Smoked carrots

Dr. Marita Lynagh of the University of Newcastle, Australia, works in the field of public health and behaviour. Here she talks to us about her recent work examining incentives as a way to lower smoking rates amongst pregnant women.

Could you discuss your background?

I have worked as an academic and health behaviour scientist for 20 plus years with research interests in number of different public health issues including smoking cessation. I first became interested in the potential of financial incentives after witnessing the success of two national incentive programs implemented over a decade ago to address falling birth and child immunisation rates. The application of incentives as a 'carrot' to encourage smoking cessation is particularly suited to pregnancy because of its defined time period and the recurring contact that occurs during routine antenatal care. Yet a trial had never been contacted in Australia to evaluate the feasibility of financial incentives for quitting smoking during pregnancy.

What was your most significant research finding so far?

One of the most interesting and 'significant' findings from this trial was the low consent rate of women willing to participate in the trial. Our belief is that it has a lot to do with the 'timing'; of being invited to participate in a smoking cessation trial quite late in the pregnancy. Most women who quit smoking in pregnancy usually do so early in the first trimester, soon after discovering they are expecting. Many will also 'cut down' on the number of cigarettes they smoke. But a certain proportion continue to smoke for the duration of the pregnancy. It was this 'hard-core' group that our trial had attempted to recruit. We suspect that a number of these women had wanted to quit, and possibly made one or more unsuccessful attempts. By the time we invited them to participate they had convinced themselves that quitting later in pregnancy won't make any difference to the baby – as any potential 'damage' would have already been done.

From your results, there is not much difference between the $20 and $40 groups in terms of quitting. Do you think this is a lack of statistical power or that the actual value of the incentive is less important?

It is difficult to draw clear conclusions about the impact of the size of the financial reward based on the results of our trial, largely due to the small sample size and lack of statistical power. Certainly the results are suggestive of reward value not greatly effecting rates of quitting, however it is also possible that the gap between the conditions (i.e. $20 versus $40 reward) was not sufficient to produce an effect. Would a $20 reward versus a $200 reward have resulted in a difference in quit rates? Further research is needed to answer this question.

Providing pregnant smokers with incentives to quit seems to be fairly controversial, do you expect that this will impact on the uptake of the method?

There has been, and still remains, a degree of controversy and concern over perceived moral issues associated with the idea of 'paying' people to change their health behaviour. Interestingly, 'pay-for-performance' initiatives implemented in a number of countries to change the behaviour of medical practitioners have received much less controversy. Our own research indicates that smokers are much more receptive to the idea of paying someone to quit compared to non-smokers. This is not an unsurprising finding. There is general caution and a reluctance by others to implement an incentive program for pregnant smokers, but this is to be expected. It would be of greater concern if such a strategy was quickly and readily adopted before we have good evidence of its effectiveness.

What was the biggest obstacle you've overcome during your research?

Gaining funding to conduct a large-scale efficacy trial was a challenge. After numerous submissions over five years, we eventually succeeded in receiving a (reduced) grant-in-aid from the National Heart Foundation of Australia to conduct a feasibility trial. The next biggest hurdle was getting ethical clearance from all the relevant research ethics committees. There were also a number of 'logistical' obstacles that were overcome – related to conducting a research trial in a busy, crowded antenatal out-patient clinic in a public hospital.

How would you like to extend your research? Have you another trial planned as a follow-on to the current one?

We have already begun discussions about potential collaborations on a trial with Aboriginal women in Australia. Rates of smoking during pregnancy among Aboriginal women and other socially disadvantaged women are higher than the national average, and the health problems associated with smoking in pregnancy (e.g. premature birth and low birthweight) are more prevalent in these populations. We are also exploring options for 'reaching' women earlier in their pregnancy, as most women don't begin their hospital antenatal care program until their second trimester. Encouraging smokers to quit earlier in pregnancy would be much better for their babies, and women may be more amenable to the idea at an earlier stage of pregnancy. We would also like to investigate 'vouchers' in place of cash rewards, as a number of participants indicated that this may be more acceptable.

What would be your 'dream' research project?

My 'dream' research project is really any project that leads to meaningful improvement to the health and/or quality of life of people. It's not just about the size of the grant or the number of publications, it has to actually make a positive difference to someone somewhere.

Of cigarettes and carrots

The University of Newcastle is a leading Australian university with a strong focus on medical research. The School of Medicine and Public Health, as the largest school within the Faculty of Medicine, marries this tradition to a focus on teaching the medical students of tomorrow.

For several thousand years, humans have cultivated and smoked tobacco for its ability to alter our mental state. This long-running history began with homemade pipes and gradually progressed to the industrially-manufactured cigarettes we know today. Beginning in the 1920s, however, doctors began to observe that smoking was detrimental to our health, leading to heart attacks, lung cancer, pulmonary disease, and numerous other illnesses. A number of studies, reports, and law-suits followed this, but once the dust had settled smoking came to be considered the leading preventable cause of death worldwide. Indeed, the majority of health authorities around the world are united in their desire to discourage smoking amongst their populations.

If the stick fails to work, what about the carrot? How incentives assist pregnant women in quitting cigarettes.

However this is easier said than done. Nicotine, the psycho-active compound found within tobacco, is highly addictive, which thus makes efforts to quit smoking exceptionally difficult. The difficulty of quitting has led to a large market of alternative nicotine-based products (patches, e-cigarettes, etc.), as well as a number of government programs designed to help smokers. These range from the financial (extra taxes on cigarettes) to the graphic (images of cancer-riddled lungs on every pack) to the, well, non-graphic (requiring plain packaging on every cigarette carton). Australia is considered to be a leader in this field, having pushed through plain packaging laws only a few years ago in the face of fierce opposition from tobacco groups.

Despite these efforts, smoking is still widespread throughout the world, it is estimated that over 1.2 billion people smoke tobacco – with the majority of these living in developing countries. The highest rates of smoking are seen in men, in residents of developing countries, or members of disadvantaged social groups. Thus the majority of countries use targeted programs to assist these vulnerable groups to quit smoking, using the often limited healthcare budget in the most effective way possible.

One major risk group is that of pregnant women. Tobacco smoke delivers a number of chemicals to the bloodstream of the mother, many of which can have detrimental effects on the unborn child. Observational studies have noted that smoking during pregnancy can lead to increased risk of complications, low birthweights, and placental abnormalities. Programs targeting pregnant women are thus commonly used by healthcare agencies, although with the same problem as seen in the wider community: quitting is really, really hard.

THE CARROT, NOT THE STICK

The majority of anti-smoking campaigns are rooted in negatives: smoking gives you cancer, your lungs are full of tar, every breath is doing you damage, etc. And yet, they still fail to work all the time – around 10% of women still smoke during pregnancy. A new type of thinking has thus slowly started to emerge: if the stick fails to change behaviour, what about the carrot? This is the basis of the idea of conditional incentives – a reward, such as money or a voucher, provided only when the patient can avoid the negative behaviour – in this case smoking.

Interesting idea, but does it work? This is where Dr. Lynagh of the University of Newcastle, Australia comes in. Her research career has lately focused on the effectiveness of incentive-based programs in helping pregnant women to quit smoking. Their most recent trial, known as Project ENtiCe, followed 42 pregnant women, all current smokers. For every scheduled antenatal clinic visit at which they were able to show that they had avoided smoking (as verified by biochemical analysis), they were given an immediate cash reward of $20 or $40 Australian dollars. The reward amounts increased incrementally by $20 or $40 each time they returned a negative test – or in the case of the control group, no money at all. They then followed these women right up to delivery to see how effective the process was.

By the end of the trial it seemed that the incentives were a definite improvement, while only 15% of the control group managed to quit during the study, the number rose to 20% and 22% for the $20 and $40 groups respectively. The incentive groups also tended to quit earlier during the pregnancy and stay cigarette-free for longer periods of time. Although the drop-out rate was relatively high at 28%, this is common to almost all smoking-cessation interventions, as participants lose faith in their own ability to succeed. Interestingly, the majority of smokers believed that vouchers would be a more appropriate incentive than the cash reward given during the trial.

From these results it seems that incentives may help people to quit. But does the size of the incentive matter? While the majority of participants thought that higher payments would make for better incentives, the study did not show any difference in quitting rates between the incentive groups. This may be simply due to the small number of participants in the study, or may indicate that incentive size is not as important. Dr. Lynagh is sanguine about the possibilities, believing that only with further, larger trials will the true answer be identified. After all, as she says, “Sometimes the most significant research finding is not the one that we have specifically searched for”.

USE CARROT? (Y/N)

If, as it seems, incentives provide a way to encourage pregnant women to quit smoking – then why don’t we just do it? One problem with this approach is that it is somewhat controversial: every dollar used needs to come from elsewhere in the budget, and many disagree with the idea of paying people to do something that is, after all, in their own interest. Furthermore, they say, is it fair to pay some to quit, when others can quit smoking by themselves? To further understand the nuances of public opinion, Dr. Lynagh’s group conducted surveys of pregnant women regarding their opinion on the matter.

The results indicated that resistance to the idea remained high. Sixty percent of respondents either disagreed or strongly disagreed that paying pregnant smokers to quit was a good idea. Only 30% of respondents thought that it would work at all, (a further 22% were undecided). And when asked how much would be a reasonable amount to pay, 62% said that zero was a reasonable number. A common viewpoint appeared to be that there was a lack of fairness – why should smokers be paid to quit when non-smokers aren’t paid to be healthy?

Despite this, however, many alternative viewpoints remained. Smokers, perhaps unsurprisingly, were much more likely to believe that incentives were a good idea. Non-smokers also believed the idea had promise, feeling that payments over a range from 50-1000 Australian dollars were a reasonable incentive to quit. Dr. Lynagh notes that many of these opinions are inconsistent with similar schemes for other target groups. “Interestingly,” she commented, “‘pay-for-performance’ initiatives implemented in a number of countries to change the behaviour of medical practitioners have received much less controversy”.

Is this then an idea which the population will come around to, as further studies indicate the effectiveness of incentive-based programs? Dr. Lynagh is relatively unconcerned, her focus is on determining what works best for patients. “It’s not just about the size of the grant or the number of publications”, she comments, “it has to actually make a positive difference to someone somewhere.” It is this approach to improving lives which serves her well in her quest to help the children of tomorrow.

Researcher Profile

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Dr. Lynagh has been researching public health for over 20 years, particularly focusing on changing population behaviour for better health. Her successful research career has led to over 30 publications in a number of fields including smoking control, alcohol harm-reduction, promoting activity, and supporting cancer survivors. She has matched this to a strong history of teaching undergraduates and postgraduates, receiving a number of awards for excellence in education.

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Preventing Disease in Babies Before Birth

Dr. Jodie Benson a specialist Obstetrician and Gynaecologist at University Hospital Geelong and a senior clinical lecturer at Deakin University in Australia. Here Dr. Benson discusses her research into vitamin D deficiency in pregnant women that might adversely affect their babies.

Why did you become interested in vitamin D deficiency in pregnancy?

My interest in vitamin D deficiency in pregnancy was almost accidental. Not until I spent time with an endocrinology colleague did I become aware of the full extent and seriousness of the problem, especially in newborns. My interest was further heightened by the patients who were at risk for this problem. In our clinic these women were often marginalized—not fluent in English and with poor social supports.

There was no consensus on how to screen for or manage the disorder in pregnancy. So in this era of evidence based medicine, the idea of studying vitamin D deficiency in pregnancy and preventing the birth of vitamin D deficient babies just jumped out at me.

What specific abnormalities in the newborn might someone see if the mother is vitamin D deficient herself?

Vitamin D deficiency impairs bone mineralization and leads to bone softening, but most vitamin D deficient newborns are asymptomatic. Softening of the skull bones may occur, but most alarmingly a neonate may suffer from hypocalcaemic seizures. Rickets—bowed long bones—is the typical manifestation of long-term vitamin D deficiency but isn't evident until weight bearing occurs. Other associated problems include thinning of bones—osteomalacia—as well as osteoporosis, increased fracture risk and muscular aches and pains. More recently, vitamin D deficiency has been linked to an increased risk of death from cardiovascular disease, as well as with cognitive impairment in older adults. Vitamin D deficiency is also associated with increased mortality from malignancies—such as colon, breast, ovarian, melanoma and prostate—as well as an increase in upper and lower respiratory tract infections. Vitamin D deficiency is also associated with an increase risk of depression and suicide. There is some evidence linking vitamin D to multiple sclerosis, type 2 diabetes, inflammation and the risk of allergies in children and adolescents.

Are there any specific racial, cultural or socioeconomic factors that predispose pregnant women to have low levels of vitamin D in their blood?

Vitamin D deficiency can result from inadequate sun exposure and/or inadequate nutritional intake, as well as from diseases that impair vitamin D adsorption or conversion into active metabolites (e.g. liver and renal disease). Typically vitamin D deficiency occurs in populations with highly pigmented skin, as well as those with clothing practices resulting in minimal sun exposure.

Increasingly we are seeing maternal vitamin D deficiency in a more diverse population. As people spend more time indoors for both work and domestically, sun exposure is decreased. This can be compounded by the "sun smart" message and use of sunscreens. Vitamin D deficiency is also increased in the obese population.

How do you identify pregnant women with low levels of vitamin D and can you effectively remedy the problem to keep the baby healthy?

A simple blood test—either prepregnancy or in early pregnancy—can detect vitamin D deficiency. Oral supplementation can commence as soon as the diagnosis is made. Our study showed that if you adequately supplement vitamin D deficient women, they and their babies will have normal levels at birth.

The option of treating postnatally is also important. Breast milk is not a very good source of vitamin D—oral supplements must be given. Generally, breastfeeding is best for both babies and their mothers, but if the mother is at risk of vitamin D deficiency this should be addressed with vitamin supplementation.

Do you think the public is aware of the potential problem low levels of vitamin D pose to the newborn? Should there be more public awareness of the issue?

I don't think that the generally public is aware of the potential problems that vitamin D deficiency poses to adults, children or newborns. There should be more public awareness in general.

Given that I'm an obstetrician and gynaecologist. I believe that the pregnant population should be targeted. Women are usually more highly motivated to change any behaviours or remedy any ills when they are pregnant. The family practitioner has the opportunity to screen and treat the entire family as well. This is important because studies have shown that if a pregnant woman is deficient, her family is also at risk and should be screened.

Do you have plans to extend this study in the future or focus elsewhere?

My interest has recently shifted to perinatal loss. I sit on the Consultative Council on Obstetric and Paediatric Mortality and Morbidity Stillbirth Subcommittee, convened by the Department of Health. We review all cases of stillbirth in Victoria. My recent research interest looked at the feasibility of CT scan versus autopsy in the investigation of stillborn babies. This is a terribly sad topic and we have to learn more to help and counsel families affected by the loss of their unborn baby.

Fighting Vitamin D Deficiency Before Birth

Vitamin D deficiency isn't just a thing of the past. Dr. Jodie Benson's research shows that identifying and treating vitamin D deficiency in pregnancy can prevent vitamin D deficiency in the newborn.

AN OLD DISEASE REARING ITS HEAD AGAIN

Rickets—fractures and bony deformities in infants and young children due to poor calcification of the bones often caused by vitamin D deficiency—was probably known at least by the first or second century A.D. However, rickets was not formally described as a specific malady until a treatise in 1645 by Daniel Whistler, an English physician. In fact, the term "orthopaedics" was coined by the French professor Nicholas Andry –derived from Greek words for "correct" or "straight" ("orthos") and "child" ("paidion")—in his 1741 book Orthopaedia: or the Art of Correcting and Preventing Deformities in Children. By 1918, Kurt Huldschinsky, a German paediatrician, successfully showed that rickets could be treated by exposure to ultraviolet lamps. This made sense, since vitamin D is produced in the skin from the conversion of 7-dehyrdocholesterol by UV radiation. By 1945, rickets had all but been eliminated in developed countries by the enrichment of food, especially of milk, with vitamin D via UV irradiation. But rickets, and vitamin D deficiency generally, seems to be making a comeback of late.

There was no consensus on how to screen for or manage the disorder in pregnancy. So in this era of evidence based medicine, the idea of studying vitamin D deficiency in pregnancy and preventing the birth of vitamin D deficient babies just jumped out at me.

Dr. Jodie Benson and her colleagues have observed that vitamin D deficiency seems to be on the rise in some populations in Australia and elsewhere. They note that lifestyle factors, such as increased time spent indoors and increasing use of UV-blocking sunscreens, effectively decrease UV light absorption, hence vitamin D production. Obesity is also a risk factor for vitamin D deficiency. The problem is compounded in darker pigmented individuals and people whose cultural practices include extensive body covering, both situations decreasing UV absorption by the skin. What most concerned Dr. Benson, an obstetrician, was the problem of vitamin D deficiency in the pregnant women for whom she cared. Since foetal vitamin D is acquired transplacentally from the mother, low maternal vitamin D levels lead to vitamin D deficiency in the foetus. Further, breastfeeding does not supply sufficient vitamin D—especially if the mother is vitamin D deficient to begin with—so babies who have low levels of the vitamin at birth cannot catch up with breast milk alone.

25 HYDROXY VITAMIN D LEVELS IN PREGNANT WOMEN

To investigate the prevalence of vitamin D deficiency in pregnancy and the possibility of prenatal treatment, Benson and her group designed and carried out a study of pregnant women attending a routine outpatient antenatal clinic in a tertiary referral centre in Melbourne, Australia. Relevant demographic information was obtained from the women, including socioeconomic status, lifestyle factors such as clothing preference and sun exposure habits. Vitamin D levels were measured by measuring serum levels of 25-hydroxy vitamin D—also known as 25-hydroxycholecalciferol, a precursor of vitamin D considered the best indicator of a person's vitamin D status—and women with low serum vitamin D levels were included in the study. Measurements were taken both initially and at intervals thereafter, including at delivery, as well as from the baby after birth. The vitamin D deficient women were initially divided into two groups and randomised to receive either vitamin D supplementation during their pregnancy versus non-treatment. Since there was no standard recommendation for vitamin D supplementation during pregnancy, the withholding of treatment was not ethically

objectionable. However, after delivery any women in the non-treatment arm were offered treatment with vitamin D for themselves and their newborns.

FACTORS ASSOCIATED WITH VITAMIN D DEFICIENCY

Benson's study initially enrolled and randomised 78 pregnant women, although at the end of the study 45 women were available for data analysis, 22 in treated and 23 not treated, some women being lost due to compliance and other issues. The women in both groups were similar in age, 28-29 years, and had only been in Australia for 6-8 years. Thus, younger immigrants represented a goodly portion of the study population. Perhaps as expected, approximately 75% of these vitamin D deficient women were dark skinned and about 15% wore veils and extensive body coverings. Both of these situations are associated with low levels of vitamin D. Also, dairy intake—including vitamin D fortified milk—was similar, at about 8-9 servings per week. Importantly, about a fourth of the women had a previous history of vitamin D deficiency.

At the first visit—between 12-16 weeks of gestation—the 25-OH vitamin D levels in these two groups of women was approximately the same and low. In the treatment group, the serum level averaged 32 nmol/l, while in the placebo group it was 33 nmol/l. Although no official recommendations existed at the time for vitamin D levels in pregnancy, Benson and her colleagues used clinical guidelines published in 2011 by American endocrinologist Michael Holick and his colleagues that considered the normal level of 25-OH vitamin D to be >75 nmol/l, while deficiency was defined as a level <50 nmol/l. Clearly, both of Benson's patient groups were significantly deficient in vitamin D, putting themselves and their babies at risk for the adverse effects of vitamin D deficiency. Benson and her co-workers found what they had expected—pregnant women with recognized risk factors having significantly low levels of vitamin D. So did the vitamin D supplementation work?

SIGNIFICANT RISE IN VITAMIN D LEVELS

When Benson analysed the 25-OH vitamin D levels over the course of the pregnancies in both groups, she found the very answer she was looking for. While both groups had levels of 32-33 nmol/l at the beginning, both in the deficiency range, when levels were drawn at 28 weeks of pregnancy—when other routine blood work was drawn—the treatment group was now up to an average of 65 nmol/l, while the untreated women had average levels of 41 nmol/l. The untreated women were still deficient, below 50 nmol/l, while the treated women were now in a borderline zone between 50-75 nmol/l. And at that point, the dosing of vitamin D supplementation in the treatment group could be doubled—from 2000 IU of ergocalciferol (vitamin D3) to 4000 IU—to see if more was better, and also to see if a higher dose was tolerated in pregnancy.

At delivery, the level in the treatment group had risen to 71 nmol/l, now in the recommended normal range, while the level in the untreated group was still in the deficiency range, at 36 nmol/l. Beyond that and most importantly, the vitamin D levels in the newborn babies correlated with the maternal vitamin D levels. In the treatment group, the 25-OH vitamin D levels averaged 81 nmol/l, compared to 42 nmol/l in the untreated group, a significant difference. The treated babies had normal vitamin D levels—presumably keeping them safe from rickets and other manifestations of vitamin D deficiency—while the levels in the untreated group were in the deficient range. Benson's hypothesis was confirmed—treating the mother with vitamin D before pregnancy significantly improves the mother's vitamin D levels, but it also raises her baby's levels so it can be born with normal levels of vitamin D. Screening and treatment appears to be a reasonable strategy to combat maternal and foetal vitamin D deficiency.

Benson admits that this is a small study and technically not extremely rigorous. However, the results speak for themselves and, combined with research by other groups, the study gives clear evidence that supplementing vitamin D in pregnancy remedies vitamin D deficiency in pregnant women themselves and prevents neonatal vitamin D deficiency. The results of Benson's study indicate that larger studies are needed to verify the results, determine the optimum levels of vitamin D in pregnancy and how much supplementation should be given, and generate guidelines that can then be applied to all prenatal patients. But the die is cast in this battle against the resurgence of neonatal vitamin D deficiency.

Researcher Profile

Dr. Jodie Benson
Obstetrician and Gynaecologist Specialist and Clinical Lecturer
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Dr. Jodie Benson received her Bachelor of Medicine & Surgery from the University of Melbourne. She completed formal specialist training in Obstetrics & Gynaecology at Monash Medical Centre, being elevated to Fellow in 2011. Her early career featured international experience including a Fellowship in Robotic Gynaecological & Endoscopic Surgery. This time abroad also ignited a passion for intrapartum care and labour ward teaching, having worked in a busy obstetrics unit in Ireland delivering close to 10,000 babies a year. She returned to Australia in 2010 and worked at South West Health care and Deakin Warrnambool, where she was the Women's Health rotation coordinator, before moving to Geelong. Dr. Benson combines her clinical role at GUH, with RANZCOG training supervision, Deakin medical student teaching as well as sitting on the Consultative Council on Obstetric and Paediatric Morbidity and Mortality (DHS).

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Water Babies

Dr. Hagit Friedman has been involved in developing Infant Neuro Aquatics, a treatment for prematurely born infants suffering from developmental disorders. In this interview Dr. Friedman elaborates on the current state-of-the-art in the field and the results of her recent work.

As a starting point, please explain how your research background led to your current interest in premature birth?

Towards the end of my PhD I realized that I wanted to change direction and work with people rather than microscopes and neuronal cell cultures, to see the humans that are behind the synaptic proteins we tracked in my PhD project.

How did Infant Neuro Aquatics, as a developmental impairment treatment of premature infants, become your specific research interest? Where does your research fit in the larger context of current medical research?

I can identify two milestones in my biography that led me to choose my research questions. The first was a seminar course I took towards the end of my PhD on 'new findings about the biological mechanisms behind Psychiatric illnesses', where I "met" the molecules I tracked with the computerized confocal system, but in their clinical context. The second was meeting families with babies who were born premature, when I took my own child to a private water class, and the impression I had about the change in these babies along the three-month class.

Have you encountered any obstacles while doing your research?

As we work with a sensitive population (young infants that were born premature), and as my research projects are ground breaking, the first challenge is the ethics approval. We have clinical trials committees in both the institute (university / hospital) and the head office of ministry of health. We overcame the difficulties by good hard work together with the committees.

Have you made any major discoveries to date, and if so, what are the potential implications?

Amongst the major discoveries are those regarding safety: there has been no report of infant illness associated to training in water. Immediate beneficial results include eliciting of an infant "quiet alert" state in the water, decreased muscle tone and spasticity, reduction in anxiety and stress levels, improved sleep and feeding, and general comfort due to decreased tension. Our developmental track results show significant long term improvement of 40 per cent and significant immediate improvement of 60 per cent in developmental tracks of babies receiving Infant Neuro Aquatics compared to babies who did not.

We conclude that Infant Neuro Aquatics adjusted for young infants born premature, can be safely applied and regarded as a suitable aquatic rehabilitation approach. Additionally, early initiation of Infant Neuro Aquatics adjusted for young infants born premature, during pick activity of cortical sub plate, may be beneficial for their neural development.

Do you envision any type of Infant Neuro Aquatics treatments emerging from your work and how they might be implemented for the benefit of the public at large?

Until now, and although water activity has been widely known and popular for toddlers, it has not yet been scientifically proven as a developmental treatment for infants and hence has not become a part of the regular "basket of treatments" recommended by medical insurance and clinicians for young infants. I hope that our results, and results of additional studies alike, will change the current approach "from the field, and upwards".

What long-term consequences would you expect your work to have on future research and potentially on healthcare, society and policy?

With additional study projects I would expect the health care establishment to acknowledge Infant Neuro Aquatics as a safe and efficient early developmental intervention, and will include it in the battery of treatments funded by the government. This will allow families from remote settlements and low income parents to reach Infant Neuro Aquatics and give their babies a better chance for good health and development.

Are you planning to extend this research further? Where might you look next and why?

I want to establish specific long term protocols for specific neural conditions / difficulties. In the present stage we built a general protocol for the most common clinical characteristics of premature young infants, and we added to it a "personal touch" to assist with specific difficulties of certain infants. Next, I will want to build long term protocols for specific neural injuries in babies.

Is there anything else you wish to add?

I wish to thank the parents of the premature infants for their trust and participation – to allow us to track their infants' development and train with them in the water.

I love my work, still exciting me every day – big present.

I hope that after all the hard work we do in this project; we will be able to give the families of babies with developmental risk and practitioners an organized "cookbook", or at least a set of recommendations in this approach, based on our scientific research.

Infant Neuro Aquatics: Good for babies, good for parents

An increasingly large number of infants are born prematurely due to modern medical interventions such as In Vitro Fertilisation. Many of these infants suffer from various developmental impairments, and Dr. Hagit Friedman work aims to treat these impairments by exposing infants to water activity, a technique which she calls Infant Neuro Aquatics.

PREMATURE BIRTH

Premature birth is the birth of a baby at less than nine months of gestation. In Europe and many developed countries the preterm birth rate is generally 5-9 per cent, and in the USA it has risen to 12–13 per cent in the last decades. A continuously growing phenomenon, prematurity is a risk factor for multisystem injury, as body systems of the premature infants are not ripe for life out of uterus. Health problems may include immediate and long term complications. In the long term, prematurity leads to high risk for brain injury and developmental impairments, ranging from Minor Neurological Dysfunction, through ASD, to Cerebral Palsy. The earlier the baby is born, the greater the risks.

Early intervention may minimise developmental deficit for infants at risk. Our […] results show 40 per cent long term and 60 per cent immediate improvement in developmental tracks of babies receiving Infant Neuro Aquatics compared to babies who did not.

Dr. Hagit Friedman has been focusing her research efforts on developing a technique named Infant Neuro Aquatics thought to aid premature infants develop normally. She explains that there has always been a "tradition" of water activity for children, mostly related to sports, and although, the nurses at the Neonatal Intensive Care Unit shared with her the positive changes in the behaviour of premature neonates after bath, there have been very few scientific papers in this field to rigorously study the effects of water activity on infant development and growth. Dr. Friedman therefore set out to do a careful scientific analysis, hoping to show that this activity is indeed good for babies at developmental risk. She also hopes to develop suitable protocols and assess which impairments can be treated in this way. Dr. Friedman is, to her knowledge, the only researcher working on the effects of water activity on very young infants who were born prematurely.

CAUSES AND TREATMENT

Preterm birth is the most common cause of death among infants worldwide. About 15 million babies are preterm each year (5 to 18 per cent of all deliveries).The causes of premature birth are frequently unknown, with diabetes, high blood pressure, twin, triplet, etc. pregnancies, obesity or being underweight, stress, etc. all contribute to an early delivery. In the last 20 years or so the fast development of IVF technology led to an increase in the number of preterm babies (many IVF pregnancies finish before term). However, recent advances in medicine enable increasingly more premature infants to survive. Indeed, complications from preterm births resulted in 0.74 million deaths in 2013 compared to 1.57 million in 1990. Many of these infants are nonetheless not healthy. Early assessment and detection of developmental impairments is crucial for early and effective intervention as early identification will support initiation of early treatment and may minimise neurological and functional deficits.

In developed countries premature infants are usually cared for in a neonatal intensive care unit (NICU). In developing countries where advanced equipment and even electricity may not be available or reliable, simple measures such as kangaroo care (skin to skin warming), encouraging breastfeeding, and basic infection control measures can significantly reduce preterm morbidity and mortality. The chance of survival at less than 23 weeks is close to zero, while at 23 weeks it is 15 per cent, 24 weeks 55 per cent and 25 weeks about 80 per cent. The chances of survival without long term difficulties when born in these early weeks are low

Some children adjust well during childhood and adolescence, although disability is more likely nearer the limits of viability. Studies of people born premature and investigated later with MRI brain imaging, demonstrate qualitative anomalies of brain structure and grey matter deficits within temporal lobe structures and the cerebellum that persist into adolescence. Throughout life they are more likely to require services provided by physical therapists, occupational therapists, or speech therapists.

INFANT NEURO AQUATICS

In many western countries children are now diagnosed at a relatively young age (1.5 - 3 years old), however diagnosis at this age may be late, due to the fast change in plasticity of brain neural circuits which regulate the infant's sensory, cognitive and emotional skills. Retrospective studies found that the majority of children diagnosed with neurodevelopmental impairment exhibited early behavioural signs during their first 12 - 15 months of life. Infant Aquatics have been found to benefit infant health, being based on the physical properties of water and their physiologic outcomes on the sensory, motor, cardio-vascular, and respiratory functions.

Preterm birth is emotionally traumatic for parents and may lead to depression, anxiety and stress, affecting parents' function, and the development of deep emotional bond with their infant. Parent-infant negative interaction in NICU may lead to non-functional parenthood. Early intervention of Infant Aquatics, starting in NICU and continuing at home and hydrotherapy pool may benefit parental resilience, function and bonding with the infant; and advance infant brain development, during an important developmental time window.

In the pool, babies are placed in warm water in vertical and horizontal positions, supported by the buoyancy of water and the caring hands of parent or hydrotherapist. Training starts with a set of pre-structured movements through which parents practise handling of the infant in the water, in a way that enables free and integrated movement, eye contact, vocal communication and increased confidence. The working technique employed is modified for premature infants and the specific needs of each infant. Training includes passive mobilisation, various rotations, relaxed floating, and 8-shape delicate mobilisation when the infant is supported under occiput and rib cage, and more. During this developmental stage infants have mainly spontaneous movements, which reflect neurodevelopment stages and abnormalities long before they are fully pronounced. Dr. Hagit has pioneering results showing that Infant Neuro Aquatics is positively correlated with better neural development indexes of the premature infants. Using the non-intrusive General Movements (GM) tool they show above 80 per cent versus about 30 per cent immediate developmental improvement without Neural Infant Aquatics and better coping parameters for the parents.

HEALTHCARE APPLICATIONS

Alongside publishing a scientific paper and participating in scientific meetings, Dr. Friedman has also been invited to share her experience with major teams of developmental clinics. It is her hope that these interactions and publishing the results, as well as this interview, will advance a more supportive approach / concept based on scientific data, and produce a position paper or even a white paper that will bring this intervention into the heart of "evidence-based treatments" for young infants. When this is achieved, and more infants can have Infant Neuro Aquatics treatments subsidised by their medical insurance, it is expected that the health and development of more infants will improve.

With additional study projects the health care establishment is likely to acknowledge Infant Neuro Aquatics as a safe and efficient early developmental intervention, and will include it in the battery of treatments funded by the government. This will allow families from remote settlements and low-income parents to reach Infant Neuro Aquatics and give their babies a better chance for good health and development.

Researcher Profile

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Dr. Hagit Friedman is a researcher at the Department of Nursing of University of Haifa and a Lecturer at Kinneret Academic College. Dr. Friedman's research projects focus on early diagnosis and early intervention in neural development, reflecting her academic knowledge and the clinical certification & training she did, in Israel and abroad. Dr. Hagit Friedman has a scientific academic background in neurobiology (MSc, HUJI) and in neurodevelopment and neuroimaging (PhD, T-IIT). Dr Friedman is also Dipl. in Acupuncture (TAU, IL), licensed in neurodevelopment assessment in multiple tools - basic and advanced problem solving level (RMUoHP, USA) and Dipl. in Infant Aquatics (Hydrotherapy unit EMC, IL). She is devoting her knowledge and academic work for the advancement of health and development of infants at risk.

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Helping children reach their fifth birthday in Sindh, Pakistan

Dr. Fauziah Rabbani of the Aga Khan University, Karachi has been at the helm of Project NIGRAAN since its inception in 2011. In this interview she discusses how the project has progressed and the measures being taken by policy makers to support the development of frontline healthcare in Pakistan.

To start, please tell us about your background.

I have been working as a public health specialist for the past 26 years and I am currently head of the department of Community Health Sciences (CHS); one of the largest departments of the medical college at the AKU Karachi, with 38 faculty and 170 staff. I am a health systems expert with a focus on implementation research. I have written several book chapters, indexed technical reports and I have more than 60 peer reviewed publications to credit.

My qualifications include MBBS (Karachi), MPH (USA), FCPS (Community Medicine) from College of Physicians and Surgeons Pakistan, FRCP (Edin) and PhD (Health Systems Research) from Karolinska Institutet Sweden.

How did Project NIGRAAN come to be?

Like other health system experts I have always felt that a critical challenge Pakistan is facing is 'governance within the health sector'. While focus on particular diseases or services is important, literature has shown that weak health systems and poor policy planning result in unnecessary child deaths; many never reach their fifth birthday.

In 2011, I participated in a meeting held by the Health Services Academy Islamabad where experts from the World Health Organisation (WHO) Alliance for Health Policy and Systems Research (AHPSR) conducted a workshop on setting priorities in health research investments using a validated systematic methodology called Child Health and Nutrition Research Initiative (CHNRI). Based on CNHRI criteria an implementation research question that we presented scored highly during this meeting. The question posited whether supportive supervision of Lady Health Workers (LHWs), a governance issue, by Lady Health Supervisors (LHSs) could bring about a decline in child mortality due to pneumonia and diarrhoea.

Later in the year we were invited to a refinement and protocol development workshop by WHO in Islamabad. It was here that Project NIGRAAN was technically conceived. A follow up meeting in 2013 was held in Montreux, Switzerland with the final proposal submitted in February 2013. Funding approval was received in May 2013 and the contract between AKU and WHO for NIGRAAN execution was signed in August 2013.

What are the main outcomes you hope to see from Project NIGRAAN?

The primary outcome is improvement in Community Case Management (CCM) of childhood diarrhoea and pneumonia in District Badin (rural Sindh). This would, in the long term, bring about a decline in childhood mortality among under-fives (Millennium Development Goal 4).

How will you judge the success of the main outcomes?

Through quantitative baseline and end-line community caregiver surveys, focus group discussions and key informant interviews with health workers/other stakeholders. The knowledge and skills of health workers will be measured via scorecards.

What are the secondary outcomes you hope to see emerge from Project NIGRAAN?

Improved knowledge, skills and supervisory processes among LHSs for CCM of pneumonia and diarrhoea in children under five. This will in turn lead to an improvement in knowledge, skills and performance of LHWs through structured supportive supervision by LHSs. Finally there will be improved knowledge of community caregivers through interactions with LHWs and LHSs during community case management of children with diarrhoea and pneumonia.

Project NIGRAAN lasts almost 24 months, what are the most significant changes you've seen in this time?

There has been improved communication and co-ordination between LHSs and LHWs. LHSs now accompany LHWs during follow up visits to the household and have more of a problem-solving approach. There has been improved knowledge and skill scores of LHSs in terms of diarrhoea and pneumonia following training

and regular monitoring through NIGRAAN. A written feedback system has helped this.

How can LHW and LHS improve their knowledge?

Regular refreshers on Lady Health Workers Programme (LHW-P) curriculum and clinical skills and mentorship training courses is the only way to improve knowledge. Unfortunately this has not been available for the last few years for LHWs and LHSs.

How has the national and regional government in Pakistan supported your work?

In January 2014 the first executive project management team meeting was held. The meeting provided a platform to bring decision makers and implementers together. A dissemination seminar in August 2015 sensitised participants regarding the use of evidence in health system decision making. Provincial and regional health departments will be incorporating the value of written feedback from LHSs to LHWs in their public policy development documents.

What can the international community do to combat child mortality?

Endorse and ratify through various conventions that access to good quality health care is a fundamental human right. Declare that any child dying due to diarrhoea and pneumonia in the presence of proven lifesaving community based interventions which can readily be delivered through peripheral health workers at their doorstep is a crime.

Overcoming the paradox: Children in Sindh dying in the presence of a vital human resource available at their doorstep

Aga Khan University, Karachi, is the leading academic institution within Pakistan. The Community Health Sciences Department has 38 faculty and 170 staff, who work on a number of projects including those focused on the improvement of health systems through implementation research. One of these is Project NIGRAAN, a WHO-funded project targeted at childhood mortality.

Diarrhoea and pneumonia are currently the leading killers of children under five worldwide. Indeed, according to the WHO, 2195 children die of diarrhoea, and 2561 will succumb to pneumonia every day. This means that in the minute or so it takes to read the next few paragraphs, three children will have died without ever having had the chance to go to school, to learn to read, or to do any of the things which we take for granted in our lives. This is a shocking statistic, and indeed one of the UN Millennium Development Goals is to reduce these deaths by at least two thirds. Unfortunately the majority of these deaths occur in rural, remote, and under developed areas, where distance and transportation time limit access to the hospitals and medical clinics which could otherwise treat the patients. Indeed, treatment for both of these diseases has been well established: antibiotics to kill the pneumonia-causing bacteria, Oral Rehydration Therapy (a solution of salts in water) to support the patient during their diarrhoea. Actually providing these treatments is another challenge – one that depends upon getting reliable medical support into remote areas.

One country that has been making major inroads into this problem is Pakistan. With almost 200 million inhabitants and a strong industrial base (as well as some exceptional cricket players), Pakistan is considered to be one of the up-and-coming economies (identified by investment bank Goldman Sachs as one of the 'Next 11'). However, the majority of the population is located along the Indus River, leaving large swathes of land to the north and west that are thinly populated and lacking in the amenities more common in the heavily populated cities. Indeed, despite their future prospects, Pakistan currently suffers from approximately 700,000 diarrhoea and 350,000 pneumonia cases annually in under-fives, only 40% of which are adequately treated. To prevent this, and to provide medical treatment across their entire population, the government of Pakistan has developed a number of programmes including those reaching out to local communities at their doorsteps.

THE LADY HEALTH WORKER PROGRAMME

One of these programmes is known as the "National Programme for Family Planning and Primary Healthcare", which is almost universally referred to as the "Lady Health Workers Programme (LHW-P)". This programme relies upon the recruitment of women from local communities to act as health care workers, able to provide basic services to women and children including vaccinations needed for polio. These women, known as Lady Health Workers (LHWs) are usually responsible for catchment areas of 100-150 households (approximately 1000 people), and are provided a salary by the government. Importantly, they must have completed at least 8 years of schooling and need to be a resident within their catchment area, thus encouraging connections with the people under their care.

These LHWs are then supervised by Lady Health Supervisors (LHSs), who are essentially experienced LHWs with additional training. Each supervisor has 20-25 LHWs working under her, and they are responsible for ensuring that treatment is adequately provided, collecting information on cases, and acting as an interface

between LHWs and the senior staff of the health care system. Overall, approximately 60 percent of Pakistan's population is covered by the Lady Health Workers.

Unfortunately there are a few problems with the programme that still need to be optimised, in particular with regards to the supervision of LHWs. An in depth evaluation indicated that slightly over 40% of supervisors never visit households covered by LHWs, as part of a joint visit or consultation. Feedback is limited: 61% of LHWs never received comments regarding their performance, while only 5% were ever informed that their performance was unsatisfactory (contrast this to reports from supervisors stating that they had personally informed LHWs in 85% of cases). This lack of supervision and feedback means that the LHWs cannot correct mistakes and develop their skills, which in turn limits their effectiveness in treating childhood diseases.

Somewhere in the world, pneumonia and diarrhoea will kill three children within the next minute. Project NIGRAAN hopes to break this deadly chain.

NIGRAAN

To fix these shortcomings, funding has been obtained from the World Health Organisation for a project known as NIGRAAN. Coordinated by Dr. Fauziah Rabbani of the Aga Khan University, NIGRAAN ('supervision' in Urdu, Pakistan's lingua franca) reflects the target aim of improving supervisor skills within the Lady Health Worker Programme. NIGRAAN is set up as a cluster-randomised trial currently running in District Badin, approximately four hours from Karachi (Pakistan's largest city, and indeed the second largest in the world). Badin has a total population of 1.1 million, covered by 1100 LHWs, who are in turn supervised by a total of 36 LHSs.

Of these health workers, 34 supervisors were chosen and randomly assigned (17 each) to either a control group or a group that would receive the enhanced 'NIGRAAN Training'. During the exploratory pre-intervention phase a questionnaire based household survey asking caregivers their opinions on LHW skills, reliability, and importance of LHW-P was conducted. A total of 170 LHWs (five for each supervisor) were then randomly selected for in depth knowledge and skill assessments using scorecards. Focus group discussions and in depth interviews were held with health workers, determining their opinions, experiences, and skills in both healthcare and supervision. Results indicated that the training level of LHWs and LHSs was sub optimal, with the overall knowledge of both diarrhoea and pneumonia falling below the desired level for the programme. Health worker knowledge was significantly better for diarrhoea than pneumonia, while 18% of the LHWs could correctly diagnose and advise treatment for diarrhoea, none could perform as well for pneumonia cases. Supervisors were slightly better, but often lacked clinical skills due to their lack of recent experience in the field. This lack of experience was also noted by households being served under the programme, as the majority of mothers surveyed (97%) would rather go directly to a doctor for treatment.

Following this initial information gathering stage, the project moved into the intervention phase. All supervisors were given a two day refresher training course, following the usual LHS curriculum, with a generalist focus. The supervisors included in the 'intervention arm' were then given a further four day course, this time with a specific goal of increasing knowledge of childhood pneumonia and diarrhoea. The course also included workshops on supervisory and communication skills, as well as 'hands on' teaching at a local hospital. Beyond this, the supervisors were given more comprehensive checklists for their supervisory roles, as well as report cards, these were intended to increase the likelihood of providing written evaluations to their LHWs.

SMS FOR A CURE

One facet of the NIGRAAN project involves steps to improve the overall flow of information between LHWs, their supervisors, and other senior staff within the healthcare system. As such, NIGRAAN also provides additional provision for increased case surveillance, essentially improving the detection and data gathering associated with each incident of suspected diarrhoea/pneumonia. A fixed protocol has been developed for obtaining information from the caregiver of the sick child by the LHW, notifying the immediate supervisor via SMS, and then planning a follow-up visit together. The LHS is then responsible for commenting on disease treatment by the LHW, including written feedback, and informing the central data manager at the same time. All cases of pneumonia or diarrhoea are logged, with GPS coordinates indicating precisely where they have occurred.

Improvements in feedback are provided by the emphasis on written performance evaluations, (which did not previously occur), intended to formalise the process of providing feedback and thus improve supervision quality. By providing every LHS of the Lady Health Worker Programme in Badin with a mobile phone (and LHWs with a communication allowance), NIGRAAN also improves the instantaneous flow of important information, removing the previous reliance on occasional face-to-face meetings or written reports. The development of this SMS based reporting system has almost doubled pneumonia and diarrhoea case reporting, with the vast majority of new cases being reported within 24 hours. Mobile phone based systems are enhancing numerous fields across many developing countries (witness the major changes caused by the African mobile banking system, M-PESA, for example), and so it seems only natural that they can be harnessed to improve healthcare outcomes as well.

EXAM WEEKS

The final six-month post intervention evaluation of knowledge and skills of LHWs and LHSs is due at the end of 2015. During repeated assessments conducted in the intervention phase it was observed that the general knowledge of pneumonia and diarrhoea increased dramatically with an almost three fold boost in test scores amongst LHSs given training. Supervisory skills were also noticeably improved, with the increase in supervisory scores in LHSs given NIGRAAN training being almost double as compared to those given no training. This is also evident in the sudden increase in feedback, with greater numbers of supervisors providing written feedback to their LHWs. This supportive supervision has translated into a two-fold increase in LHWs' knowledge and skills pertaining to diarrhoea and three-fold for pneumonia. Most importantly, there has been a boost in the level of community interaction observed. Supervisors are now more likely to accompany LHWs into the field, while the project has, in the words of one LHS, "helped us in restoring our contact with the community".

ONWARDS AND UPWARDS

So where to from here? Preliminary results from this study are, as previously mentioned, very promising. Those involved at all levels are interested in expanding the programme to larger parts of Pakistan, although a few kinks remain to be worked out first. Training systems need to be modified slightly to include more hands on work, and to encourage LHSs to be more direct in their mentoring of LHWs. The salaries available for LHWs and LHSs need to be regularised, as does the availability of transport within the areas of responsibility. And finally the availability of medical supplies needs to be enhanced, many caregivers of children commented that they would not bother seeing a LHW due to the likelihood that there would be no suitable medicine available.

Once these problems have been solved, however, the lessons learned from NIGRAAN will be extended across Pakistan and possibly into other countries. Dr. Rabbani is looking forward to the moment when this will occur; one of her deep-seated beliefs is that "access to good quality health care is a fundamental human right". Through the hard work of those such as her and the many LHWs and LHSs involved in the project, this universal access to healthcare may be closer than we think.

Researcher Profile

Dr. Fauziah Rabbani
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Dr. Fauziah Rabbani is the Head of the Community Health Sciences Department at Aga Khan University in Karachi. The department is one of the largest in the Medical College with 38 faculty and 170 staff. Dr Rabbani also simultaneously heads the health systems and policy research group at CHS, she teaches at undergraduate MBBS level and directs courses in Health Systems Research and Quality Management at graduate level. Dr. Rabbani is a visiting scholar at the Karolinska Institutet, Sweden and has academic collaboration with the Royal Tropical Institute of Amsterdam, DokuzEylul University, Turkey and the Chinese University of Hong Kong to name but a few. She is a visiting professor at ISTUD Fundazone Italy. Her work has won many accolades including a gold medal in research from the Pakistan Academy of Medical Sciences, the Commonwealth Award of Excellence and the WHO UNISOL prize. Dr. Rabbani is a health systems expert with a focus on implementation research, her work has been published in over 60 peer reviewed publications and several book chapters.

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Project NIGRAAN

In the frontiers of emotionally intelligent parenting

When most parenting programs sang the praise of behavioural reinforcement strategies, Dr. Sophie Havighurst and Ms Ann Harley realized there was a critical aspect missing – considering the role of children's emotions.

How did your work preceding the development of the Tuning in to Kids® program influence you to start working with emotional socialization in children?

S.H: During my training as a clinical psychologist I was working in forensic mental health, which made me aware that problems in understanding and regulating emotions played a large role in offenders criminal behaviour. I became interested in prevention and explored what forms of early intervention seemed effective.

I also spent a brief period with Professor Matt Sanders and Triple P, a behavioural parenting program. This program was one of the first to establish evidence for preventing behavioural difficulties in young children. I wanted to develop a similar program that had a greater focus on children's skills in understanding and regulating emotions, and that addressed the responsiveness of parents to children's emotions.

I moved to Melbourne in 1999 to do a PhD exploring this topic and during this time I was introduced to Ann Harley.

A.H: I had worked in schools and community organisations as a teacher, parent educator and counsellor for 15-20 years. Over this time I developed and facilitated parenting and support programs for parents, and conducted training workshops on parenting for professionals.

The experience taught me that most parents love their children and want to do their best. I noticed that the area parents struggled with most, was communicating effectively around emotions, especially during times of stress. They often lacked the understanding and the skills to manage such times and the solutions offered from the literature at the time were to focus on managing children's behaviour, without regard for the emotions driving the behaviour.

How did you come to realize that you shared a common interest in emotion coaching, and how this interest made you join forces to produce a parenting programme?

A colleague who noticed that we shared an interest in Dr. John Gottman's work around 'Emotion Coaching' introduced us in 1999. We realized that with our shared interest and our respective backgrounds, we could develop a program that was based on Gottman's research.

S.H: Ann and I shared an interest in the work of the pioneers who had conducted a landmark study examining the way parents respond to children's emotions. This study also investigated how parental response affected the way children manage emotions.

What excited Ann about Gottman's work was that he was the first to provide a clear and accessible framework for teaching emotion communication to parents. Most importantly, however, he also provided strong evidence showing that when parents were emotionally attuned to their children, the children were more likely to do well academically, socially and emotionally, with fewer behaviour problems and improved health outcomes. Conversely, children with difficulties regulating emotion have a more difficult path ahead, with a higher risk of developing conduct disorder and other mental health difficulties or experience substance abuse, poor peer relations, criminal behaviour and violence as they grow older.

A.H: We saw that traditional parenting programs aiming to handle difficult behaviour were not always successful. Recognising the importance of the recent research, we realized that there was an aspect missing in the current parenting programs – an aspect that if included might increase the success rate for interventions used with children with difficult behaviour.

When we met and started to collaborate on the Tuning in to Kids® parenting program, incorporating the research on emotion coaching, with all we had learned over the years, I felt truly excited. I began to feel that we had arrived at something that would really benefit families.

The Tuning in to Kids® program is based on previous research that you have translated into practice, backed up by a rigorous evidence base. How did you become convinced that you needed to provide an evidence base for your program?

S.H: My training in clinical psychology, PhD studies and my experience with Triple P, taught me that the best way to give a parenting program legitimacy was to provide evidence of its effectiveness. When Ann and I started working together, these ideas were still pretty 'fringe' and not encouraged by the establishment.

We were both very clear that this was a direction we wanted to take. We decided that we would not disseminate our program until we had enough evidence from randomized controlled trials to give our program the rigorous empirical backing that only a small number of programs had achieved.

Lending an ear to the emotional state of your children.

Developing emotional competence is a crucial step towards successful socialisation of children. Realising there was a gap between research and practice on this topic, Dr. Havighurst and Ms Harley turned the research behind emotion socialisation into an extensive evidence based parenting program – Tuning in to Kids®.

A NON-BEHAVIOURAL APPROACH TO BEHAVIOUR

Behavioural problems in children are not a new phenomenon; they are likely to have existed since time immemorial. The way we deal with difficult behaviours in the youngest members of our society has, however, widely shifted with the social influences of the time at hand.

For our predecessors, strict discipline was often seen as a virtuous path to good behaviour. During the 19th century Psychology, instead, turned to research underscoring the value of reinforcement contingencies – awarding desirable behaviour while punishing less desirable behaviour. As the scientific tide again turned, research saw a shift in focus towards the importance of emotions influencing child development and behaviour.

The early research on this theme springs from the notion that emotions are integral to human relationships, and hence greater focus should be placed on emotions in child development. The research by Gottman, Katz and Hooven that excited Dr. Havighurst and Ms Harley alike showed that behavioural problems in children were often tied to poor emotion socialisation in parenting. This research underscored the importance of the parent's socialisation practices on the emotional development of the child.

Gottman and colleagues described a number of different parenting styles. Some parents are emotionally dismissive, often warm and involved, but avoid or shut down emotions in their children and themselves. Others are emotion disapproving, believing emotions to be harmful, manipulative or unnecessary. Yet other parents have a laisse-faire parenting style, allowing all emotions but without setting boundaries around behaviour, not assisting with problem solving and not teaching children to understand and manage their emotions. Lastly, there are parents that are emotion coaching, noticing emotions in their children and seeing these moments as opportunities to be close and to teach their children. These parents also assist children in labelling their emotions, are empathic and accepting of the child's emotions, and if necessary they help their child to problem solve or to set limits around behaviour.

BRINGING THE RESEARCH TO LIFE

Tuning in to Kids® was born out of the realisation that this new research was not reaching those who needed it most. Starting out on a small scale, Dr. Havighurst and Ms Harley set out to create a parenting program that helped parents to emotion coach their children and also integrated aspects of attachment theory, emotion-focused cognitive behavioural therapy, mindfulness, child development theories and the neuroscience on brain-behaviour relationships. Together Dr. Havighurst and Ms Harley shaped the program to teach parents how to be emotionally responsive to their children as well as to help them understand and manage their own emotions.

It has made me a calmer parent and my child is calmer and we feel closer to each other. He is also better able to manage his behavior.

In order to gather the evidence for whether the program was effective in helping parents and improving children's emotional development as well as their behaviour, they assembled a battery of questionnaires and observation tools that allowed them to assess how parents responded to emotions in their children. Whether and how parents emotion coach proved to be challenging to measure. The

questionnaires available were often not accurate about what parents were doing when they or their children were really emotional.

"People often think they are emotion coaching, but when kids become emotional, it often brings out automatic reactions in parents that shut down the child's expression of emotions or escalates it. Consequently, questionnaires are not always that accurate at capturing whether people are emotion coaching." Working out these problems in evaluating this aspect of parenting required many years of trial and error to determine the best ways of measuring whether parents were learning to emotion coach.

CONSTRUCTING AN EVIDENCE BASE

Starting out on a small scale, Dr. Havighurst and Ms Harley set out to compile a pilot study assessing the effectiveness of their new program. The data from the pilot was encouraging enough to launch the first randomized controlled trials with parents in the community as well as parents with children who had clinical-level behaviour problems.

The first studies proved to be highly successful, showing significant improvements on nearly all outcome variables. Parents who received the Tuning in to Kids® program improved their ability to respond to children's emotions in supportive and teaching ways. Parents were also much less likely to be dismissive or critical when their children were emotional. "Our

research often shows that parents reduce emotion dismissing with statistically large effect sizes while we get small to moderate effect sizes in increasing emotion coaching. Empathy usually also increases more than parents ability to scaffold and teach about emotions. However, we believe, and other research also shows, that if you reduce the critical and negative environment this makes a really big difference to children's development and wellbeing."

Furthermore, children of parents participating in the program experienced significant reductions in behavioural problems. This effect was most clear for the children who experienced behavioural difficulties at the onset of the study and who had clinical level problems, however Dr. Havighurst and Ms Harley emphasize that Tuning in to Kids® is a program for all parents, promoting optimal development of all children. They want to avoid stigmatization of the program focusing only on children with behavioural problems. Since those first studies the program has been further adapted and disseminated to more than 3500 professionals who have been trained to deliver the program in a wide range of community and clinical settings.

EVER EXPANDING ADAPTATIONS

Since the first controlled trials, Dr. Havighurst and Ms Harley have completed another four randomized controlled trials studying the effectiveness of the program in different contexts with their colleagues Dr. Katherine Wilson and Dr. Christiane Kehoe as well as many other research assistants and students. The program has now been expanded in a variety of directions, including programs aimed at parents of toddlers and teenagers (for which they are currently conducting randomised controlled trials), a program tailored to suit fathers, and programs targeting childcare workers. The program has also been evaluated in different settings to prove that the program retains its validity when used in a community, school or clinical setting. Modifications for children with anxiety, chronic illness and children who have experienced complex trauma have also been conducted. Future studies are planned with teachers, students and parents in a whole of school approach to Tuning in to Kids/Teens, and the team are also collaborating with international colleagues to evaluate the program for use in other countries.

Dr. Havighurst and Ms Harley believe that emotion coaching is a style of communication that allows the recipient to feel understood, no matter their age. A colleague at the University of Canberra has begun a study to investigate the effectiveness of the program in the aged care setting.

WITHOUT WESTERNISED LIMITATIONS

With the help of international efforts, the program materials have now been translated into German, Turkish, Persian, Hebrew, Somali, Vietnamese, Amharic, Arabic, Norwegian and Chinese. These translations are valuable not only for using the program outside Australia, but they are also invaluable tools in the local setting. Many parents attending the Tuning in to Kids® programs have a culturally heterogeneous background. These translations take into account the different needs of parents from other cultural circumstances, assuming a profound respect for each culture. However, the idea to equip parents with tools to handle emotions remains the same.

"All of us, from whatever cultural background, experience emotions the same way. However, our attitudes and beliefs about emotion are culturally prescribed and affect how we behave towards our children and ourselves."

Dr. Havighurst and Ms Harley are on a mission to improve child and family psychological health in the world: "We believe we can make a difference in how people treat children on a much larger scale." Considering what they have achieved in only fifteen years, they are likely to succeed in their mission.

Researcher Profile

Dr. Sophie Havighurst
Senior Lecturer at Mindful, Centre for Training and Research in Development Health, Department of Psychiatry, University of Melbourne.

Dr. Havighurst is the Principal Investigator of the Tuning in to Kids® research program and a program author. She holds a diploma in clinical psychology and a PhD focusing on child psychology. She coordinates and teaches courses for child and adolescent mental health professionals and has a small private practice.

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Tuning in to Kids

SCIENCE APPLIED TO DAILY LIFE

> Application of basic science findings to our daily lives has become very common. These include commonly known facts such as: eating greasy food increases one's risk for cardiovascular diseases, an active brain decreases the chance of developing dementia at old age, physical activity has numerous health benefits, protecting the skin against sun exposure decreases the risk of skin cancer, and many more. One very good example that shows how basic science can impact our lives is the aging population phenomenon, which is a consequence of the increase in life expectancy observed in the last century. This is a direct result of the findings of scientists that have examined factors related directly to aging as well as other pathophysiological alterations that can

> contribute indirectly to the process of aging. This section will examine the work of five dedicated scientists that aim to translate basic science concepts to improve our quality of life. The first article examines Dr. Margot Skinner's work on the importance of posture for a good night's sleep, especially for the management of obstructive sleep apnea hypopnea syndrome. The second is from Dr. Stephen Lloyd, who has proposed a protective enzyme, cv-PDG, for DNA repair after UV damage. The use of this enzyme could be an alternative for sunscreen and could importantly decrease the development of skin cancer. The last two articles demonstrate different solutions for eye problems and diseases. Dr. Hideto Sagara comments on his combined treatment approaches for the control of bleb leak in patients with glaucoma, and Dr. Mark Willcox discusses his contact lens disinfecting solution to prevent contamination and infectious diseases in the eye.

The Power of Sleep

Dr. Margot Skinner is a physiotherapist and senior academic at the School of Physiotherapy, University of Otago, New Zealand. Her research has focused on the management of obstructive sleep apnoea hypopnea syndrome.

To aid our readers better understand your work, please tell us how your research background led to your interest in sleep and obstructive sleep apnoea?

As a physiotherapist, my focus is all about optimising people's physical function to improve their quality of life. My particular area of practice and teaching has always been centred around people with acute conditions especially those with respiratory and cardiac compromise. People with obstructive sleep apnoea (OSA) are hugely compromised during the day from respiratory events that happen during the night. At the time I was searching for a topic for my PhD, a new sleep laboratory had just been established in the respiratory department at the local hospital. It seemed as if the door had opened for me to begin some research in an area which is focused on respiration, or lack of it as happens in OSA, where there were still many unanswered questions.

What treatments and technologies do you believe are most promising for OSA?

The evidence is clear that continuous positive airway pressure (CPAP) is the preferred management, particularly for those with moderate to severe OSA. However compliance can be a big issue as CPAP involves wearing a mask over the nose, which is attached to a machine that blows air down the throat to keep the upper airway walls open during sleep. Another device some find works for them is wearing a splint that fits over the teeth. The splint is designed to pull the lower jaw forward in order to open the upper airway, but there can be side effects relating to the constant pressure on the teeth every night. For mild OSA, or for those where use of nasal CPAP is unsuitable, we have found that conservative management such as use of an elevation pillow or application of the anti-supine device may work well. We have demonstrated these have none of the side effects or compliance issues associated with the other devices.

Many people are probably not aware of the long-term potential consequences of sleep apnoea. What would you like our audience to know about the disorder?

OSA is largely underdiagnosed and undertreated but when associated with daytime symptoms it is actually present in 4% of men and 2% of women across the world. There is a high risk of dying from untreated OSA compared to no OSA. In people with OSA during sleep the upper airway closes, or partially closes, causing breathing pauses and the lack of air being drawn in leads to a drop in blood oxygen levels. The body responds by waking and as the breathing starts again there is a swing in blood pressure. The disturbed sleep (often more than 30 awakenings each hour) has daytime consequences such as morning headache, sleepiness, impaired memory and concentration, depression and lethargy and is associated with an increased risk of stroke, heart attack and heart rhythm disturbances.

You've conducted significant research into the use of anti-supine devices. How can patients use these devices effectively in their daily lives?

For some people OSA is position-dependent. That means the airway obstructions during sleep occur in the supine position, or while sleeping on the back. It is estimated that around 55% of OSA is position-dependent. Thus the avoidance of sleep on the back seems a logical way to manage position-dependent OSA. Anti-supine devices are designed with that in mind. Besides preventing sleep in the supine position our anti-supine device has novel but simple design features to aid compliance. It is lightweight, comprising foam covered stockinet and a polystyrene ball, and Velcro fasteners. It is made to measure, inexpensive, washable and can be easily applied by the individual.

How has your previous work in cardio-pulmonary physiotherapy influenced your current research?

The leading cause of mortality in the world today is as a result of non-communicable diseases sometimes referred to as 'diseases of lifestyle'. One of the major contributors is physical inactivity. Cardiopulmonary physiotherapy is focussed on wellness, and improving quality of life through physical activity and education. Many people with OSA are physically inactive, obese, and have diabetes and high blood pressure, which are also all hallmarks of non-communicable diseases.

Have you worked with any other researchers?

The concept to undertake research on conservative approaches for the management of OSA was mine but I had a really supportive team of supervisors and advisors during my PhD, one of whom was undertaking research into mandibular appliances for the management of OSA at the time. In another study that was similar to the one with the anti-supine device versus nasal CPAP, I used an elevation pillow. At the moment we are considering a study on the relationship between OSA and physical inactivity.

Use of Anti-supine Device in the Management of Positional OSA

Sleep health is an important component of health in general. Most people spend a third of their life sleeping and yet it is the component that is often overlooked and undervalued both by the individual and by health professionals.

SO MUCH MORE THAN SLEEP

Emerging research is linking sleep deprivation with a long list of 'diseases of lifestyle,' but sleep quality is often overlooked by healthcare professionals. Sleep disordered breathing ranges from mild snoring to severe obstructive sleep apnoea (or OSA). With OSA, the upper airway either partially closes by 50% or more (hypopnea) or fully closes (apnoea) and inhibits breathing.

Obstruction of the airway typically creates these pauses in breath. This obstruction can be a large tongue, or large tonsils, seen mainly in children, or throat muscles that relax too much. All of these obstructions fail to allow normal breathing during sleep and can block the flow of air. Once the airway is blocked, the body's natural survival mechanism takes over and the individual may choke or gasp to wake and resume normal breathing. Unfortunately, the airway becomes blocked again moments later and the cycle continues throughout the night.

In OSA during sleep, the hypopnoeas and apnoeas result in a decrease in oxygen saturation in the blood. When the breath pauses are associated with daytime symptoms it is technically called obstructive sleep apnoea hypopnea syndrome (or OSAHS) and can cause a variety of health conditions. People with OSA are at risk for diabetes, stroke, high blood pressure, depression, headaches, and daytime sleepiness.

Over time the receptors that sense chemical stimuli related to blood pressure are put on heightened alert with the result that overall daytime blood pressure increases. Also, the body's sensitivity to insulin and blood sugars is negatively affected, leading to an increased risk of diabetes.

OSA/OSAHS is largely underdiagnosed in the adult population, but based on the results of the renowned Wisconsin Sleep Cohort Study it is known that 4% of middle aged men and 2% of women would meet OSAHS diagnostic criteria.. According to Dr. Skinner: "If OSA is subsequently diagnosed then it needs to be managed because the long-term consequences clearly show there is a higher risk of dying compared to those without OSA, and the risk is independent of factors such as age and obesity."

In adults with OSA, the condition usually cannot be cured. Therefore, OSA needs to be managed on a nightly basis in order to reduce daytime symptoms. Although studies have shown that regular physical activity can help with daytime symptoms, the upper airway obstruction will not go away because someone loses weight around the neck, even though symptoms may improve.

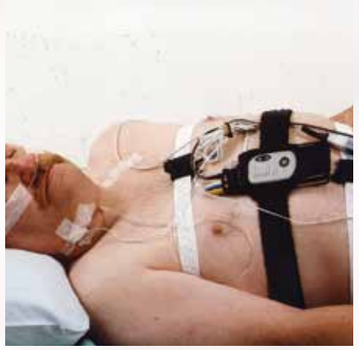
Over the years there've been many devices that have been designed to help patients whilst they sleep. Unfortunately many of these devices lack comfort and durability and therefore compliance becomes a serious issue. We know the device that works best is the nasal CPAP but it is not always well tolerated.

People with OSA are at risk for diabetes, stroke, high blood pressure, depression, headaches, and daytime sleepiness.

IMPORTANCE OF POSTURE

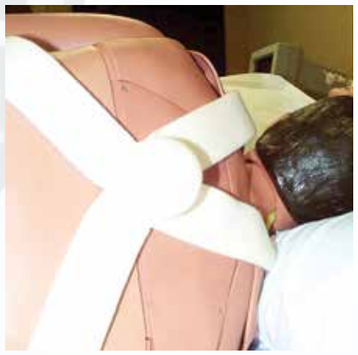
Research has indicated that posture can influence the severity of OSA. There is a subgroup of patients that have positional OSA. In these adults, the number of apnoea and hypopnoea events is twice as high, or more, when sleeping on the back when compared to lying on the side or stomach. It is therefore best for these patients to avoid sleeping in the supine (lying on the back) position.

Dr. Skinner's research has focused on the efficacy of anti-supine devices and how they manage positional OSA. Dr. Skinner believes that for the majority of position-dependent patients, an anti-supine device is an effective and simple way to manage their condition. This is especially the case when the nasal CPAP is not tolerated. With mild positional OSAHS, an anti-supine device most importantly prevents sleep in the supine position and reduces the night time obstruction and physiological effects (such as increased blood pressure), as well as the associated daytime symptoms. An anti-supine device is easy to assemble and can be tailored to the individual for optimum comfort.



Setup for home sleep monitoring

Antisupine band



Antisupine band in side lying

For adults with moderate to severe OSA, studies have shown nasal CPAP is superior to anti-supine devices. However, it's vital to remember that in order to be effective, the nasal CPAP must be worn for four or more hours during sleep. Nasal CPAP therapy is a non-surgical device, similar to an air compressor that is placed near the patient. A tube connects to a mask that fits over the patient's nose and delivers a steady flow of air. The air flow has enough pressure to keep upper airway muscles from collapsing and blocking the passage. As comfort and compliance issues can be associated with the use of nasal CPAP this has led to the exploration of alternative therapies.

Dr. Skinner has studied alternative devices to the nasal CPAP for OSAHS. Although the physiological outcomes with nasal CPAP were better, the use of an elevation pillow has a place in the management of mild OSA. The pillow works by elevating the head and torso to reduce the weight of gravity on the upper airway. This may be a cost effective way of managing OSA when compliance or side effects from other devices are an issue.

Additional research has been performed to test the efficacy of other devices such as: a neck collar designed to hold the head in a neutral position (cervico-mandibular support collar), a mandibular advancement splint (to pull the lower jaw forward), elevated posture using an elevation pillow, and a device to prevent the supine position (thoracic anti-supine device).

HOW HEALTH PROFESSIONALS CAN HELP

Thankfully there has recently been a heightened level of interest in the health literature regarding the association between OSA and daytime symptoms. This had led to more healthcare professionals being aware of the importance of diagnosing OSA. However, a major problem to overcome is that society is generally not aware of the fact that daytime symptoms of sleepiness, including poor memory, mood swings and lethargy may be a result of a condition that actually happens during sleep.

People, who live alone are not likely to have breathing pauses witnessed, so are unaware they have a sleeping disorder. In addition, those with witnessed breathing pauses are often reluctant to bring the matter up during a consultation with a healthcare professional. "I always urge my students and colleagues when assessing a patient to routinely include a simple question about sleep health just as we ask a patient about their normal level of physical activity and whether they smoke. In this way physiotherapists can be proactive about referring on if there are some pointers arising from the patient's history" says Dr. Skinner.

Where OSA remains undiagnosed and untreated, there are potential negative consequences. For the subgroup with mild position-dependent OSA at least, simple management with an anti-supine device may be all that is required to manage the OSA and reduce daytime symptoms.

TESTING FOR OSA

An adult who has breathing pauses during sleep and daytime symptoms may wish to undertake a home trial of sleeping off the back. A subsequent reduction in daytime symptoms can be indicated by improved (lower) scores on a standardised sleepiness questionnaire, such as the Epworth Sleepiness Scale. It is important for people to know that the physiological consequences of OSA that occur during sleep do not always match the daytime symptoms, therefore a risk assessment by a medical doctor and then referral to a sleep clinic for a diagnosis, needs to be undertaken in order to confirm a diagnosis and to determine the optimal long term management.

THE FUTURE OF OSA RESEARCH

Dr. Skinner hopes that medical and other health professionals and patients will realise that OSA is a common type of sleep disordered breathing as it is both underdiagnosed and undertreated. With the global epidemic of non-communicable diseases predicted to get worse over the coming years, diabetes, obesity, physical inactivity and other factors will be even more prevalent. Untreated OSA will only add to this burden unless we translate the knowledge we have to better inform our global community about such consequences.

There are some studies that have shown that physical activity and structured exercise can have a benefit on daytime symptoms in people with OSA, thus improving quality of life. Dr. Skinner plans to explore this concept in more depth, in particular with regard to the timing of exercise during the day.

Researcher Profile

Dr. Margot Skinner
Senior Lecturer
School of Physiotherapy, University of Otago, New Zealand

Dr. Skinner is a researcher and senior lecturer at the School of Physiotherapy at the University of Otago in New Zealand. She is also Vice President of the World Confederation for Physical Therapy. Her research interests have been led by her desire to optimise people's quality of life and have centered on acute respiratory and cardiac conditions. Currently she is testing the efficacy of treatments and management devices for obstructive sleep apnoea (OSA). She has published many studies on OSA and has presented in multiple international physiotherapy and health related conferences.

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Nuclear sunscreen

Dr. Stephen Lloyd has been working on the mechanism of DNA repair after UV damage. Here he talks about the development of a protective enzyme, cv-PDG.

To begin, could you tell us more about yourself? What brought you into this field?

As a graduate student at MD Anderson Hospital and Tumor Institute, I worked on the mechanism of action of the anticancer drug, bleomycin, which creates a plethora of DNA damage leading to effective tumour cell killing. After learning about an agent that creates so much DNA damage, I wanted to learn how cells respond to this damage and their strategies to repair it. As a postdoctoral fellow with Philip Hanawalt at Stanford University, I began studies to characterize enzymes that would potentially augment DNA repair of ultraviolet light-mediated DNA damage. These investigations led to a career-long pursuit to ultimately develop these UV repair enzymes into therapeutically relevant molecules.

Thirteen years passed between the characterisation of cv-PDG in 1998 and the cv-PDG-NLS-TAT variant reported in 2011. What made you decide to come back to the protein and continue development?

The progression and timing of research investigations are largely driven by successes in obtaining peer-reviewed funding. For more than two decades, our NIH R01 funding from the National Institute of Environmental Health Sciences had UV-induced carcinogenesis as a central focus. However, these investigations were primarily directed toward understanding the structure-activity relationships for this class of enzymes, and the contexts in which activation of the repair pathway for pyrimidine dimers could be beneficial to eukaryotic cells. In none of those studies were we specifically trying to develop a drug for therapeutic trials.

However, given the clinical successes of AGI Dermatics in using the bacteriophage T4 pyrimidine dimer glycosylase in xeroderma pigmentosum patients, and knowing that the Chlorella virus enzyme was superior in catalytic efficiency, thermal stability, and substrate specificity, we considered that this enzyme might be significantly more effective than their T4 enzyme. Further, data showed that when the T4 enzyme was introduced into cells, very little of it became localized to the nucleus where it needed to function.

To address this problem, we genetically modified the enzyme to contain nuclear localization sequences and, to overcome challenges associated with the transdermal delivery of an intact enzyme, we adopted the TAT delivery peptide as a modification for efficient delivery to cells. However, to move these types of studies from the basic to the applied sciences, it was necessary to form Restoration Genetics, Inc. and avail ourselves of NIH-sponsored small business grants. Through these funding mechanisms, we have been able to guide studies that take these enzymes through large-scale fermentation, purification, and encapsulation, with preclinical efficacy, pharmacology and toxicology trials.

Do you think the Cv-pdg protein will be incorporated into products such as sunscreen for general audiences, or will it be a prescription product for those at higher risk of skin cancer?

As a genetically modified form, containing the nuclear localization sequence or the TAT delivery peptide, these enzymes would be classified by the FDA as drugs. Thus, they would not be sold in an over-the-counter formulation. Even if the enzyme was purified from virally infected green algae, with no genetic modifications, it is unclear that it could be sold in a sunblock formulation. The reason for this is that the function of the enzyme is to change the structure of DNA from a damaged form (the cyclobutane pyrimidine dimer) to its original, undamaged state – and the FDA classifies any molecule that alters the structure of DNA as a drug. We believe that it will be important for the FDA to consider whether natural product-based therapeutics that restore DNA to its previous undamaged state warrants classification as a drug. If this hurdle could be overcome, then commercial sunscreens could contain this purified repair enzyme.

You have started a spin-off company, Restoration Genetics, to commercialise this discovery. Could you tell us more about your thoughts and experiences during this process?

Even though there are many parallels between directing and managing a basic research laboratory and the founding and operations of a small business such as RGI, there are unique challenges to maintaining the success of the company. The first is that there are distinctly different goals for our company versus the specific aims of a basic research grant: milestones versus hypothesis-driven investigations are significantly different and required changes in management that we had not previously experienced in academic research.

Also, the timeframe and cost to bring a fundamental basic science discovery to the first clinical trial is intimidating. In this regard, although there are multiple funding mechanisms to launch ideas, the processes to further refine lead compounds, define their markets, and the competition for the next major infusion of funding have been a continuous challenge. In this entrepreneurial environment, tenacity to contribute to the betterment of human health and wellbeing is essential.

Restoration Genetics has been successful in obtaining government development grants. Where to from here?

The governmental grants that we have obtained have been absolutely instrumental in achieving the successes that have accrued to date. The outcomes of the preclinical toxicology and efficacy studies will largely inform and guide the remaining preclinical investigations required by the FDA. We are actively seeking partnering opportunities to complete the preclinical studies and obtain financing for the phase I clinical trials and beyond.

Beyond sunscreen – solar repair

Founded in 1887, the Oregon Health & Science University is a world-class teaching hospital and research institution with the causes and cures of cancer amongst their top research priorities.

Most of us will have had this experience at some stage in our lives, usually right after spending a glorious day at the beach, in the park, or hiking in the mountains. That evening we look in the mirror, realising, as we look at our incandescent red glow, that sunscreen would probably have been a good idea after all. The days following, in which we progress from tomato-red to zombie-skin-flaking, act as a strong reminder of the importance of UV protection. Unfortunately, the effects of sunburn are far worse than the merely aesthetic. Too much exposure can damage skin, witness the leathery look of aged sun-bathers, and in a number of cases, can lead to skin cancer. Indeed, skin cancer is one of the most common types, making up over 40% of all cancer diagnoses, with 1.5 to 3.5 million new cases diagnosed each year in the USA alone with a cost of over 8 billion dollars.

An algal virus may hold the key to repairing UV-related DNA damage.

The majority of damage comes from ultraviolet (UV) radiation, which is energetic enough to cause a number of unwanted chemical reactions directly within our cells. The most dangerous of these, those which act as the precursor to cancer formation, involve reactions between atoms inside DNA strands. One of the most common UV-related consequences is known as pyrimidine dimers, in which two of the molecular letters of our DNA code (C & T, out of the usual bases: GATC), cross-link to each other, forming a distortion in the usual DNA structure. This damaged section in turn interferes with the usual DNA reading and replication processes that can ultimately lead to mutations in the genetic code. Over time, mutations in the genome can give rise to the uncontrolled cell growth which is characteristic of cancer.

Given the danger of UV radiation, and the relative ubiquity of sunlight, it is no wonder that living organisms have a number of methods available to repair this kind of DNA damage. The version that mammals such as humans use

is known as Nucleotide Excision Repair (NER). In this process, the misshapen section of DNA is recognised by enzymes and then a relatively short section of one strand is cut out. As DNA is double stranded, the other undamaged strand can then be used as a template to accurately fill in the remaining gap, leaving a complete and accurate stretch of genome.

SUNBURNT THUMBS

While this process is effective, it involves a number of steps and can thus be somewhat slow. In contrast, a subset of bacteria and viruses, having no protective 'skin' and thus being far more sensitive to UV radiation, use a faster process known as Base Excision Repair (BER) in addition to their own NER system. Here, the unnatural base pair is detected and then 'flipped', being rotated by enzymes such that it points outside the DNA strand rather than, as usual, in towards the core. Poking out like a sore (possibly sunburnt) thumb, these flipped bases are readily detected and cut out by damage control enzymes, after which the remaining gap is refilled with the correct bases. BER is fast and effective, but the first step, detecting and removing UV-damaged DNA, requires specific enzymes to proceed. Enzymes which, curiously enough, are completely lacking in humans.

This is where recent work from Stephen Lloyd and Amanda McCullough of Oregon Health & Science University comes into play. It has long been known that humans have an effective and complete BER system for many forms of DNA base damage but lacks only an enzyme

to begin the process of UV damage repair. However these enzymes, known as pyrimidine dimer glycosylases (PDGs), are often found in other forms of life, such as bacteria and viruses. The question Drs. McCullough and Lloyd are answering is: can these enzymes act to fill the gap in the human DNA repair system?

The answer began in 1998, when Drs. Lloyd and McCullough, in collaboration with Dr. James van Etten published the discovery and characterisation of a PDG protein from a Chlorella virus (viruses which infect the single-celled aquatic algae known as Chlorella). This protein, cv-PDG, was unique compared to other PDGs in that it had a wider range of possible substrates (damaged DNA sections which it could recognise). This flexibility, combined with the general stability of the protein, made for significant potential.

A BETTER PAIR OF SCISSORS

Flash-forward to 2011, when a new publication comes out, this time describing a new and improved version of the cv-PDG protein. Firstly, the protein has an extra piece attached, a "TAT" peptide sequence derived from a harmless HIV protein. This peptide allows the protein to pass through the cell membrane, effectively jumping over the fence which separates inside and outside. This is where the second attached peptide comes into play. Unlike bacteria, humans corral their DNA strands into a specific compartment in the cell, known as the nucleus. Proteins can be delivered to the nucleus only if they have the correct 'address' written on them, the nuclear localisation sequence (NLS). By attaching this sequence to the cv-PDG protein, it is automatically sent to the correct location to start repairing DNA.

The important question, then, is: does this protein actually work? Yes, yes it does. Initial tests were performed with cultured keratinocytes, essentially free-living skin cells in a flask, while later work involved tests on lab-grown skin. When these cells are hit with ultraviolet light, there is immediate formation of damaged DNA such as pyrimidine dimers, damage that can be seen over 24-72 hours later. But when the modified cv-PDG protein was added to the mix, it rapidly soaked into the skin cells and was then promptly transported to the nucleus. Upon repeating the ultraviolet blast the damage was repaired extremely rapidly, rapidly enough that very few pyrimidine dimers could be detected even after the time needed to harvest the cells or tissue. Cv-PDG was thus able to enter skin cells and rapidly initiate repair of the UV-induced DNA damage, all without further effort from the researchers.

Given the success of this protein in repairing UV-related DNA damage, Dr. Lloyd began to suspect that it may have a use as a therapeutic for those with higher risk of skin cancer. To follow up on this idea, he and his long-time research and life partner, Dr. Amanda McCullough, set up a spin-off company to develop the technology. Restoration Genetics Inc., as it is known, has successfully raised over 1.5 million dollars in funding from the US government. Their current goal is to extend their knowledge of the protein in various model systems, with an eye to beginning the long process of gaining approval to turn their discovery into a therapeutic.

A long process, to be sure, but a rewarding one. As Dr. Lloyd comments, "melanoma and non-melanoma skin cancers are escalating at alarming rates worldwide, with exposure to harmful UV irradiation being at the root of this epidemic. Decreasing the cellular burden of UV-induced DNA damage is an important component for disease prevention." Through their work with the cv-PDG, Restoration Genetics hopes to assist in this last, challenging step. Perhaps, one day, summer will no longer be a time of incandescent sunburn and worry over skin cancer.

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Having completed his BS (Biology) with a major in marine pollution biology, Dr. Lloyd found his interest turning towards chemotherapy and soon gained a PhD in Molecular Biology from the University of Texas. With a career containing stints at Stanford and Vanderbilt Universities, as well as industry employment and two directorships via the University of Texas, Dr. Lloyd has had significant success along the way. He is currently joint-head of a laboratory with his wife, Dr. McCullough, their efforts focusing on the mechanisms of DNA damage and repair.

KEY COLLABORATORS:
Dr. Amanda McCullough, Oregon Health & Science University
Dr. James Van Etten, University of Nebraska

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Counteracting Complications of Glaucoma Filtration Surgery

Dr. Hideto Sagara is an interventional ophthalmologist, who is interested in testing new treatments for cataract and glaucoma. Here we discuss the background and motivation of Dr. Sagara, together with the outlines of his current study on a new treatment approach to control one of the most common complications of glaucoma filtering surgery.

To start, what is your academic background and how did you pursue the early steps of your career in medical research?

I have graduated from Fukushima Medical University, Japan in 1993. In 1999, I was appointed as the director of Marui Eye Clinic, a clinical centre where more than 500 patients a year undergoing operations such as cataract and glaucoma. I have obtained my doctoral degree in ophthalmology in 2002, and my area of expertise is glaucoma and other diseases affecting the eye surface. I am also involved in academic teaching activities, since I work as a part-time instructor at Fukushima Medical University since 2007.

What has been your motivation for choosing ophthalmology as a domain of specialization?

Since I was a medical student, I have been intrigued by the eye as a sophisticated organ in which a variety of anatomical and physiological functions are amazingly synchronized. I am also passionate about delicate hand work, which I have been good at since I was young. Therefore, ophthalmology was the right domain for my interests and passion.

You are currently testing a new treatment regime combining two different eye drops to treat a condition known as 'bleb leak'. Can you describe to the reader what bleb leakage is and its potential complications?

Bleb leak is a possible complication of the filtering surgery, which is one of the most common treatments for glaucoma. Patients with glaucoma suffer varying intensities of blurred vision, usually due to an increase in the eye's inner fluid, which in turn places pressure on the optic nerve. The filtering surgery allows drainage of aqueous humor from within the eye to underneath the conjunctiva where it is absorbed. Bleb leaks occur almost directly from the anterior chamber (AC) of the eye ball, resulting in a shallow AC with severe hypotony, which usually occurs due to a defect in epithelial regeneration at the thin and avascular bleb surface. Bleb leaks may lead to severe damage and inflammation of the eye tissue, which can eventually lead to blindness if remains untreated.

How many patients are participating in the clinical trial and what are its primary goals?

So far, the trial involves more than 50 patients, who do not only suffer a bleb leak following filtrating surgery and mitomycin C treatment, but also severe eye surface disorder, to which the combined treatment strategy is also highly relevant. The primary goal of the study is the efficacy of the treatment strategy in terms of epithelial healing and the complete cessation of bleb leak over a long period of time.

In case of a positive outcome, could the findings of the clinical trial motivate a change in the current treatment guidelines for bleb leakage?

Currently in Japan, patients with bleb leak are usually treated only with separate sodium hyaluronate and autologous serum eye drops, a method that has been proposed and verified by my research group. However, it is difficult to continue the treatment for more than a few months, because patients often fail to adhere to the treatment regimen, which involves daily frequent application of the eye drops. If the combined treatment regimen under test in the ongoing trial shows promising results, this will strongly motivate a change in the current treatment guidelines of bleb leak, at least in Japan.

New Clinical Trial on Combined Treatment Approaches to Control Bleb Leak

Bleb leak is a serious complication of the filtering surgery, one of the most common treatments for glaucoma resulting from the increase in the eye's fluid pressure. Here we introduce Dr. Sagara's current clinical trial in which he tests the efficacy of combining multiple treatment approaches for the control of bleb leak.

GLAUCOMA AND THE FILTRATION PROCEDURE

Glaucoma refers to a group of eye disorders that cause damage to the optic nerve responsible for vision. If untreated, glaucoma causes blurred vision which may eventually progress to blindness. Glaucoma is currently the leading cause of irreversible blindness worldwide. A recent study estimates a current global prevalence of 64.3 million cases among people aging 40-60 years, and expects the number to reach 76 million in 2020, and 112 million in 2040.

In most of the cases, glaucoma is caused by an increase in the fluid pressure inside the eye. Our eyes are filled with a clear fluid called the aqueous humor, which maintains their shape and provides nourishment to the lens and the cornea. In healthy eyes, the aqueous humor is produced and drained at nearly equal rates in order to keep the fluid pressure within physiological ranges. However, in some disease conditions where the aqueous humor is produced in excessive amounts or inefficiently drained, glaucoma can develop.

The modern approaches in glaucoma management aim to avoid damage of the optic nerve, and to preserve vision and total quality of life for patients, with minimal side effects. Among these approaches is a common surgical intervention known as 'glaucoma filtration surgery'. The latter involves making a partial thickness flap in the white of the eye (sclera), where a tiny window opening can be made underneath the conjunctiva. The flap is then loosely sutured back in place to allow the excessive fluid to escape through the opening, which restores normal eye pressure. The surgical procedure typically results in

Bleb leak

the formation of a small fluid 'bleb' on the sclera, the reason why the overall structure is commonly known as the 'filtering bleb'.

Scarring can occur around or over the flap opening, causing it to become less effective or totally ineffective. This outcome is traditionally avoided by the topical application of chemotherapeutic agents, such as the famous 5-fluorouracil and mitomycin C, which inhibits the proliferation of the scleral matrix cells to prevent scarring. However, the use of these anti-proliferative agents may lead to defective epithelial regeneration at the filtering bleb surface as a side-effect. If these defects remain untreated, aqueous oozing, known as 'point-bleb leak', occurs. Bleb leak due to epithelial defects usually occurs within three months to a few years after the filtration surgery and is therefore commonly described as late-onset bleb leak. Indeed, studies have reported an increased incidence of late-onset bleb leak and blebitis since the introduction of mitomycin C in glaucoma filtering surgery. In severe cases, point-bleb leaks may progress to profuse leaks, which can result in vision-threatening complications. Another risk factor for the occurrence of late-onset bleb leak is the tear dysfunction, since a well-functioning tear film plays a role in promoting epithelial healing which helps resolve the defective bleb wall. The tear film consists of an aqueous layer and a lipid layer, which both are essential for the formation of a healthy tear film. The lipid layer is secreted by the meibomian gland, whose secretions are termed the 'meibum'. The most common cause of tear film dysfunction is obstructive meibomian gland dysfunction (OMGD), which can further promote the late-onset bleb leak, if concurrently occurs in patients who underwent filtering surgery.

MANAGEMENT OF LATE-ONSET BLEB LEAK

Over the past 15 years, Dr. Sagara and his research team has been investigating treatments for late-onset bleb leak. Previous findings by Dr. Sagara as well as other researchers have shown that the aqueous layer of the tear film can be augmented by Sodium hyaluronate eye drops and autologous serum eye drops. In 1998, Dr. Sagara and his colleagues have tried to stop bleb leakage with autologous blood injection into the leaking bleb. Although, the leakage had stopped for a while, it recurred in only a few days. However, it was found that the epithelial defect of the bleb wall was slightly improved after the blood including serum injection. As mentioned above, Dr. Sagara suspected that serum eye drops would be useful to treat the bleb leak by augmenting tear film volume and improving the tear film condition. The autologous serum includes almost all of the same components as the aqueous layer of the tear film, just not the lipid component. Therefore, Dr. Sagara has speculated that autologous serum cannot be possibly used as a stand-alone treatment, and that combining other treatments targeting the augmentation of the lipid component might be advantageous. Indeed, current trials are underway to investigate the efficacy of treatments for OMGD, such as eyelid massage and warm compresses in the management of severe epithelial failure arising from tear film dysfunction. These treatments particularly aim to stimulate the secretion of the meibum in order to improve the quality of the tear film.

A CLINICAL TRIAL ON COMBINED TREATMENT-APPROACHES

At first, Dr. Sagara and his fellow researchers have tested a treatment regimen involving the separate application of sodium hyaluronate, autologous serum, and antibiotic eye drops to treat bleb leak. However, while the epithelial defects were resolved for a few months, this treatment method was not effective for a complete cessation of leakage in most patients. This may have been due to the difficulty for some patients to adhere to the regimen, which involves frequent administration of multiple eye drops every day. However, Dr. Sagara and his colleagues are currently running a clinical trial to test the efficacy of combined treatments, preserving both aqueous and lipid components of the tear film, in preventing bleb point-leak. They first determine the presence of OMGD and tear dysfunction in patients with late-onset point-leak following filtration surgery and mitomycin C treatment, and evaluated the long-term outcome of eye drops containing both Sodium hyaluronate and autologous serum (to augment the aqueous layer of the tear film) and eyelid massage and warm compresses (to facilitate secretion of meibum to counteract the OMGD). This treatment strategy could be described as an approach to stop aqueous leakage through the promotion of wound healing of the defective bleb surface by ensuring a well-functioning tear film. Eye drops combining both sodium hyaluronate and autologous serum are practically convenient and patient-friendly. Moreover, sodium hyaluronate is known for strong adherence to the body muocus membranes, including the eye's, which prevents the quick evaporation of the eye drops, allowing a prolonged action.

PRESENT AND FUTURE OF BLEB LEAK TREATMENT

Despite the inconvenience and modest efficacy, the regime based on the application of sodium hyaluronate and autologous serum separately is currently approved in Japan for the treatment of late-onset bleb leak. Therefore, in case of a positive outcome of the current trail on combined eye drops as well as combined treatment approaches, the current treatment guidelines shall be adapted accordingly. Dr. Sagara is currently planning more clinical trials to test therapies again other disorders of the ocular surface.

Researcher Profile

Dr. Hideto Sagara
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Dr. Sagara is an interventional ophthalmologist who is interested in exploring various treatment methods eye cataract and glaucoma. He currently works at the Glaucoma Clinic of Fukushima Medical University, while being the Director of Marui Eye Clinic. Dr. Sagara is the executive director of Fukushima Ophthalmologist Society, and a member of a number of other scientific societies such as Japanese Ophthalmological Society, the Japan Society of Ophthalmic Surgeons and the Japanese Glaucoma Society. In 1999, Dr. Sagara won The Young Researcher's Award of the Japanese Society of Ophthalmological Optics, and in 2010, he won the Chairman's Award of The 64th Annual Congress of Japan Clinical Ophthalmology.

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Contact lens Hygiene: Importance and tools

Professor Mark Willcox is a medical microbiologist who is particularly interested in studying the microbial colonization and the pathogenesis of the adverse events associated with contact lens wear. Here we introduce his background and motivation, with a highlight on his current research interest and activity.

To begin, what is your academic and research background and how did your career in scientific research start?

I obtained my undergraduate degree in Applied Biological Science from the University of the West of England (formerly Bristol Polytechnic). During my undergraduate studies, I had the great opportunity to work in industry for one year, and I was lucky enough to be placed at a Veterinary Investigation Centre in Reading, UK. This placement sealed my interest in microbiology – specifically medical microbiology and its role in disease diagnosis, monitoring and treatment. After obtaining my degree in 1983, I moved to the University of Manchester to start my PhD studies with Dr. David Drucker, studying the bacteria causing dental caries. This prompted my interest in microbial colonisation of surfaces. In 1988, I was lucky enough to be offered a Research Fellowship at the Institute of Dental Research in Sydney, Australia. There, I continued my research on oral microbiology for five years before moving to the School of Optometry and Vision Science, UNSW to study microbial colonisation of contact lenses. I have been in this field ever since, as well as broadening my research into fields of ocular biochemistry and immunology, and clinical trials.

What has been your primary motivation as a scientist, and why did you choose medical microbiology as a domain?

My primary motivation is discovery and the application of discoveries for the benefit of people. I am particularly interested in aspects of applied or translational science. My research has examined, for example, methods for combating microbial adhesion to dentures, contact lenses, cochlear implants and other materials used in or on the human body. Also, I am interested in the development of microbial resistance to antibiotics and disinfectants, and the emergence of microbial diseases. Medical microbiology allows me to investigate all of these issues, and has allowed me to develop new ways of combating diseases. I am a strong advocate of interdisciplinary research. For example, my research path has involved interactions with dentists, optometrists, ophthalmologists, epidemiologists, immunologists, biochemists, chemists, and material scientists. I have been fortunate also to collaborate with industry. One aspect of this has been the development of new contact lens materials, and I am currently working with Professor Naresh Kumar at UNSW in Australia and the LV Prasad Eye Institute in Hyderabad India to test new antimicrobial surfaces on contact lenses in clinical trials.

What are the potential health hazards that might result from poor contact lens hygiene?

Contact lenses are a very effective form of vision correction. They give better all-round vision than glasses. However, since they come in contact with a very delicate organ, they need to be properly cleaned and disinfected. After use, contact lenses should be kept in special storage cases filled with disinfecting solution to eliminate microorganisms. Failure of the disinfection process can lead to the growth of microbes in the storage cases and on the lens surface. These microbes can infiltrate the eye leading to corneal inflammation and in severe cases ulceration. Despite the advents made in the development of contact lens disinfecting solutions, eye infections continue to occur among contact lens users, indicating that an efficient, easy-to-use disinfection system is still lacking.

You are currently performing a clinical trial on a new contact lens disinfecting solution. What is the nature of the new disinfectant, and what are the goals of the clinical trial?

The product currently under test is called Cleadew, a disinfectant based on povidone iodine, developed by a Japanese company, Ophtecs. Povidone iodine has an excellent spectrum and intensity of antimicrobial activity, and is known as a powerful wound and skin disinfectant, however, it is currently being used for the first time as a contact lens disinfection system. The current trial aims to test the efficacy of Cleadew to eliminate microbial colonization of contact lens and their storage cases, as well as its effect on the safety and comfort of the eye when used on a daily basis.

Following the completion of the clinical trial, are you planning to extend your research on contact lens care further? What might be the scope of your next step?

I have established collaboration with Professor Fiona Stapleton from the School of Optometry and Vision Science UNSW, through which we will jointly perform a clinical trial to test the efficacy of silver-containing contact lens cases in eliminating microbial contamination. The trial is based on the known antimicrobial activity of silver, as well as our previous findings for storage cases made of a particular type of silver, which showed to prevent microbial colonisation.

Contact Lens disinfecting Solutions for Proper Hygiene

Professor Mark Willcox is currently performing a clinical trial to test a unique contact lens disinfecting solution. Here we discuss the importance of maintaining good contact lens hygiene and adverse consequences of lens contamination, before detailing the properties of the new disinfecting solution and the goals of the clinical trial.

CONTACT LENS HYGIENE: WHAT CAN GO WRONG?

Soft contact lenses were first released around the world in the 1970s and silicone hydrogel lenses were released in the early 2000s. Nowadays, more than 150 million people around the globe are using contact lenses, either for medical or cosmetic purposes. Daily wear of contact lenses requires the regular use of a storage case, which contains an antimicrobial solution to disinfect the lenses overnight. Storage cases play an essential role in contact lens hygiene. However, without proper maintenance, the case may become contaminated with microorganisms during handling. Microbial contamination of contact lens cases can compromise contact lens wear and lead to serious adverse events. Furthermore, scientists have isolated identical strains of bacteria from corneal ulcers and contact lens storage cases, suggesting the case being a reservoir for the microorganisms responsible for the ulcer. Several studies have shown that lens case contamination is common, ranging from 30 to 80 percent. Despite good compliance, lens case contamination can still occur. Further compounding the problem is the formation of what is called 'microbial biofilms', which are aggregates of microbes held together within a self-produced matrix. These biofilms render microbes more resistant to the effects of disinfectants. Therefore, Professor Willcox's advises contact lens users to follow the hygiene instructions of the lens and disinfecting solution's manufacturers. This ideally includes the total replacement of the disinfectant solution usually each month and air-drying the storage case after each use. He also recommends the frequent replacement of the storage case, advisably when a new disinfecting solution is purchased.

Failure to maintain the hygiene of contact lenses or contact lens storage cases may result in contact lens–related complications, mainly due to the infiltration of the cornea by microorganisms. This causes inflammation of the cornea (scientifically known as keratitis), which is a potentially sight-threatening condition. A recent epidemiology study estimates at least 50 percent less risk of developing corneal keratitis if case hygiene is appropriately maintained.

LENS CARE SOLUTIONS TO IMPROVE HYGIENE

Contact lens disinfecting and cleaning systems are essential elements in contact lens care. Most soft contact lens users use multipurpose disinfecting solutions for antimicrobial protection. The most widely used contact lens disinfecting and cleaning solutions contain a variety of disinfectants and various cleaning agents including surfactants. The usage rate of multipurpose disinfecting solutions has gradually increased to account for 90 percent of disinfection types in the United Kingdom and Australia. However, over the past 20 years, epidemiological studies of contact lens wear showed an almost constant rate of microbial keratitis associated with lens wear, even with the release of new disinfecting solutions and new lens types. Surprisingly, the initial estimates, published in 1989, for the rates of microbial keratitis during daily wear are almost identical to those published in 1999 and 2008.

A UNIQUE DISINFECTING SOLUTION UNDER CLINICAL INVESTIGATION

Professor Willcox and researchers at the School of Optometry and Vision Science, UNSW are currently testing a new contact lens disinfecting solution called Cleadew. The solution is developed and manufactured by Ophtecs Corporation (Kobe, Japan), which is also the sponsor of the clinical study. Cleadew is based on 'povidone iodine', a fast-acting disinfectant with a wide antibacterial spectrum. To many of us, povidone iodine is well-known as a topical antiseptic in minor skin cuts, grazes and abrasions in order to prevent infections.

Failure to maintain the hygiene of contact lenses or contact lens storage cases may result in contact lens–related complications, mainly due to the infiltration of the cornea by microorganisms.

While iodine is poorly soluble in water, Ophtecs has developed a unique water soluble preparation suitable for disinfection of contact lens. Cleadew is provided in a package containing small tablets and a dissolving solution. These tablets are composed of a rapidly acting outer layer of povidone iodine and a late-acting inner layer containing an iodine neutralizing agent and a proteolytic enzyme (breaks down protein particles). The contact lenses to be disinfected are placed into the storage case, and then the provided solution is added to the case along with one of the iodine tablets. As the tablet is placed in the solution, the outer layer dissolves quickly, releasing the iodine, which disinfects both the contact lenses and the storage case. Over time, the inner layer eventually dissolves to neutralize the iodine and to release the proteolytic enzyme which helps to remove lens deposits. This system allows the contact lens to be disinfected within 5 minutes, which is significantly faster than other solutions. However, the manufacturers recommend keeping the lenses in the case for four hours, allowing sufficient time for the action of the cleaning enzyme.

The main goal of the current clinical trial on Cleadew is to evaluate the status of microbial contamination in lens storage cases during daily wear. Professor Willcox and his fellows are investigating the level of microbes in cases using traditional microbial culture techniques as well as non-culture techniques which can detect microbial DNA in the storage cases. Additionally, the eyes of the participants are being examined for comfort and absence of irritation during the use of the disinfectant. Professor Willcox and his colleagues have shown in previous clinical trials that contact lens cases can be contaminated by a variety of microbes, and that some disinfecting solutions allow Gram-negative bacteria to grow in storage cases. ‘Certain types of disinfecting solution result in around 50 percent of the examined cases being colonized by Gram-negative bacteria’, said Professor Willcox. Furthermore, users of the disinfecting solutions that allowed the growth of Gram-negative bacteria experienced a higher incidence of keratitis. The current clinical trial is important to validate the efficacy and safety of Cleadew for use as contact lens disinfectant.

Prior to market release, and in addition to success in clinical trials, all contact lens disinfecting solutions need to comply with governmental regulatory requirements of efficacy and safety. The International Organization for Standardization (ISO) 14729 ‘stand alone’ disinfection test is often recommended to demonstrate efficacy. The test requires qualifying contact lens disinfecting solutions to show a specific spectrum and intensity of antimicrobial activity at the manufacturer’s recommended disinfection time. Cleadew has successfully passed these tests, as well as other tests that demonstrated excellent efficacy against Gram-negative bacteria known to resist other disinfecting solutions. With regard to safety, Cleadew has been proven safe in multiple tests examining the toxicity of its solution to mammalian cells in the laboratory. This is in addition to limited trials on volunteers to assess potential irritation to the eye during use. The findings of these regulatory tests were presented at many international conferences such as the ‘British Contact Lens Association Annual Meeting’, recently held in 2015. Interestingly, Cleadew has recently acquired the CE mark, which authorizes its commercialization in the European markets.

THE NEXT STEP IN CONTACT LENS HYGIENE RESEARCH

Professor Willcox is planning to continue research and clinical trials on contact lens disinfecting solutions. He is currently collaborating with Professor Fiona Stapleton from the School of Optometry and Vision Science, UNSW in studying some further aspects of microbial contamination of contact lens cases and the efficacy of silver-containing contact lens storage cases. Silver has a good antimicrobial activity and can kill many different types of microbes. ‘We have previously shown that one particular type of silver lens case had significantly less microbes in the case than normal control cases’, said Professor Willcox. This work will be mainly done by a dedicated doctoral researcher, Ananya Datta, who is planning to test the feasibility of other silver-containing storage cases to minimize lens microbial colonisation and the associated adverse effects in a clinical trial.

Researcher Profile

Professor Mark Willcox
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Professor Willcox is interested in contact lens research, specifically studying the microbial causes of adverse events during contact lens wear. He has established several animal and cell-based models for studying contact lens adverse events and biomaterial infections. He developed new techniques that allow researchers, for the first time, to assess the contribution of particular proteins or lipids to the production of adverse responses during lens wear. He also investigated the use of tears as the source of biomarkers for diabetes, breast and prostate cancer. His research has led to the publication of 12 patents and over 350 peer reviewed papers.

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